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Cynthia Alkhoury Abboud

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THÈSE EN COTUTELLE PRÉSENTÉE

POUR OBTENIR LE GRADE DE

DOCTEUR DE

L'UNIVERSITÉ DE BORDEAUX

ET DE L'UNIVERSITÉ DU SAINT ESPRIT KASLIK

ÉCOLE DOCTORALE DES SCIENCES DE LA VIE ET DE LA SANTE

ÉCOLE DOCTORALE DE L'USEK

SPÉCIALITÉ NEUROSCIENCE

Par Cynthia ALKHOURY ABOUD

**CONTRÔLE PEPTIDERGIQUE DE LA DOULEUR :
MODULATIONS DES VOIES DESCENDANTES PAR LES
SYSTÈMES RELAXINE.**

Sous la direction de : Pr. Marc LANDRY
Co-directeur : Pr. Walid HLEIHEL

Soutenue le 31 mars 2021

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Titre : Contrôle peptidergique de la douleur: modulation des voies descendantes par les systèmes relaxine.

Résumé :

La douleur chronique souvent accompagnée d'anxiété et de dépression est un fléau mondial. La modulation de la douleur par les neuropeptides (NP) est bien connue au niveau des afférences primaires et de la moelle épinière. Peu de données sont toutefois disponibles sur leur rôle dans la douleur dans le cerveau. La famille relaxine comprend la relaxine, présente dans le système nerveux central (SNC) et qui possède des propriétés antifibrotiques, et la relaxine-3, strictement exprimée dans le SNC et qui présente des effets anxiolytiques et antidépresseurs. Notre objectif est d'étudier la modulation de la douleur par les neuropeptides relaxine et relaxine-3 dans un modèle de douleur inflammatoire persistante chez la souris.

Nos résultats démontrent que non seulement le système relaxine-3 / RXFP3, mais aussi le système relaxine / RXFP1 encore très peu exploré dans le SNC, ont des effets analgésiques en conditions de douleur inflammatoire. Les sites d'action de ces systèmes peptidergiques comprennent des régions corticales (cortex cingulaire, claustrum) et sous-corticales (amygdale) qui régulent les voies descendantes et l'intégration sensorielle dans la moelle épinière. Nos données mettent en évidence le potentiel thérapeutique de cette famille peptidergique dont les rôles dans la douleur n'avaient jamais été testés.

Mots clés : douleur chronique, amygdale, cortex cingulaire antérieur, relaxine, relaxine-3

Unité de recherche

Institut des maladies neurodégénératives CNRS UMR 5293

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Title: Peptidergic control of pain: modulation of descending pathways by the relaxin systems

Abstract:

Chronic pain, often accompanied by anxiety and depression, is a global scourge. The modulation of pain by neuropeptides (NP) is well known at the level of primary afferents and the spinal cord. However, little data is available on their role in pain in the brain. The relaxin family includes relaxin, which is present in the central nervous system (CNS) and has antifibrotic properties, and relaxin-3, which is strictly expressed in the CNS and has anxiolytic and antidepressant effects. Our objective is to study the modulation of pain by the neuropeptides relaxin and relaxin-3 in a mouse model of persistent inflammatory pain.

Our results show that not only the relaxin-3 / RXFP3 system, but also the relaxin / RXFP1 system, which is still poorly explored in the CNS, have analgesic effects in conditions of inflammatory pain. The sites of action of these peptide systems include cortical (cingulate cortex, claustrum) and subcortical (amygdala) regions that regulate descending pathways and sensory integration in the spinal cord. Our data highlight the therapeutic potential of this peptide family, whose role in pain has never been tested before.

Keywords: Chronic pain, amygdala, anterior cingulate cortex, relaxin, relaxin-3.

Unité de recherche

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Cette thèse de doctorat a été réalisée au sein de l'Institut Interdisciplinaire de Neurosciences CNRS UMR5297 dirigé par Dr. Daniel Choquet dans l'équipe « Mécanisme central de la sensibilisation à la douleur » dirigée par le Pr. Marc Landry, et dans l'Institut des Maladies Neurodégénératives CNRS UMR 5293 dirigé par Dr. Thomas Boraud dans l'équipe « Signalisation purinergique, inflammation et troubles neurologiques » dirigée par le Pr. Eric Boué Grabot et le Pr. Marc Landry. Cette thèse a été conduite sous la direction du Pr Marc Landry et du Pr Walid Hleihel. Ce travail a été financé par une bourse du CNRS-libanais ainsi que par le Labex Brain. Les résultats obtenus au cours de ce projet ont donné lieu aux publications et communications suivantes.

Publications:

Animal models of pain: Diversity and benefits.

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J Neurosci Methods (2020) 108997

Analgesic effect of relaxin receptor (RXFP-1) activation on persistent inflammatory pain in mice: behavioral and neurochemical data.

Abboud C., Brochoire L., Drouet A., Akhter Hossain M., Hleihel W. and Landry M.
En soumission pour Pain report

Communications orales :

The effect of relaxin-3 on pain: behavioral and morphological data

Abboud C., Hleihel W. and Landry M.

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The effect of relaxin-3 on pain: behavioral and morphological data

Abboud C., Hleihel W. and Landry M.

Relaxin meeting, **October 2019-Paris**

The effect of relaxin-3 on pain: behavioral and morphological data

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Je dédie ce travail à mon pays, en espérant qu'il renaitra de ses cendres tel un phœnix

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Liste des abréviations

5-HT : 5-hydroxytryptamine ou somatostatine
ACC: cortex cingulaire antérieur
aCSF: liquide céphalo-rachidien artificiel
ADNc: acide désoxiribonucléique complémentaire
AMP: amplificateur
AMY: amygdale
AN: lobule ansiforme
AON: noyau olfactif antérieur
ATP: adenosine triphosphate
BLA: amygdale basolatérale
BMA: amygdale basomédiane
CaMKII: calcium/calmodulin-dependent protein kinase II
CeA: amygdale central
CFA: adjuvant complet de Freund
CGRP : calcitonine-gene related peptide
CLA: claustrum
CPSE: courants postsynaptiques excitateurs
DRG : Dorsal root ganglia
ENTl: entorhinal area, partie latérale
FN: noyau fastigial
GABA: acide gamma-aminobutyrique
GAD: glutamate décarboxylase
H2: relaxine humaine-2
HIP: region hippocampale
IASP : international association for the study of pain
IC: cortex insulaire
Icv: intracérébroventriculaire
IGF-1: insulin growth factor 1
IGF1R: récepteur d'IGF-1

kDa: kilodalton
LDLa: low density lipoprotein A
LH: habenule latérale
LRR: leucine rich repeat
LS: noyau latéral septal
MAPK : Protéine kinase activée par un mitogène
MD: noyau médiodorsal du thalamus
ME : moelle épinière
MEA: noyau amygdalien médial
Mgr : Mas related G protein coupled receptors.
MH: habenule médiane
MPO: medial preoptic area
mRNA: acide ribonucléique messenger
NaCl: acide chloridrique
NDB: noyaux de la bande diagonale de Broca
NGF : nerve growth factor
NI : nucleus incertus
PAG: substance grise périaqueducule
PBS: phosphate-buffered saline
PC: commissure postérieure
PERI: perirhinal area
PFA: paraformaldehyde
PFC: cortex préfrontal
PIR: cortex piriform
PKC: protéine kinase C
PN: noyau paranigral
PnR: noyau pontine raphe
PO: complexe postérieur du thalamus
PWL: paw withdrawal latency (seuil de retrait de la patte)
PWT: paw withdrawal threshold (latence de retrait de la patte)
Rln-3: relaxine-3

RN: noyau rouge

RT: noyau réticulaire du thalamus

S1 et S2 : cortex somatosensiel

SALRP: somatostatin and angiotensin like peptide receptor

SEM: erreur standard des moyennes

SNC: système nerveux central

SNP: système nerveux périphérique

SRT : spinoréticulothalami tract

SSp: cortex somatosensiel primaire

SST: cortex somatosensiel primaire

SST: somatostatine

STT: spinothalamic tract

SUB: subiculum

TrKA : tyrosine kinase A receptor

TT: taenia tecta

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Introduction

1. Douleur

1.1. Définition et épidémiologie

La douleur est définie, depuis 1979, par l'association internationale pour l'étude de la douleur (IASP) comme étant « une expérience sensorielle et émotionnelle désagréable, associée à une lésion réelle ou potentielle, ou décrite dans ces termes » (1979). De là, la douleur est caractérisée par ses 3 composantes : la composante sensorielle-discriminative qui permet de déterminer l'intensité et la localisation de la douleur, la composante affective qui permet de déterminer la réponse émotionnelle à la douleur de manière assez subjective puisqu'elle est relative à chaque individu, et la composante cognitive qui permet d'évaluer la douleur et par conséquent moduler sa perception (Crofford, 2015; Ellison, 2017).

La douleur peut être aussi classée en fonction de la durée de l'évènement, ainsi la douleur est qualifiée d'aigue si la durée est inférieure à 3 mois, et de chronique si la durée est supérieure à 3 mois (Bouhassira et al., 2008; Treede et al., 2015). La douleur aigue est bénéfique et nécessaire puisqu'elle joue un rôle protecteur vis-à-vis de l'organisme contre tous types de stimuli (mécanique, thermique, chimique) (Orr et al., 2017). Cependant la douleur chronique est désormais considérée comme un fléau mondial qui dégrade la qualité de vie (Orr et al., 2017; Treede et al., 2019). En effet 20% de la population mondiale souffre de douleur chronique, ce pourcentage augmente pour atteindre 30% en France (Queneau et al., 2018; Treede et al., 2015). La douleur chronique est souvent accompagnée de dépression, ainsi 85% des personnes souffrantes de douleur chronique souffrent également de dépression (Bair et al., 2003; Williams et al., 2003). Malheureusement la douleur chronique reste mal gérée cliniquement avec 40% des patients n'ayant pas recours à une gestion adéquate de leur douleur (Breivik et al., 2006; van Hecke et al., 2013).

1.2. Types de douleur

On peut distinguer 3 types majeurs de douleur en se basant sur la physiopathologie complexe de la douleur.

1.2.1. Douleur nociceptive

La douleur nociceptive constitue effectivement le système d'alarme protecteur de l'organisme qui se déclenche en réponse aux lésions tissulaires engendrant des sensations douloureuses assez désagréables au point qu'elles ne peuvent pas être ignorées (Costigan et al., 2009). Les lésions tissulaires en réponse aux stimuli de type mécaniques, thermiques ou irritants peuvent avoir lieu aux niveaux des organes, muscles ou os (Orr et al., 2017). Par conséquent la douleur nociceptive est subdivisée en 2 groupes : d'une part on a la douleur nociceptive somatique qui constitue la réponse à une atteinte qui peut être soit superficielle (au niveau de la surface de la peau) soit profonde (aux niveaux des os), et d'autre part on a la douleur nociceptive viscérale qui correspond à une réponse vis-à-vis d'une atteinte des organes internes et qui peut être ressentie indirectement (douleur différée) (Ellison, 2017; Orr et al., 2017).

1.2.2. Douleur neuropathique

La douleur neuropathique est considérée comme étant une douleur chronique pathologique résultant d'une lésion ou d'un dysfonctionnement du système nerveux central (SNC) ou du système nerveux périphérique (SNP) (Bouhassira et al., 2008; Yam et al., 2018). Les principales causes de ce type de douleur sont les maladies métaboliques (diabète), les traumatismes, les infections (herpès), la sclérose en plaques, les accidents vasculaires cérébraux et les tumeurs... (Costigan et al., 2009; Ellison, 2017; Yam et al., 2018). La douleur neuropathique est caractérisée par une augmentation de la sensibilité centrale et périphérique vis-à-vis des stimuli douloureux (hyperalgie), et des stimuli non douloureux (allodynie) (Ellison, 2017) et elle est souvent accompagnée d'une sensation de brûlures, d'engourdissements, de picotements et de douleurs lancinantes (Orr et al., 2017).

1.2.3. Douleur inflammatoire

La douleur inflammatoire surgit après une lésion tissulaire engendrant une réaction inflammatoire. Ce type de douleur est aussi caractérisé par une augmentation de la sensibilité périphérique et centrale causée par la libération des cellules du système immunitaire d'un ensemble de médiateurs inflammatoires, connu sous le nom de soupe inflammatoire (partie développée ultérieurement). Par conséquent la douleur inflammatoire est souvent accompagnée d'hyperalgie et d'allodynie. Il est important de signaler que ce type de douleur dure aussi longtemps que

l'inflammation et cesse donc quand celle-ci n'y est plus (Ciaramella, 2019; Costigan et al., 2009; Yam et al., 2018)

1.3. Nociception

La nociception correspond au processus physiologique qui mène à la détection consciente de la douleur. Ce processus renferme 4 étapes (Fig.1) : la 1^{ère} étant la transduction du stimulus douloureux qu'il soit mécanique, thermique ou chimique, suivie par la transmission, puis la perception et finalement la modulation (Ellison, 2017; Renn and Dorsey, 2005)

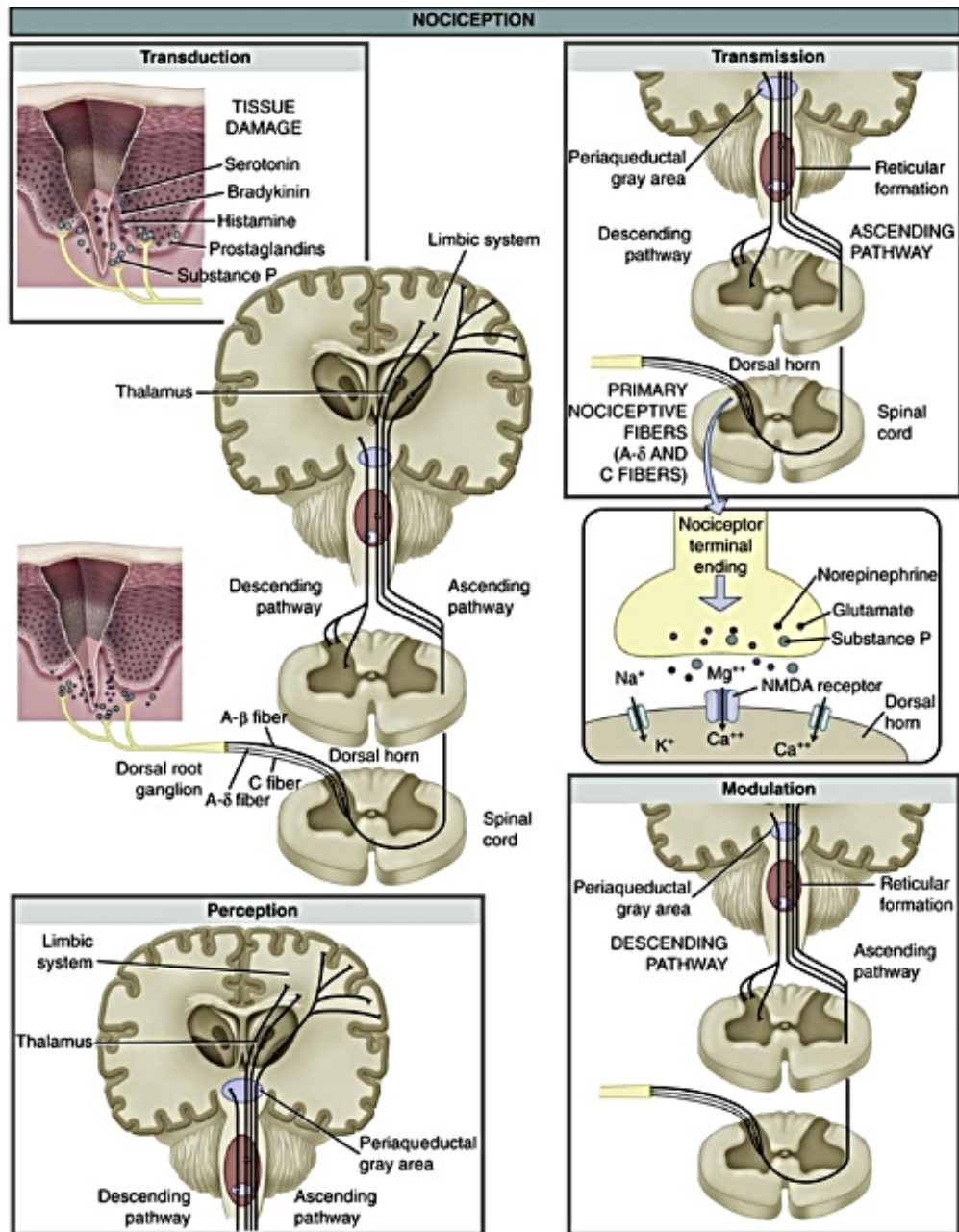


Figure 1: les 4 étapes de la nociception (Ellison, 2017)
 Les 4 étapes de nociception renferment la transduction, la transmission, la perception et la modulation

1.3.1. Transduction : de la périphérie à la moelle

La transduction correspond à la conversion des stimuli nocifs en potentiel d'action, par les nocicepteurs.

1.3.1.1. Généralités sur les nocicepteurs

Les nocicepteurs ou fibres afférentes primaires nociceptives sont des neurones spécialisés ayant une forme pseudo-unipolaire. En effet le corps cellulaire de ces neurones est localisé dans les ganglions de la racine dorsale (DRG) pour les nocicepteurs du corps et dans les ganglions du trijumeau pour les nocicepteurs du visage. De ce corps cellulaire émane une tige axonale qui se divise en 2 branches : d'une part la branche périphérique qui innerve les organes cibles et qui est dotée de terminaisons libres et d'autre part la branche centrale qui innerve la moelle épinière (ME). Les nocicepteurs ne sont excités que si l'intensité du stimulus (mécanique, thermique ou chimique) est assez élevée pour provoquer des dommages tissulaires, c'est pourquoi on parle toujours de stimuli nocifs (Basbaum et al., 2009; Yam et al., 2018).

1.3.1.2. Classification

On distingue 2 grands types de nocicepteurs : les fibres afférentes primaires A δ et C.

Les fibres A δ sont myélinisées de diamètre moyen compris entre 2-5 μ m, avec une vitesse de conduction de signal de 30 m/s. Ces fibres projettent vers les couches lamina I et V de la moelle épinière (Fig. 2) et sont responsables de la première sensation douloureuse, décrite comme étant une douleur aigue, rapide, piquante et localisée. Les fibres A δ sont divisées en 2 groupes : les fibres de type I qui répondent aux stimuli mécaniques et aux stimuli thermiques de très forte intensité (supérieur à 50 °C), alors que les fibres de type II répondent aux stimuli thermiques de moindre intensité et aux stimuli mécaniques de très haute intensité (Basbaum et al., 2009; Julius and Basbaum, 2001; Yam et al., 2018).

Les fibres C quant à elles, elles sont amyéliniques avec un plus petit diamètre et une vitesse de conduction de 2m/s. Ces fibres projettent vers les couches lamina I et II de la moelle épinière (Fig.2) et sont responsables de la deuxième sensation de douloureuse, décrite comme étant tardive, lente, brûlante et diffuse. Les fibres C sont divisées soit en fibres polymodales, répondant aux stimuli mécaniques, thermiques et chimiques, soit en nocicepteurs silencieux répondant aux stimuli thermiques et non mécaniques sauf dans le cas de blessure. Les fibres C peuvent être aussi divisées en fibres peptidergiques et fibres non peptidergiques. Les fibres C peptidergiques expriment le récepteur TrKA (tyrosine kinase A receptor) qui se lie au NGF (nerve growth factor) et libère la substance P et le CGRP (calcitonine-gene related peptide). Les fibres non-peptidergiques expriment les récepteur c-RET, les récepteur de la famille Mgr (Mas related G

protein coupled receptors) et le récepteur purinergique P2X3 (Basbaum et al., 2009; Julius and Basbaum, 2001; Yam et al., 2018).

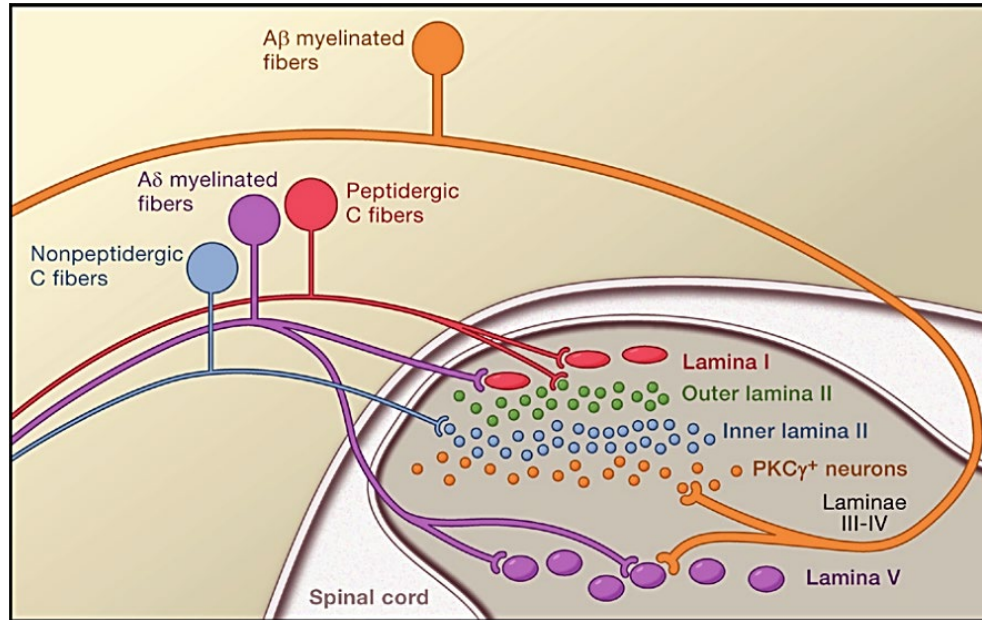


Figure 2: Projections des fibres afférentes primaires (Basbaum et al., 2009).

Les fibres C-peptidergiques (en rouge) projettent vers lamina I, les fibres C-non-peptidergiques projettent vers la lamina II, les fibres Aδ (en mauve) projettent vers lamina I et V.

1.3.1.3. Neurochimie de la douleur

Afin de pouvoir remplir leur rôle de transducteur d'information nociceptive, les nocicepteurs traduisent les différents stimuli en signaux électriques grâce à la présence de différents canaux ioniques sur leur surface. Les canaux du potentiel transitoire de récepteur (TRP) sont activés par les stimuli thermiques, par exemple TRPV1 (canal TRP vanilloïde-1) est activé par une chaleur douloureuse (seuil de 43°C) alors que TRPM8 et TRAP1 sont activés par le froid. Les stimuli mécaniques activent les canaux DEG/ENaC, ASIC, TRPV2, TRPV4, et les canaux potassiques KCNK. Les stimuli chimiques quant à eux ils activent une panoplie de canaux et de récepteurs : l'ATP se fixe sur P2X, NGF se fixe sur TrKA, les acides se fixent sur ASIC3 (Fig.3)

L'activation de ces différents récepteurs engendre un potentiel électrique au niveau du nocicepteur, connu sous le nom de potentiel du récepteur. Ce potentiel active les canaux ioniques voltage dépendant. Les canaux sodiques voltage dépendant et les canaux potassiques voltage dépendant génèrent le potentiel d'action (PA) par dépolarisation de la membrane cellulaire. Les

canaux calciques voltages dépendant influencent la libération des neurotransmetteurs (nt) aux niveaux périphérique et central (Basbaum et al., 2009; Julius and Basbaum, 2001).

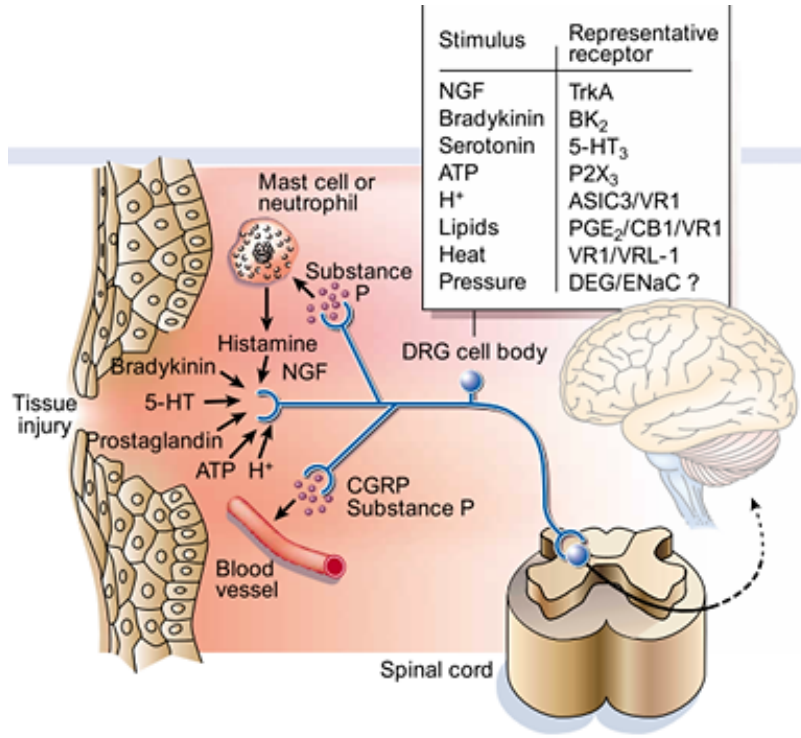


Figure 3: Activation des différents récepteurs par les stimuli nociceptifs. (Julius and Basbaum, 2001).
 Suite à une lésion tissulaire, l'activation des nocicepteurs est due à l'activation des récepteurs présents sur la surface membranaire des nocicepteurs. NGF se fixe à TrkA, bradykinine se fixe sur BK₂, sérotonine se fixe sur 5-HT₃, ATP se fixe sur P2X₃, H⁺ se fixe sur SIC3, lipides se fixent sur PGE₂.

1.3.2. Transmission et voies ascendantes : de la moelle au cerveau

La transmission correspond au passage du potentiel d'action depuis l'extrémité périphérique du nocicepteur jusqu'à l'extrémité centrale pour être conduit dans un premier temps dans la ME et par la suite aux structures supra-spinales.

Au niveau de la corne dorsale de la ME, les fibres Aδ et C interagissent soit directement avec les neurones de projections, soit indirectement via les interneurons spinaux qui à leur tour interagissent avec les neurones de projections, pour atteindre les structures supra-spinales à travers le tractus spinothalamique (STT) ou spinoréticulothalamique (SRT) (Fig.4)(Ellison, 2017; Renn and Dorsey, 2005). Le tractus spinothalamique assure le relais de l'information jusqu'au thalamus et est impliqué dans la composante sensorielle-discriminative de la douleur, alors que le SRT assure le relais jusqu'au tronc cérébral et est impliqué dans la composante émotionnelle-affective de la douleur (Basbaum et al., 2009; Reddi et al., 2013).

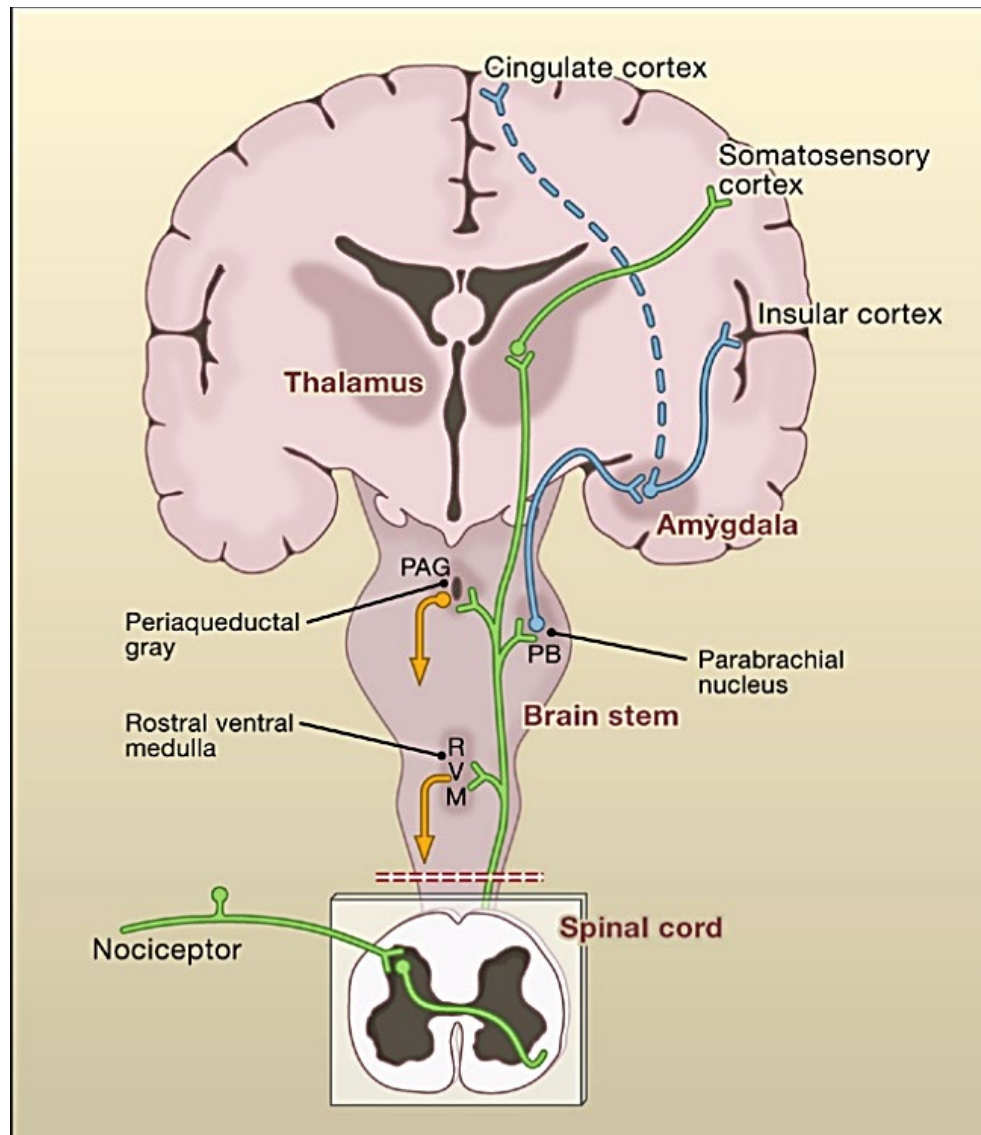


Figure 4: Les voies ascendantes de la douleur. (Basbaum et al., 2009).
 L'information nociceptive atteint la corne dorsale de la ME et est transmis aux structures supra-spinales via le tractus spinothalamique (vert) ou spinoréticulothalamique (bleu). Le STT transmet l'information au thalamus qui projette vers le cortex somatosensoriel (composante sensori-discriminative). Le SRT transmet l'information au tronc cérébral qui projette vers l'amygdale (composante émotionnelle)

1.3.3. Perception et intégration cérébrale

Les voies ascendantes acheminent donc l'information nociceptive depuis la corne dorsale jusqu'aux structures supra-spinales, par conséquent la perception de la douleur ne revient pas à l'activité d'une région cérébrale distincte mais plutôt d'un ensemble de régions prénommé « matrice de la douleur ». La matrice englobe entre autre les régions suivantes : thalamus, cortex somatosensoriel (S1 et S2), cortex cingulaire antérieur (ACC), cortex insulaire (IC), cortex

préfrontal (PFC), amygdale (AMY), hippocampe et substance grise périaqueducule (PAG) (Brodin et al., 2016).

Une région clé de cette matrice est le thalamus. Le thalamus reçoit les projections souvent glutamatergiques depuis la corne dorsale qui interagissent avec les neurones de 3^{ème} ordre des noyaux du thalamus (Ralston, 2005). Ces neurones projettent à leur tour vers le cortex somatosensoriel ce qui permet de déterminer l'intensité et la localisation de la douleur, ces neurones projettent aussi vers le cortex limbique (ACC, IC, PFC) et l'amygdale, régions impliquées dans la composante émotionnelle de la douleur (Bourne et al., 2014).

En ce qui concerne la composante affective, les régions les plus impliquées sont l'ACC et l'amygdale (Gao et al., 2004). Les études électrophysiologiques menées sur les animaux et les humains valident l'implication de l'ACC dans la composante affective (Davis et al., 1997; Koga et al., 2010; Porro et al., 1998; Rainville et al., 1997; Sikes and Vogt, 1992; Yamamura et al., 1996). De plus l'ACC a un réseau de connectivité assez complexe (Fig.5), puisqu'il reçoit des projections ou inputs nociceptifs depuis le thalamus (Dum et al., 2009; Shyu and Vogt, 2009), l'amygdale (Han et al., 2015; Ma and Peschanski, 1988) et le cortex (S1 et IC) (Eto et al., 2011) puis à son tour émet des projections ou outputs vers le PAG (Bragin et al., 1984), l'amygdale (Apps and Strata, 2015; LeDoux, 2000), le PFC (Medalla and Barbas, 2012) et même des projections directement à la ME (Chen et al., 2014).

L'AMY est une des régions les plus impliquée dans l'émotion (Janak and Tye, 2015; Pape and Pare, 2010), de plus les études électrophysiologiques ainsi que le fMRI chez l'humain ont mis en évidence l'implication de l'AMY dans la douleur (Becerra et al., 2001; Bernard and Besson, 1990; Bornhövd et al., 2002; Neugebauer and Li, 2002). Par conséquent l'AMY est impliquée dans la composante affective de la douleur (Thompson and Neugebauer, 2017). En effet la CeA (amygdale centrale) reçoit l'information nociceptive (Fig.6) soit directement via le tractus spino-parabrachio-amygdaloïde, soit indirectement depuis la LA/BLA (amygdale latérale et amygdale basolatérale) qui reçoit des inputs sensoriels de la part du thalamus et des régions corticales auxquels LA/BLA rajoute l'information émotionnelle (Bernard and Besson, 1990; Janak and Tye, 2015; Neugebauer, 2015; Neugebauer et al., 2004, 2009; Veinante et al., 2013). La CeA projette par la suite vers les régions impliquées dans la peur et l'anxiété d'une part et vers les voies descendantes de l'autre (Bourgeois et al., 2001; Jongen-Rêlo and Amaral, 1998; Pape and Pare, 2010; Pomrenze et al., 2015; Sah et al., 2003).

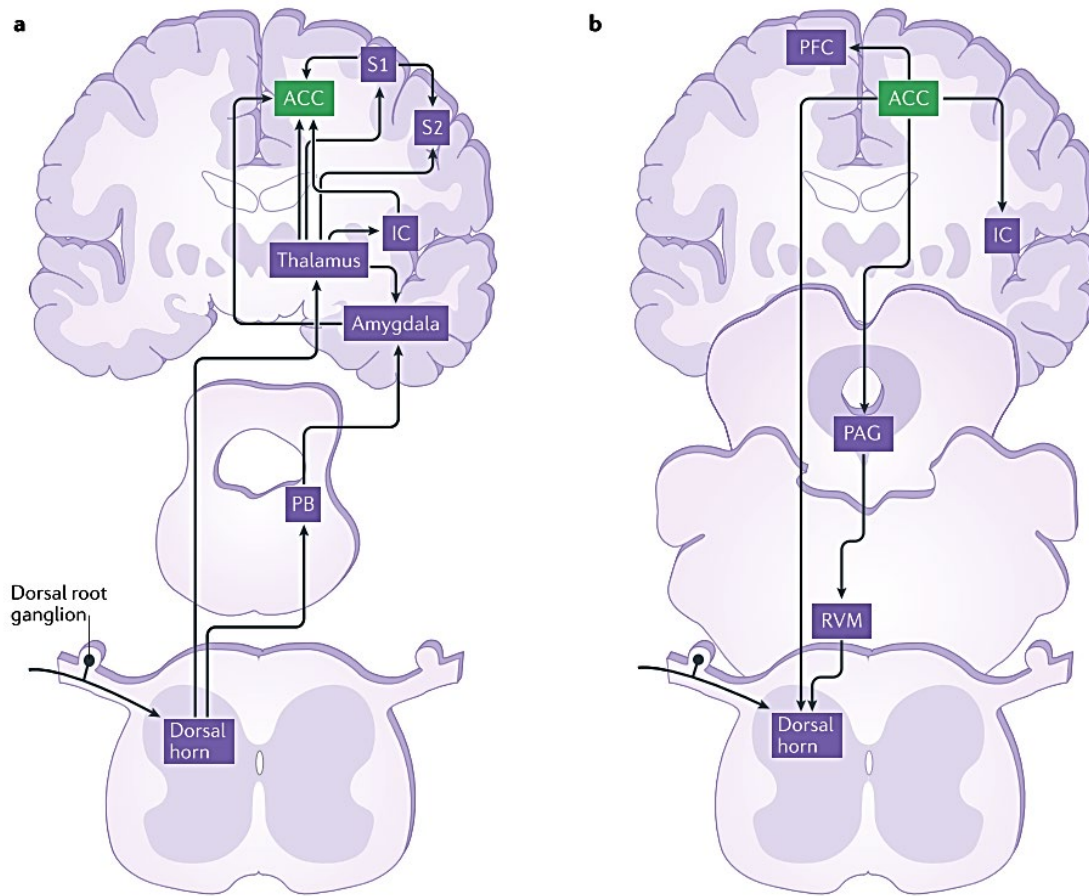


Figure 5: Réseau complexe de l'ACC. (Bliss et al., 2016)
 L'ACC reçoit des inputs nociceptifs de plusieurs régions cérébrales (a) : thalamus, amygdale et cortex. ACC projette (b) vers le PAG, l'amygdale et la ME

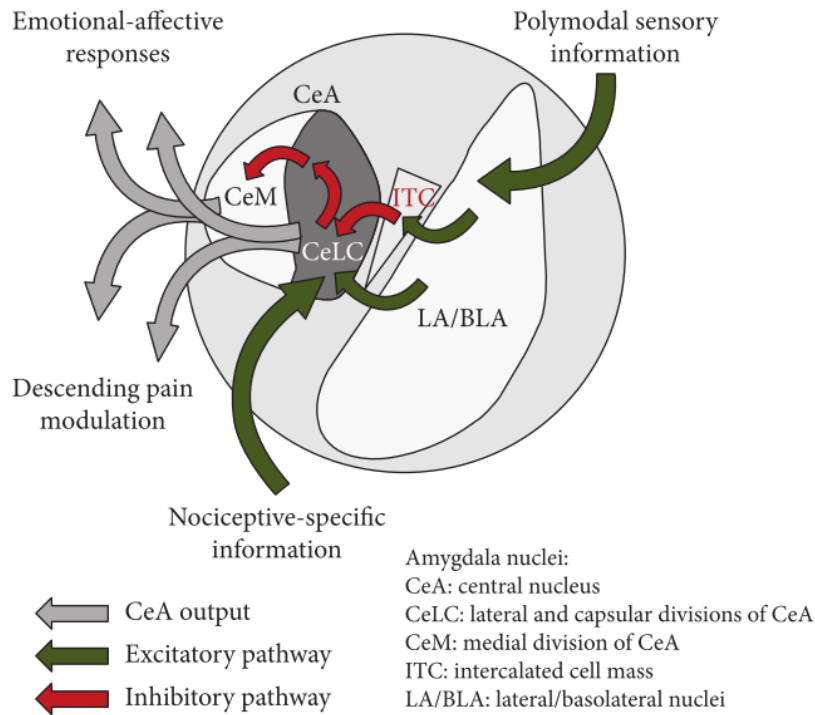


Figure 6: Neurocircuitrie de l'amygdale (Thompson and Neugebauer, 2017)

LA/BLA reçoit l'information sensorielle depuis le thalamus et les régions corticales, auxquelles elle rajoute l'information émotionnelle et les relaie au CeA. Cette transmission peut-etre inhibée par les interneurons GABAergiques de l'ITC. La CeLC reçoit l'information nociceptive via le tractus spino-parabrachio-amygdaloïde. La CeA projette soit vers les régions impliquées dans la peur et l'anxiété, soit vers les voies descendantes.

1.3.4. Modulation et voies descendantes

La perception de la douleur est modulée par les voies descendantes. Ainsi les structures supra-spinales projettent directement vers la ME ou indirectement en projetant dans un premier temps vers le tronc cérébral qui envoie des projections à la ME (Kwon et al., 2014). Les voies descendantes généralement projettent vers les lamina I et II, et les modulations seraient due en majorité aux voies mono-aminergiques (sérotonine, dopamine et norépinephrine)(Millan, 2002; Pertovaara, 2006; Zhao et al., 2007). Une des voies descendantes les plus importantes est la voie PAG-RVM (Rostral Ventral Medulla) (Fig.7) (Ossipov et al., 2010). Le PAG reçoit des inputs de plusieurs régions supra-spinales (cortex frontal, IC, AMY, hypothalamus) et renvoie des projections vers la RVM (Beitz, 1982; Mantyh, 1983; Ossipov et al., 2014). Outre les inputs du PAG, la RVM reçoit aussi des inputs du noyau parabrachial et du thalamus (Heinricher et al., 2009; Ossipov et al., 2010; Vanegas and Schaible, 2004). Puis la RVM projette directement vers la ME pour soit inhiber la nociception, soit au contraire pour l'augmenter. Ce contrôle bidirectionnel est du à la présence des cellules « on » et « off » dans la RVM. Ainsi les cellules « on » activent la nociception alors que les cellules « off » l'inhibent (Heinricher et al., 2009; Kwon et al., 2014; Ossipov et al., 2014).

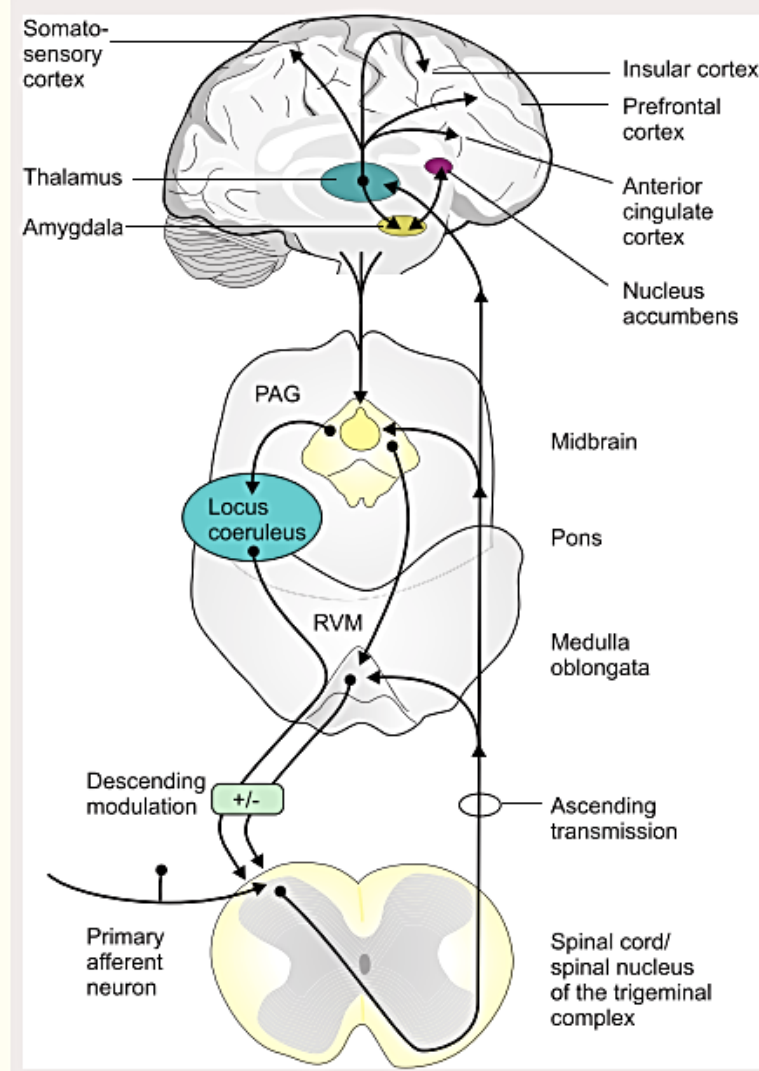


Figure 7: Voies descendantes (Brodin et al., 2016)

Le PAG reçoit des inputs des régions supra-spinales et envoie des projections vers la RVM qui influence la nociception au niveau de la ME, soit en l'inhibant soit en la stimulant

1.4. Sensibilisation et chronicité de la douleur

1.4.1. Sensibilisation périphérique et soupe inflammatoire.

La sensibilisation périphérique résulte dans la majorité des cas d'un changement lié à l'inflammation dans l'environnement chimique des nocicepteurs (McMahon and Bevan, 2005). Ce changement d'environnement conduit à l'hyperalgie en réduisant le seuil d'activation des nocicepteurs et par conséquent augmente leur sensibilité vis-à-vis de la douleur. En effet suite à la lésion tissulaire, il y a libération de plusieurs médiateurs inflammatoires, connue sous le nom de

soupe inflammatoire, par les mastocytes, plaquettes et autres cellules inflammatoires (Fig.8). Ces médiateurs se fixent sur leurs récepteurs respectifs, ce qui active différentes voies de signalisations intracellulaires (PKA, PKC, MAPK, p38). L'activation de ces voies stimule la régulation génétique dans les corps cellulaires des nocicepteurs des gènes codant les récepteurs de ces médiateurs ainsi que les gènes codant les canaux ioniques et ceux codant les TRP. D'autre part cette soupe inflammatoire stimule la libération des neuropeptides tels que la substance P et CGRP aux niveaux périphérique et central des nocicepteurs, processus connu sous le nom d'inflammation neurogénique. Ces neuropeptides sont de puissants vasodilatateurs, par conséquent il y aura un afflux de mastocytes, plaquettes et cellules inflammatoires, donc une augmentation de la libération des neuromédiateurs (Brodin et al., 2016).

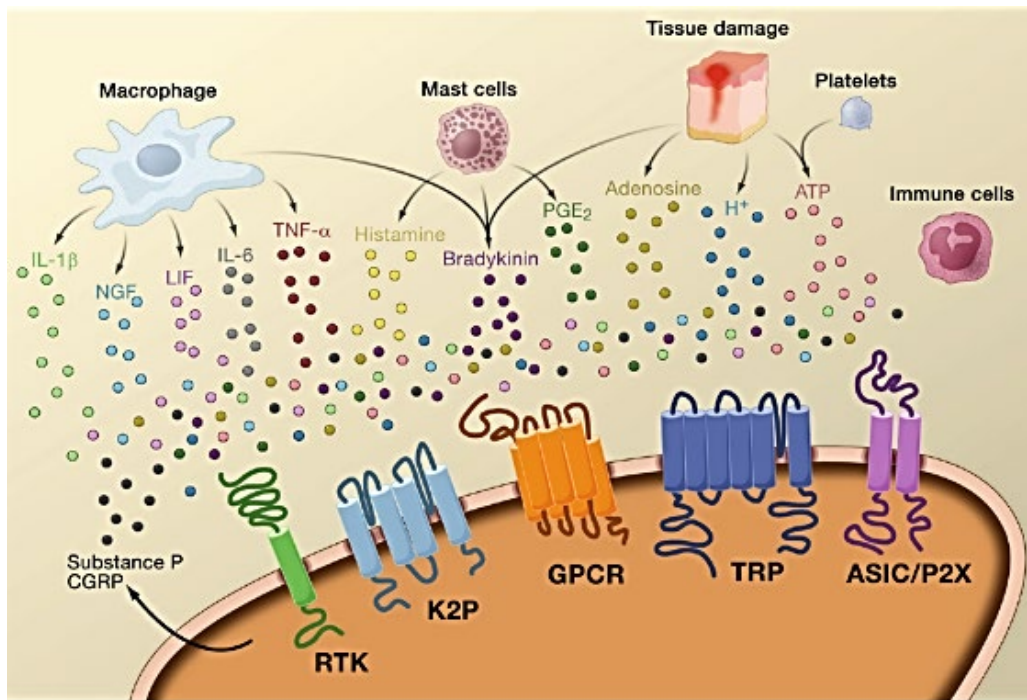


Figure 8: Soupe inflammatoire (Basbaum et al., 2009)

Suite à une lésion tissulaire, les macrophages, les mastocytes, les plaquettes et les cellules immunitaires sécrètent une soupe inflammatoire : interleukine, TNF α , histamine, NGF, bradykinine, adénosine, protons, ATP. Ces médiateurs modulent l'activité du nocicepteur en se fixant sur les différents récepteurs présents sur la surface : TRP, ASIC (canaux ionique sensible à l'acide), canaux potassiques (K2P), récepteur de la tyrosine kinase (RTK), les récepteurs couplés à la protéine G (CGRP).

1.4.2. Sensibilisation centrale

La sensibilisation centrale mène à un état d'hyperexcitabilité du SNC conduisant à une augmentation du traitement de messages nociceptifs (Woolf, 1983). On peut distinguer 3 mécanismes à la base de la sensibilisation centrale : l'altération de la transmission glutamatergique, la désinhibition et l'activation microgliale (Fig.9).

1.4.2.1. Altération de la transmission glutamatergique

Au cours d'une douleur aigue, le glutamate libéré par les nocicepteurs au niveau de la ME se fixe sur les récepteurs AMPA et génère des courants postsynaptiques excitateurs (CPSE). La sommation des CPSE mène à l'émission de PA activant les neurones spinaux. Cependant dans des conditions pathologiques chroniques le glutamate se fixe aussi sur les récepteurs NMDA normalement silencieux car bloqués par le magnésium. Cette fixation mène à une augmentation de l'influx calcique, ce qui par conséquent active les voies de signalisation calcium dépendante (MAPK, PKC), facilitant ainsi la transmission des messages nociceptifs au cerveau (Basbaum et al., 2009; Latremoliere and Woolf, 2009; Lu et al., 1999; Woolf and Salter, 2000).

1.4.2.2. Désinhibition

La désinhibition correspond à la perte de la libération continue de GABA et/ou glycine par les interneurons inhibiteurs de la ME, libération observée dans les conditions physiologiques. Ce tonus inhibiteur permet donc de diminuer l'excitabilité des neurones spinaux. Dans le cas pathologique ce tonus est perdu et une hyperalgie s'installe. De plus la désinhibition permet aux fibres afférentes primaires A β de s'intégrer dans le circuit de la douleur en transmettant des stimuli normalement inoffensifs et qui seront perçus comme douloureux (allodynie) (Basbaum et al., 2009; Melzack and Wall, 1965).

1.4.2.3. Activation microgliale

Les lésions nerveuses périphériques mènent à la libération de l'ATP et de la chimiokine fractalkine qui stimulent la microglie (par activation de ses récepteurs membranaires) à libérer le BDNF qui favorise l'excitabilité des neurones spinaux menant à l'hyperalgie et l'allodynie. De plus les microglies activées libèrent un éventail de cytokines qui contribuent à la sensibilisation centrale (Basbaum et al., 2009).

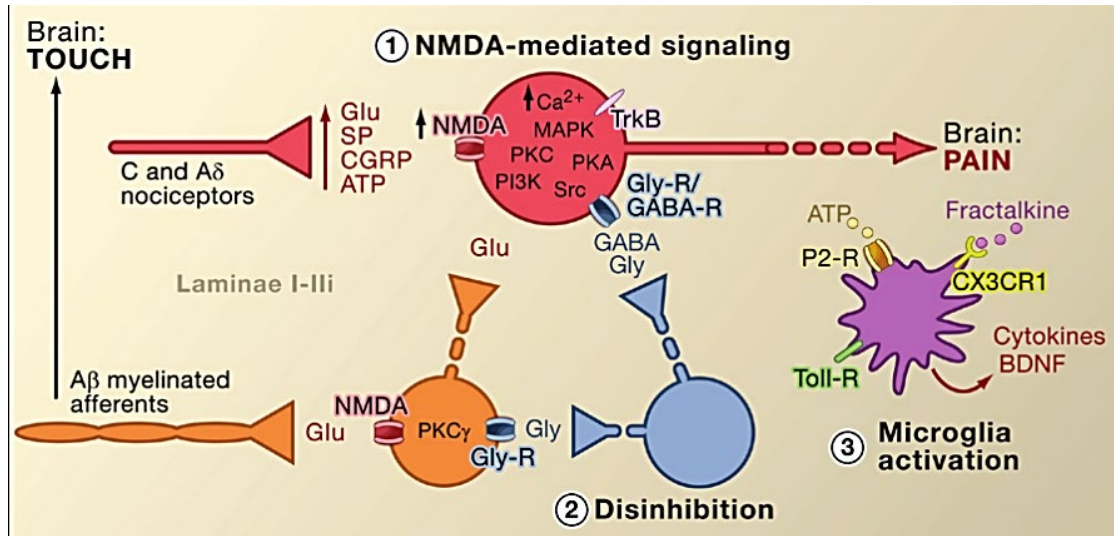


Figure 9: Mécanismes de sensibilisation centrale (Basbaum et al., 2009)

1.4.3. Douleur chronique

La sensibilisation périphérique ainsi que la sensibilisation centrale persistent même après la guérison périphérique suite à un trauma ou une intervention chirurgicale, et dans la plupart des cas on a un retour à la normale progressif. Cependant quand la stimulation nociceptive perdure et devient intense, on observe des changements au niveau de la plasticité des neurones qui engendrent une continuité de sensibilisation (périphérique et centrale) menant ainsi à une douleur chronique caractérisée par une hyperalgie, allodynie et douleur spontanée (Kwon et al., 2014; Sandkühler, 2009).

La douleur chronique reste à ce jour mal gérée cliniquement malgré son impact sur la vie quotidienne des personnes qui en souffrent. Les travaux menés au sein de mon équipe ont mis en évidence l'effet analgésique de la relaxine-3 dans un modèle de douleur inflammatoire persistante. La deuxième partie de cette introduction s'attardera alors sur ce neuropeptide.

2. Modèles animaux

La douleur est un phénomène complexe, afin de mieux comprendre la physiopathologie de la douleur ainsi que d'essayer de trouver de nouveaux traitements pour mieux gérer la douleur, les chercheurs ont eu recours aux modèles animaux. La revue en annexe (Abboud et al., 2020), soumise au Journal of neuroscience methods décrit les techniques utilisées pour évaluer la douleur, les modèles animaux de douleur et les modèles animaux ayant un succès translationnel.

Cette thèse étudie la modulation de la douleur chronique par les systèmes relaxine, c'est pourquoi on a eu recours à un modèle de douleur inflammatoire persistante et plus précisément aux souris injectées de CFA (adjuvant complet de Freund), puisque les rongeurs injectés de CFA représentent un des modèles les plus fréquemment utilisés de douleur chronique inflammatoire. Cependant la douleur chronique n'est pas une condition unique, mais plutôt un terme utilisé pour faire référence à un état de douleur qui dure dans le temps et qui a différentes étiologies résultantes de différentes pathologies et ayant différents signes cliniques (Burma et al., 2017). Par conséquent, il serait intéressant de s'attarder sur les différents modèles animaux, représentatifs des différents types de douleur chroniques. De plus les modèles animaux sont les techniques de base qui permettent d'étudier les mécanismes de douleur et d'élaborer et de tester les nouveaux traitements, de ce fait cette revue s'attarde aussi sur les succès translationnels ainsi qu'aux limitations rencontrées.

3. Relaxine

3.1. La famille relaxine

La famille relaxine est une famille de peptides apparentés à l'insuline, en effet ces peptides ont divergé de l'insuline au cours de l'évolution des vertébrés pour former une famille à part (Halls et al., 2007). Chez l'être humain, la famille relaxine renferme 7 membres : la relaxine-1 (H1), la relaxine-2 (H2), la relaxine-3 (H3), les peptides apparentés à l'insuline (INSL)-3, INSL-4, INSL-5, INSL-6. Tout comme l'insuline, les peptides relaxine sont sécrétés premièrement en tant que prohormone avec 3 chaînes peptidiques A, B et C. La chaîne C permet le repliement des chaînes A et B et aide donc à la formation des 3 ponts disulfure. Suite à un clivage enzymatique *in vivo*, les peptides relaxine acquièrent leur forme hétérodimérique mature (Fig. 10) (Chan et al., 2011).

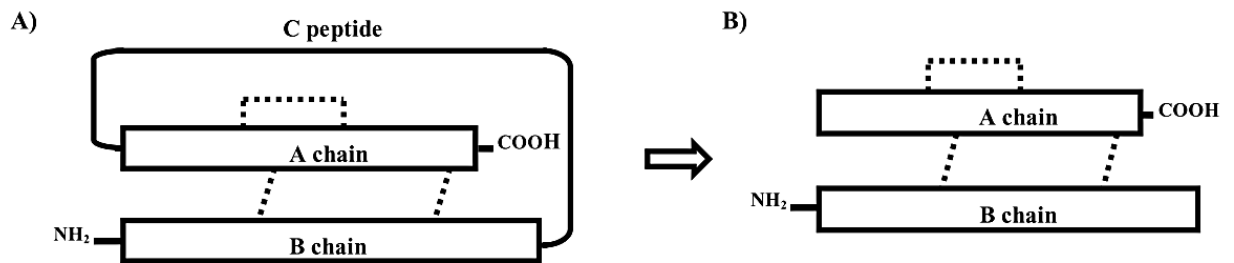


Figure 10: Structure de la relaxine. (Chan et al., 2011)

La relaxine est sécrétée sous forme d'une prohormone avec 3 chaînes (A), suite à un clivage enzymatique *in vivo*, la relaxine retrouve sa forme active avec 2 chaînes peptidiques et 3 ponts disulfure

H1, H2 et H3 ont un même motif liant Arg-XXX-Arg-XX-Ile/Val au niveau de la chaîne B. (Hsu, 2003; Wilkinson et al., 2005a, 2005b). Le rôle de la relaxine-1 humaine est inconnu pour le moment. La relaxine-2 humaine correspond à la relaxine-1 chez les autres mammifères, donc pour éviter toute confusion, ce peptide sera appelé relaxine, et elle joue un rôle hormonal antifibrotique. La relaxine humaine 3 est majoritairement impliquée dans le système nerveux (Bathgate et al., 2013), et c'est elle qui constitue l'intérêt primordial de cette thèse.

3.2. Système Relaxine-3/RXFP-3

3.2.1. Relaxine-3

3.2.1.1. Structure

La relaxine-3 a été découverte en 2001 en recherchant les bases de données de Celera Discovery System et de Celera Genomics pour des homologues de la relaxine (Bathgate et al., 2002). En effet la relaxine-3 répond aux caractéristiques des membres de la famille relaxine : en étant d'une part un peptide de 5 KDa formé de 51 acides aminés qui s'étalent sur 2 chaînes peptidiques A et B avec 3 ponts disulfure, et d'autre en ayant le motif liant Arg-XXX-Arg-XX-Ile/Val présent au niveau de la chaîne B (Bathgate et al., 2002).

Le gène relaxine-3 est considérée comme étant l'ancêtre des peptides relaxine et il est hautement conservé à travers les espèces (Wilkinson et al., 2005b; Yegorov et al., 2009).

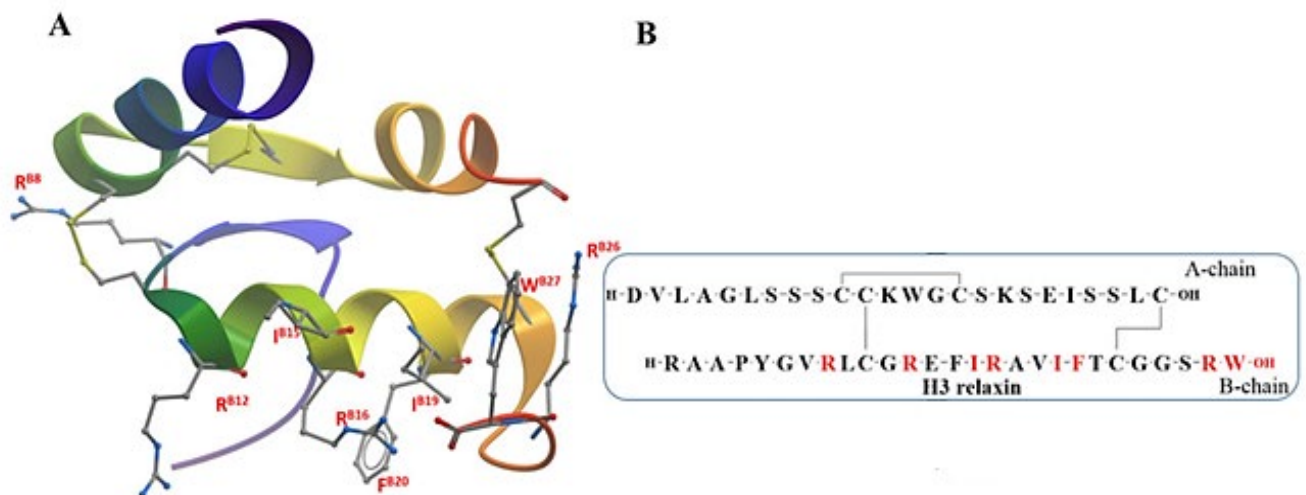


Figure 11: Structure de relaxine-3 (Patil et al., 2017)

Comme l'insuline, la relaxine-3 est formée de 2 chaînes de peptides reliées par 2 ponts disulfures avec un troisième sur la chaîne A (A). Les 51 acides aminés formant la relaxine-3 (B).

3.2.1.2. Distribution.

L'expression de relaxine-3 est strictement limitée au cerveau, par conséquent rln-3 est qualifiée de neuropeptide (Smith et al., 2010). La distribution des fibres rln-3 a été étudiée chez le rat et la souris et elle est assez similaire, cependant la distribution n'a pas encore été étudiée chez l'être humain (Bathgate et al., 2013). Ainsi les études ultrastructurales menées sur cerveaux de rats ont montré que la rln-3 est aussi bien présente dans le corps cellulaire des neurones, et plus

spécifiquement dans l'appareil de golgi et dans le réticulum endoplasmique, qu'aux niveaux des vésicules des terminaisons nerveuses (Ma et al., 2009; Tanaka et al., 2005). Le mapping a montré que la majorité des neurones rln-3 se trouve au niveau du nucleus incertus (NI) (2000 neurones), néanmoins on retrouve aussi la rln-3 aux niveaux du noyau pontine raphé (PnR) (350 neurones), du PAG (550 neurones), d'une zone dorsale à la substance noire (350 neurones) (Ma and Gundlach, 2007; Ma et al., 2007; Smith et al., 2010; Tanaka et al., 2005). Les fibres rln-3 du NI projettent vers différentes régions cérébrales telles que : PFC, cortex cingulaire, hippocampe, thalamus, hypothalamus et le tronc cérébral (Goto et al., 2001; Ma and Gundlach, 2015; Olucha-Bordonau et al., 2003). De plus le NI est assez proche du 4^{ème} ventricule, par conséquent c'est une cible pour les signaux neurohumoraux (Ma and Gundlach, 2015). L'analyse immunohistochimique (IHC) des neurones rln3 a mis en évidence leur nature GABérgique. En effet les neurones rln3 tout comme les neurones GABA expriment la glutamate décarboxylase (GAD), enzyme de synthèse du GABA (Ford et al., 1995; Ma and Gundlach, 2007; Olucha-Bordonau et al., 2003). De plus chez les rats, les neurones rln3 du NI expriment aussi le récepteur CRF-1, le récepteur à la sérotonine 5HT1A et les récepteurs orexines et hypocertines (Smith et al., 2011).

3.2.2. *RXFP-3*

3.2.2.1. Structure

RXFP-3 est le récepteur apparenté à la relaxine-3 (Bathgate et al., 2006a). Il a été premièrement découvert en 2000 au cours d'un screening de librairie d'ADNc du cortex de cerveau humain à la recherche des récepteurs couplés à la protéine G. Il fut nommé premièrement SALRP (somatostatin and angiotensin like peptide receptor) à cause de l'homologie des séquences avec le récepteur à la somatostatine SSTR5 et avec le récepteur à l'angiotensine II (AT1), ainsi on observe une similitude de 35% avec SSTR5 et de 31 % avec AT1 (Matsumoto et al., 2000). Par la suite il fut nommé aussi GPCR 135. En effet RXFP-3 est un récepteur couplé à une protéine G inhibitrice, avec 7 domaines transmembranaires et un domaine extracellulaire court (Halls et al., 2007) (Fig.12). Il est vrai que la relaxine-3 peut se fixer sur RXFP-1, RXFP-3 et RXFP-4, mais RXFP-3 est le récepteur apparenté à relaxine-3 puisque d'une part il y a un chevauchement entre la distribution de rln-3 et RXFP-3, ce qui n'est pas le cas avec RXFP-1 et RXFP-4 qui n'est même pas exprimé dans le cerveau. D'autre part RXFP-3 est uniquement activé par rln-3 ce qui n'est pas

le cas pour RXFP-1 (activé par relaxine) et RXFP-4 (activé par INSL5)(Callander and Bathgate, 2010; Liu et al., 2003; Sudo et al., 2003; Sutton et al., 2005) .

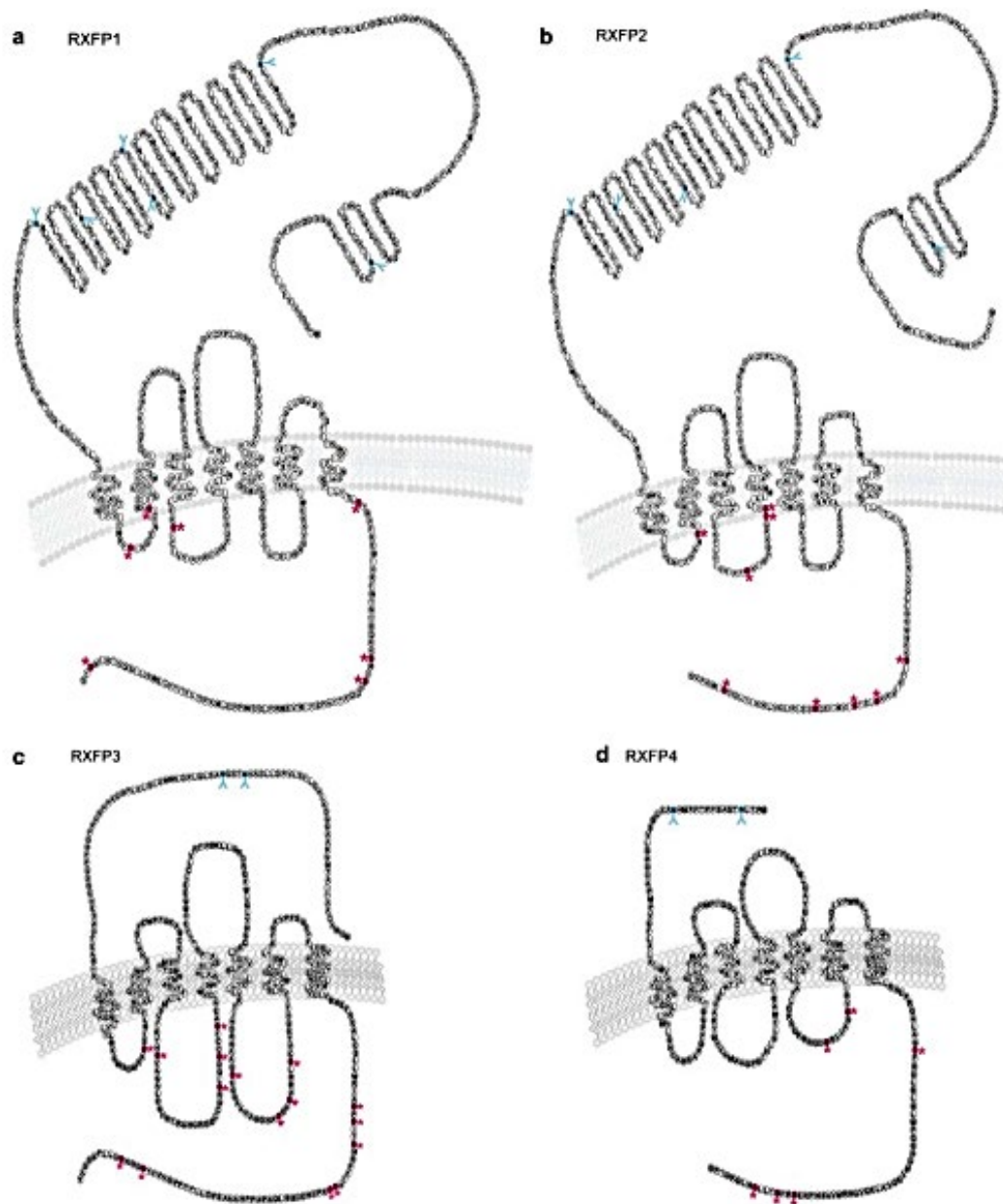


Figure 12: Structure des récepteurs de la famille relaxine (RXFP1-4) (Halls et al., 2007).
Tous les récepteurs (a RXFP-1, b RXFP-2, c RXFP-3, d RXFP-4) ont 7 TM. La taille du domaine extracellulaire est plus grande chez RXFP-1 et 2 que chez RXFP-3 et 4. Les sites de glycosylation sont marqués en bleu et les sites de phosphorylation en rose

3.2.2.2. Distribution

RXFP-3 est exprimé majoritairement dans le cerveau, cependant on peut le retrouver aussi aux niveaux des testicules, des glandes salivaires et du thymus (Chen et al., 2005; Liu et al., 2003; Matsumoto et al., 2000).

La distribution cérébrale de RXFP-3 a été étudiée en détail chez les rongeurs, et un grand chevauchement a été observé entre la distribution de RXFP-3 et celle de rln-3. Parmi les régions où on observe le plus de chevauchement entre les fibres rln-3 et RXFP-3, on peut citer : hypothalamus, septum, hippocampe, raphé médian, PAG, amygdale (centrale et médiane), Néanmoins dans certaines régions cérébrales, ce chevauchement n'est pas observé, par exemple au niveau du cortex les fibres rln-3 sont surtout observés dans le cortex retro spinal et médian alors que rxfp-3 est plutôt exprimé aux niveaux des régions latérales et ventrales. Ce qui peut sous-entendre que RXFP-3 peut être aussi activé par un transfert de volume depuis le site de libération de rln-3. En effet les travaux de Smith et al. ont permis d'avoir une répartition cérébrale détaillée des fibres rln-3, de RXFP-3 et de la densité de site de fixation chez la souris C57Bl6, (Fig. 12-1 et Fig. 12-2, tableau 1)(Kong et al., 2010; Ma et al., 2017; Smith et al., 2010, 2011).

Même si la distribution cérébrale a été détaillée chez souris et rats, quelques études ont été menées sur cerveaux humains et ont permis de déterminer la présence d'un taux élevé de RXFP-3 dans le cerveau de patient souffrant d'Alzheimer (Lee et al., 2016).

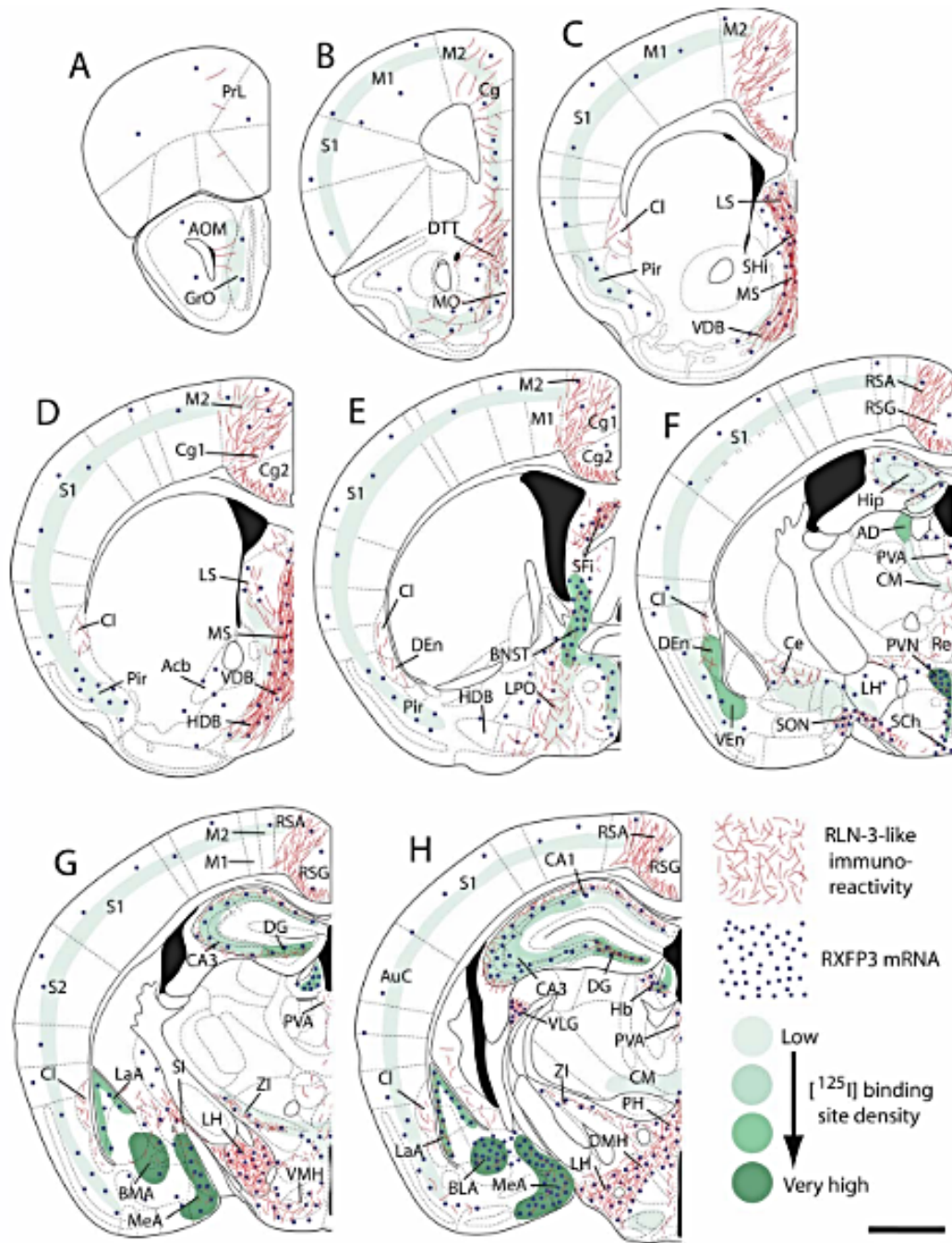


Figure 13: Illustration schématique (partie 1) de la distribution des fibres *rln-3* (*rln3*-like immunoreactivity), des ARNm de RXFP-3 et de la densité des sites de fixations de RXFP-3, sur coupes coronales de cerveau de souris C57Bl6/J (Smith et al., 2010)

Une série de dessins coronaux, adaptée de l'atlas stéréotaxique de (Paxinos et Watson, 2007), illustrant la distribution et la densité relative du RLN3-LI (fines lignes rouges), de l'ARNm de RXFP3 (points bleus) et des sites de liaison (zones vertes, avec un ombrage clair indiquant une faible densité et un ombrage sombre indiquant une densité élevée de sites de liaison).

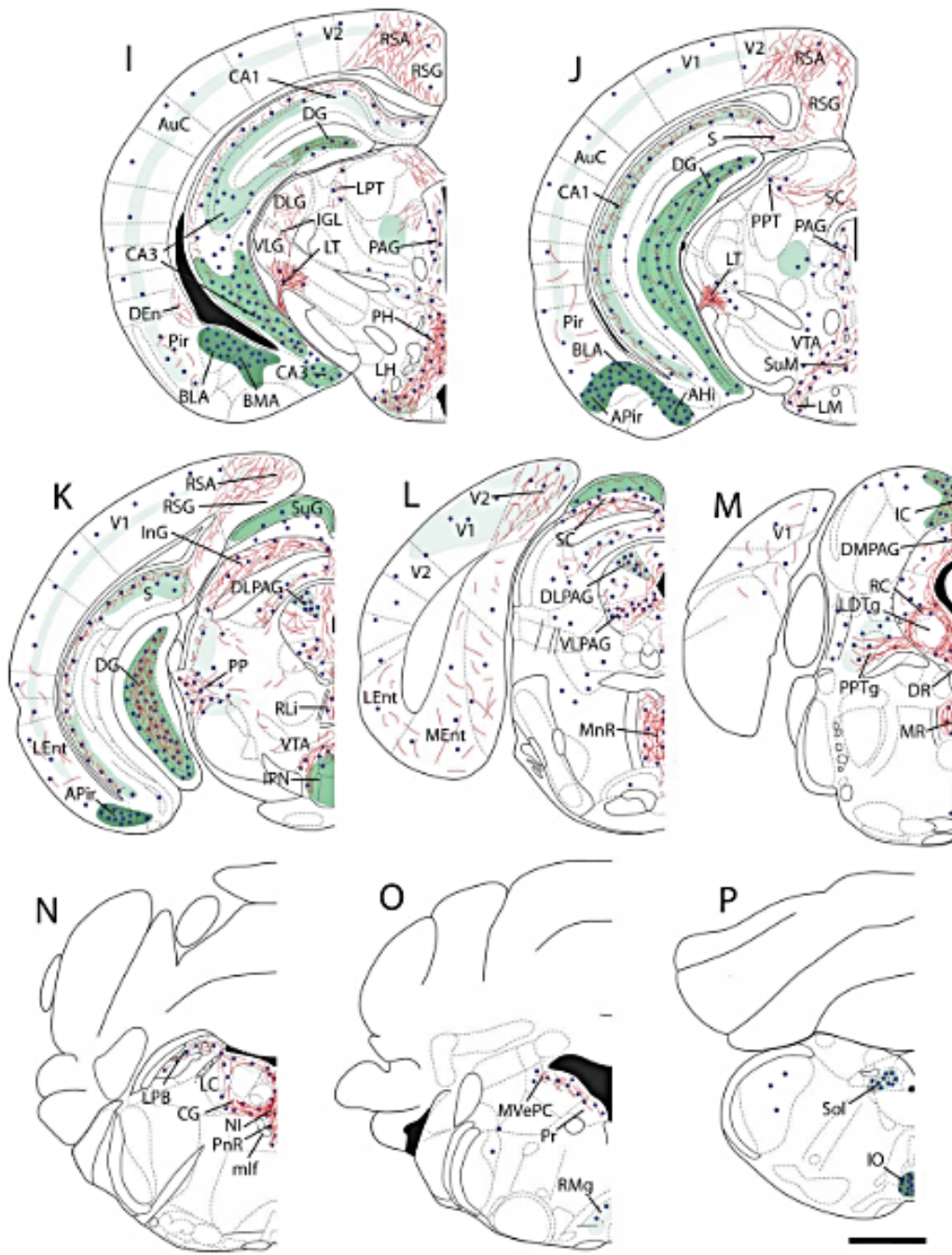


Figure 14: Illustration schématique (partie 2) de la distribution des fibres rln-3 (rln3-like immunoreactivity), des ARNm de RXFP-3 et de la densité des sites de fixations de RXFP-3, sur coupes coronales de cerveau de souris C57Bl6/J (Smith et al., 2010)

Une série de dessins coronaux, adaptée de l'atlas stéréotaxique de (Paxinos et Watson, 2007), illustrant la distribution et la densité relative du RLN3-LI (fines lignes rouges), de l'ARNm de RXFP3 (points bleus) et des sites de liaison (zones vertes, avec un ombrage clair indiquant une faible densité et un ombrage sombre indiquant une densité élevée de sites de liaison).

3.2.2.3. Voies de signalisation

L'activation de RXFP-3 par la relaxine-3, plus spécifiquement par la chaîne B de rln3 mène à l'inhibition de l'adénylate cyclase par les protéines G inhibitrices $G_{\alpha i2}$ et G_{0B} , par conséquent il y a diminution de l'AMPc. De plus l'activation de ces protéines G inhibitrices mène à l'activation de plusieurs voies de signalisation nécessitant l'internalisation de RXFP-3 (Fig.13). D'une part on a l'activation de la voie de signalisation PI3K, qui déclenche une cascade d'activation des voies MAPK, menant à la phosphorylation de MEK1/2 qui par conséquent mène à la phosphorylation d'ERK1/2. De l'autre, on a l'activation de la voie PLC qui mène aussi à l'activation de la voie MEK1/2 et à la phosphorylation d'ERK1/2. De plus l'activation de la voie PLC mène aussi à l'activation de la voie MKK4/7 des MAPK, menant à l'activation et la phosphorylation de p38 et de JNK. La phosphorylation d'ERK1/2, JNK et p38 active la transcription du facteur AP1. Finalement l'activation de la voie PLC mène aussi à la phosphorylation I κ B, ce qui permet d'activer le facteur de transcrption NF κ B (Bathgate et al., 2013).

RXFP-3 peut aussi être activé par relaxine, mais dans ce cas les conséquences de l'activation de ce récepteur se limitent à l'inhibition de l'AC et à l'activation de la voie de signalisation PLC.

Il est important de mentionner que les études de l'activation de RXFP-3 sont déroulées sur les lignées cellulaires exprimant RXFP-3, telles que CHO-K1, HEK 293 et SN93 (Kong et al., 2010).

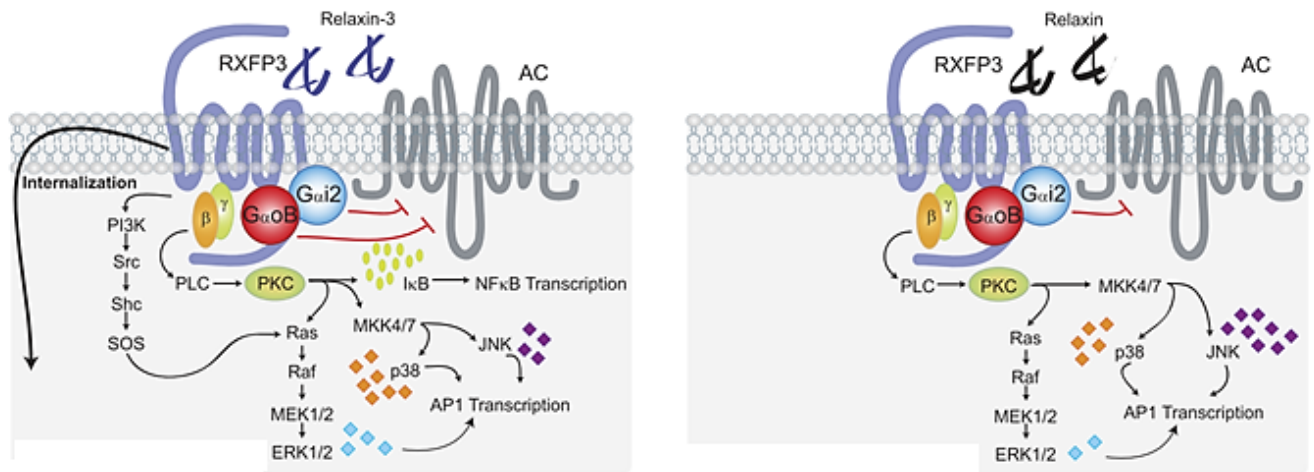


Figure 15: Activation de RXFP-3 par relaxine-3 et par relaxine (Bathgate et al., 2013).

3.2.3. Effets pharmacologiques du système *rln3/RXFP-3*

La distribution cérébrale du système *rln3/RXFP-3* (Fig.14) est à la base des différents effets observés dans les études précliniques menées chez les rongeurs. Ainsi le système *rln-3/RXFP-3* est impliqué dans l'éveil, la prise d'aliment, l'anxiété, la motivation....

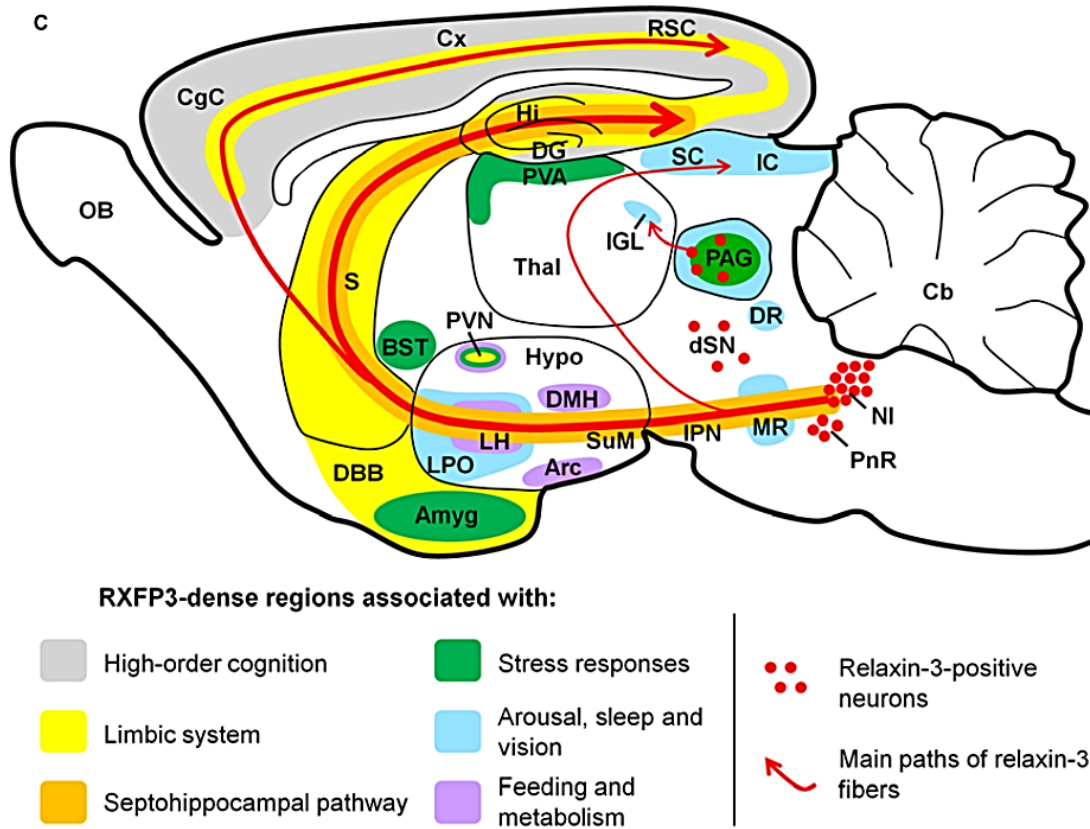


Figure 16: Illustration schématique de la distribution du système *rln3/RXFP-3* chez les rongeurs (Smith et al., 2014).

Rln3/RXFP-3 et éveil

En effet le système *rln-3/RXFP-3* est impliqué dans l'éveil puisqu'il est présent en grande quantité dans le septum, l'hippocampe et le raphé médian, régions clés du système du tractus septohippocampal (Smith et al., 2011). Le tractus septohippocampal génère le rythme thêta de l'hippocampe, qui est impliqué dans la vigilance, l'exploration, orientation, control locomoteur et la mémoire (Smith et al., 2011; Vertes, 2005). La signalisation de RXFP-3 module le rythme thêta aux niveaux de rats éveillés et sous anesthésie en modulant l'activité neuronale aux niveaux de l'hippocampe et du septum médian, les études ont montré que suite à l'injection de

l'agoniste de RXFP-3, il y a eu une augmentation du rythme thêta (Ma et al., 2009). De plus le système rln3-RXFP-3 influence aussi la locomotion des rongeurs (Bathgate et al., 2013).

Rln3/RXFP-3 et appétit

D'autre part la présence de RXFP-3 au niveau du noyau paraventriculaire de l'hypothalamus (PVN), suggère un rôle du système rln-3/RXFP-3 dans l'appétit (Liu et al., 2003). Les études ont montré que l'injection de rln-3 au niveau du ventricule latéral (VL) ou au niveau du PVN induit une augmentation de l'appétit en augmentant le taux de leptine dans le plasma (McGowan et al., 2006). D'autres études ont montré que dans le cas d'une administration chronique de rln3 dans le PVN ou VL a mené à une augmentation de l'appétit en diminuant le taux d'ocytocine et de vasopressine (Ganella et al., 2013). En cas d'administration chronique de rln-3, les rats ont développé une hyperleptinémie et une hyperinsulinémie, ce qui mime le profil des patients obèses (Leon-Cabrera et al., 2013).

Rln3/RXFP-3 et stress

Rln-3/RXFP-3 est aussi impliqué dans le stress, puisque la distribution de rln-3 et RXFP-3 passe aussi dans des régions cérébrales impliquées dans le stress, telles que : l'amygdale et le PVN (Smith et al., 2011). Les études ont montré que les rats stressés ont un taux de c-fos élevé au niveau du NI avec augmentation de l'expression de rln-3 et que ces effets ont été annulés par l'addition de l'antagoniste de récepteur CRF-1 (Banerjee et al., 2010). En effet le CRF (corticotropin releasing factor) augmente en cas de stress, et les neurones rln-3 expriment le récepteur CRF-1. D'autres études ont montré que l'injection de rln3 humaine aux niveaux du VL ou du PVN a induit une augmentation du taux de corticostérone, hormone adrénocorticotrope et prolactine, normalement secrétées en temps de stress (McGowan et al., 2014). Le système rln3/RXFP-3 module le stress en modulant les neurones CRF. Cela dit il faut encore plus d'études pour mieux comprendre la liaison entre CRF et relaxine-3.

Rln3/RXFP-3 et anxiété et dépression

Par ailleurs le système rln3/RXFP-3 intervient dans l'anxiété et la dépression, vu qu'il est présent dans les régions impliquées dans l'anxiété et la dépression, notamment le PFC, hippocampe ventriculaire et l'amygdale (Kumar et al., 2017). Les études de Ryan et al ont révélé un effet anxiolytique de l'agoniste de RXFP-3 suite à son administration centrale (Ryan et al.,

2013). Cependant cet effet dépend du taux d'anxiété de l'animal, ainsi l'effet anxiolytique n'est observé que quand le taux d'anxiété dépasse le taux basal (Zhang et al., 2015). Par conséquent on peut s'attendre à moins d'effet secondaires de point de vue translationnel. D'autre part la relaxine-3 peut influencer l'axe hypothalamus-pituitaire-adrenal impliqué aussi dans la dépression. Au cours de la dépression il y a diminution de la neurogenèse et de la maturation neuronale, les études ont montré que les souris KO rln-3 ont des perturbations au niveau de la neurogenèse et de la maturation neuronale (Kumar et al., 2017).

3.3. Système relaxin/RXFP-1

3.3.1. Relaxine

3.3.1.1. Structure

La relaxine (rln) a été premièrement découverte en 1926 par Dr. Frederick Hisaw qui a injecté le sérum de cochon-dindes portantes à des cochon-dindes vierges ce qui a induit une relaxation du ligament interpubique de ces dernières, de ce fait elle fut considérée une hormone de grossesse (Hisaw, 1926). Il va falloir attendre les années 1970 pour que la structure primaire de la relaxine soit déterminée chez le cochon (Schwabe et al., 1976), les années 80 pour le rat et l'humain (Hudson et al., 1984; John et al., 1981) et les années 1990 pour la souris (Evans et al., 1993).

Relaxine est un peptide de 6kDa avec 53 acides aminés, qui tout comme la relaxine-3 a une structure hétérodimérique avec 2 chaînes A et B et 3 points disulfure (Fig.15).



Figure 17: Structure de relaxin humaine (Patil et al., 2017)

3.3.1.2. Distribution

La principale source de relaxine est le corps jaune chez les mammifères femelles, qu'elles soient ou non enceintes (Bathgate et al., 2006b; Sherwood, 2004). Cela dit le taux de relaxine est au maximum au cours de la grossesse. Pour les mammifères males, c'est plutôt la prostate qui constitue la source majeure de relaxine (Gunnensen et al., 1995; Samuel et al., 2003). Contrairement à relaxine-3, la relaxine est considérée comme circulante et très peu d'études ont été faites pour explorer la distribution cérébrale de relaxine. En effet les études ont été menées majoritairement chez le rat, avec une similarité de distribution chez la souris. Ces études ont mis en évidence la présence de l'ARNm de relaxine aux niveaux du noyau olfactif antérieur, du cortex orbital latéral, du cortex piriforme, du néocortex, du gyrus denté, de l'hippocampe (CA1) et de l'ACC (Ma et al., 2005; Osheroff and Ho, 1993; Scott et al., 2004).

3.3.2. RXFP-1

3.3.2.1. Structure

RXFP-1 ou LGR7 (leucine rich repeat containing G protein coupled receptor 7), découvert en 2002, est le récepteur apparenté à la relaxine (Hsu et al., 2002). RXFP-1 est un récepteur couplé à la protéine G, plus spécifiquement il appartient à la sous-famille C des récepteurs couplés aux protéines G contenant LRR (LGR) (Hsu et al., 2002). RXFP-1 est caractérisé par un domaine extracellulaire assez volumineux puisqu'il renferme le domaine LRR, le linker et le module LDL_a (Fig.15) (Chen et al., 2020). Le domaine LRR renferme 10 LRRs qui constituent le site de fixation primaire à haute affinité de la relaxine (Hoare et al., 2018). Une fois la relaxine fixée, le domaine LRR induit un changement de la position du module LDL_a afin qu'il puisse interagir avec le domaine transmembranaire (Hopkins et al., 2007, 2007). Le module LDL_a renferme 3 ponts disulfures et nécessite un ion calcium pour qu'il reste stable. Même si la relaxine ne se fixe pas sur ce module, sa présence est nécessaire pour l'activation de la signalisation relaxin/RXFP-1 (Diepenhorst et al., 2014; Hoare et al., 2018; Hopkins et al., 2005, 2007). Le linker il permet de créer un site de fixation essentiel pour renforcer la fixation de la chaîne A au niveau du domaine transmembranaire (Scott et al., 2006; Sethi et al., 2016). En effet l'activation de RXF P-1 nécessite 3 étapes : la fixation de la chaîne B de relaxine sur le site de liaison de haute affinité présent au niveau du domaine LRR, la fixation de la chaîne A sur le site de fixation de faible affinité présent au niveau du domaine transmembranaire et finalement la présence du module

LDL_a puisque les études ont montré que son ablation inhibe l'activation de RXFP-1 (Chen et al., 2020; Halls et al., 2007; Scott et al., 2006).

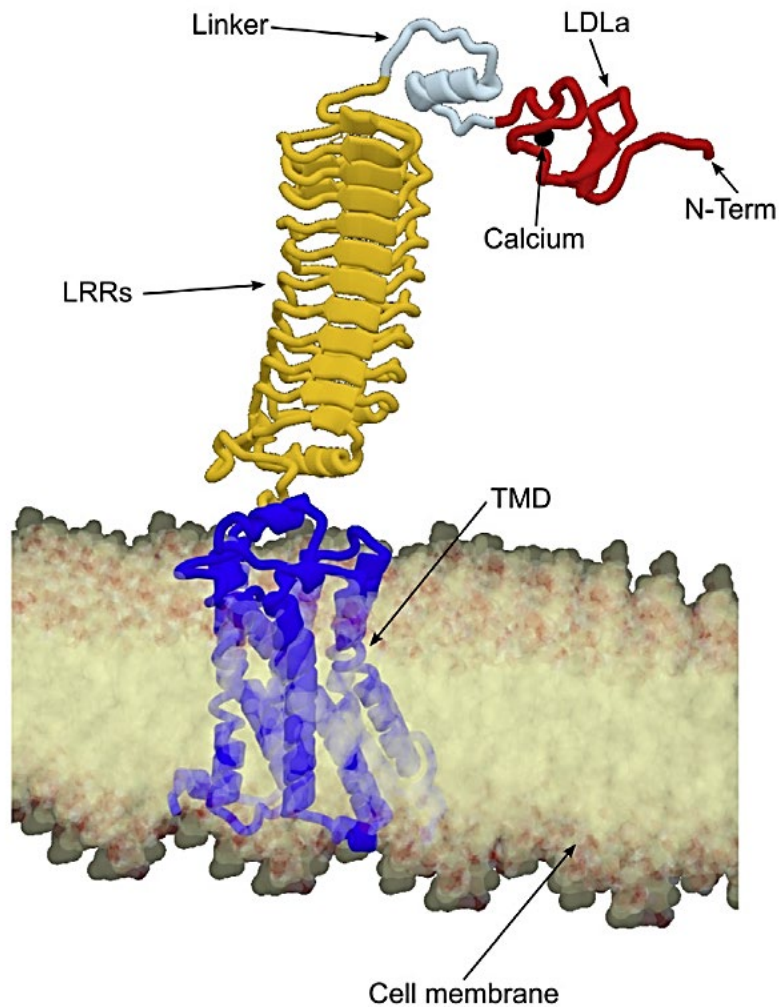


Figure 18: Représentation schématique de RXFP-1 (Bathgate et al., 2018)

3.3.2.2. Distribution

La distribution de RXFP-1 a été étudiée chez l'humain, le rat et la souris. Une similarité de distribution a été observée entre les 3 espèces (Kong et al., 2010). Les chercheurs ont eu recours à la RT-PCR et au northern blotting pour étudier la distribution de l'ARNm de RXFP-1, et à l'autoradiographie des récepteurs pour étudier la distribution de la protéine RXFP-1 (Bathgate et al., 2013; Kong et al., 2010). Les études ont montré que RXFP-1 est présent dans plusieurs organes, notamment les reins, les testicules, le placenta, l'utérus, les ovaires, la prostate, la peau, le cœur et

le cerveau (Bathgate et al., 2013; Hsu et al., 2002; Kong et al., 2010). Dans cette thèse on s'intéresse plus à la distribution cérébrale de RXFP-1. Ainsi on retrouve RXFP-1 aux niveaux du système olfactif, du néocortex, de l'hypothalamus, de l'hippocampe, du thalamus, de l'amygdale et du mésencéphale. De plus les sites de fixation de RXFP-1 sont surtout observés aux niveaux de PVN, du noyau thalamique supraoptique, de l'organe subfornical (SFO) et de la lamina terminalis (OVLT : organum vasculosum of the lamina terminalis) (Bathgate et al., 2013; Osheroff and Phillips, 1991; Tan et al., 1999).

3.3.2.3. Voies de signalisation

Les voies de signalisation activées par RXFP-1 (Fig.16) mènent à l'augmentation du taux d'AMPC et de NO (oxyde nitrique), tout dépend du type cellulaire exprimant le récepteur (Bathgate et al., 2013; Kong et al., 2010).

L'augmentation du taux d'AMPC est la conséquence de l'activation de plusieurs voies de signalisation. Premièrement l'accumulation rapide de l'AMPC est médiée par la sous-unité $G_{\alpha s}$ et inhibée par la sous unité $G_{\alpha OB}$. Puis l'accumulation de l'AMPC est médiée par la sous-unité $G_{\alpha i3}$ qui libère les sous-unités $G_{\beta\gamma}$, qui activent la voie PKC ζ via l'activation de PI3K. PKC ζ subit une translocation membranaire et active AC5, ce qui stimule l'accumulation de l'AMPC (Halls et al., 2006, 2009). Il est important de signaler que quand la concentration de relaxine est de l'ordre de l'attomolaire (10^{-18}) de la relaxine, une autre voie de signalisation est activée, la voie de signalosome. Le signalosome est constitué de RXFP-1, d'AC2 et d'AKAP79. Le signalosome facilite ainsi l'activation efficace de l'AC par les sous-unités $G_{\beta\gamma}$. La production d'AMPC est régulée par PDE4D3 et β arrestine 2 (Halls et al., 2006). Cette voie s'arrete quand la concentration de relaxine est de l'ordre du nanomolaire (Halls and Cooper, 2010). D'autre part en cas l'accumulation de l'AMPC peut aussi être indépendante de la protéine G et être le fruit de la cascade de l'activation de la tyrosine kinase qui aboutit à l'inhibition de l'hydrolyse de l'AMPC en inhibant la PDE (phosphodiesterase)(Zhang et al., 2002) .

L'augmentation du taux de NO est, tout comme l'AMPC, la conséquence de l'activation de plusieurs voies de signalisation. Ainsi l'augmentation du taux d'AMPC stimule et active PKA, qui induit la phosphorylation d'I κ B, ce qui permet la translocation de NF κ B vers le noyau. Cette translocation permet d'activer la transcription du gène de NOS, ce qui induit l'augmentation du taux de NO. Une autre voie de signalisation impliqué est celle de PI3K, ainsi quand PI3K est

activée, elle stimule Akt qui active NOS endothéliale, ce qui permet d'augmenter le taux de NO. D'autre part l'activation de RXFP-1 induit aussi l'activation des voies MAPK, qui induisent la phosphorylation d'ERK1/2, ce qui joue aussi un rôle dans l'accumulation de NO (Bathgate et al., 2013; Kong et al., 2010; Nistri and Bani, 2003).

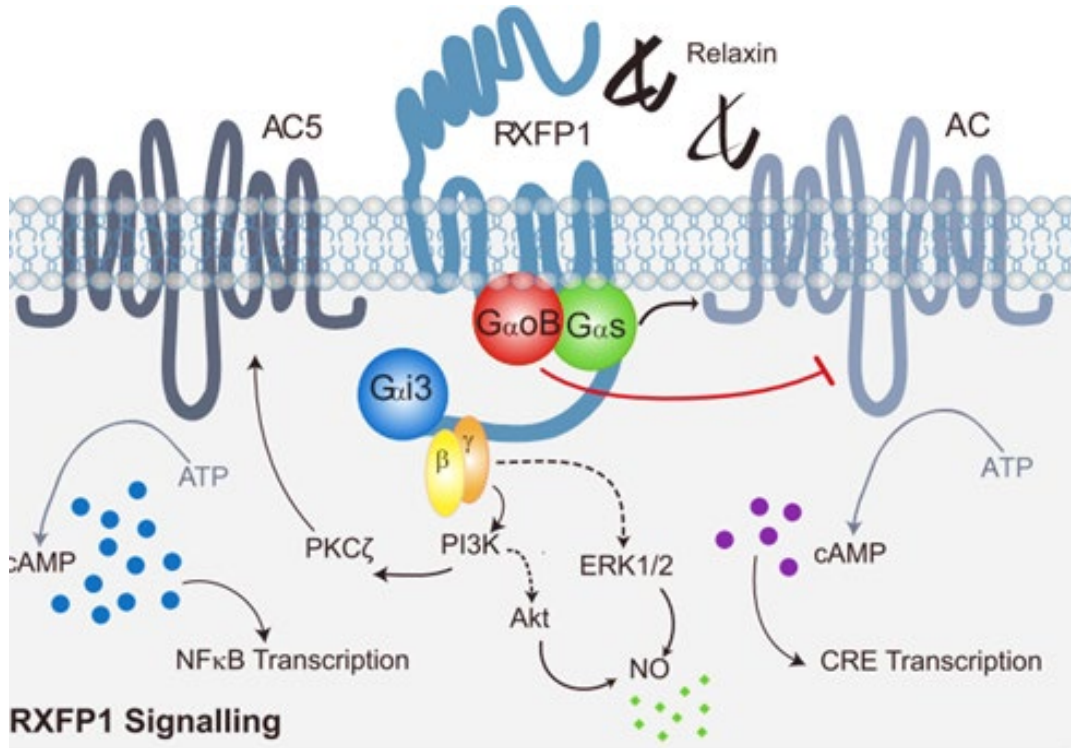


Figure 19: Illustration schématique des voies de signalisation activées par RXFP-1 (Bathgate et al., 2013)

3.3.3. Effets pharmacologiques du système relaxine/RXFP-1

La présence du système relaxine/RXFP-1 dans tout l'organisme sous-entend son implication dans plusieurs fonctions.

Relaxine et système reproductif

La relaxine est impliquée chez les rongeurs femelles dans la maturation cervicale et dans l'augmentation de la flexibilité et de l'élasticité du ligament interpubique (O'Connor et al., 1966). Malheureusement la serelaxine (obtenu par clonage de H2) a échoué à démontrer ces effets aux niveaux des études cliniques (Brennand et al., 1997; MacLennan et al., 1986). De plus les études ont montré que la relaxine facilite l'implantation de l'embryon (Johnson et al., 1991). D'autre part la relaxine est également produite dans l'appareil reproducteur masculin, et est présente dans le

sperme et augmente la motilité et la pénétration des spermatozoïdes dans les ovocytes (Weiss, 1989).

Relaxine et système cardiovasculaire

La relaxine est considérée comme un agent antifibrotique (Lam et al., 2018). En effet les études précliniques ont montré que la relaxine reverse la fibrose observé dans les cardiomyopathies (Parikh Ashish et al., 2013; Sun Junhui et al., 2019). De plus la signalisation relaxine/RXFP-1 mène à l'augmentation du taux de NO qui est un agent vasodilatateur permettant ainsi de diminuer la pression. De ce fait plusieurs études cliniques ont été lancées pour étudier l'effet de la relaxine en cas d'insuffisance cardiaque aigue, dont la plus récente est en phase III (Teerlink et al., 2013). Cependant d'autres études ont mis en évidence une corrélation positive entre le taux plasmiq de relaxine et la sévérité de l'insuffisance(Han et al., 2017; Pintalhao et al., 2017; Qu et al., 2019) .

Relaxine et reins

Les études précliniques menées chez les animaux ont montré que la relaxine diminue le taux de créatine plasmiq, la protéinurie et la fibrose (McDonald et al., 2003). Cependant les études cliniques ont montré que le taux de relaxine circulante est associé au taux de mortalité chez les patients en stade avancé d'insuffisance rénale (Hochoer et al., 2004).

Relaxine et cancer

Le système relaxine/RXFP-1 est impliqué dans le cancer des seins, de la prostate, de la thyroïde (Bathgate et al., 2013; Chen et al., 2020). Ainsi les patients souffrants de cancer de sein, ont un taux plasmiq élevé de relaxine. De plus le traitement des cellules cancéreuses du sein par la relaxine induit une augmentation de leur potentiel invasif (Binder et al., 2002). D'autre part les études menées sur les rongeurs ont montré que l'inhibition de RXFP-1 induit une diminution de la tumeur prostatique (Feng et al., 2010).

Relaxine et cerveau

Peu d'études se penchent sur le rôle de relaxin au niveau du cerveau. D'une part les études ont montré que la relaxine, en se fixant sur RXFP-1 présent aux niveaux de SFO et d'OVLTL, induit une diminution de l'osmolarité plasmiq (Bathgate et al., 2013). Cette diminution est observée en phase terminale de grossesse chez les rats et souris (Sunn et al., 2002; Zhao et al., 1999). De

plus l'injection intracérébroventriculaire de relaxine augmente la sensation de soif chez le rat, aussi en agissant sur RXFP-1 présent aux niveaux de SFO et OVLT (Omi et al., 1997; Summerlee et al., 1998). D'autre part l'injection de relaxine au niveau de l'amygdale basolatérale (région riche en RXFP-1) mène à une altération de la mémoire liée à la peur chez le rat (Ma et al., 2005).

Objectifs

La douleur chronique est débilite et accablante du fait qu'elle est souvent accompagnée d'anxiété et de dépression. Plusieurs traitements existent sur le marché pour traiter soit la douleur, soit ses comorbidités avec un risque de dépendance élevé (Ciaramella, 2019). Les neuropeptides, avec leurs voies de signalisation ciblées représentent une piste thérapeutique intéressante avec moins d'effets secondaires par rapport aux modulateurs des systèmes glutamatergique, GABAergique, monoaminergique ou cholinergique (Hoyer and Bartfai, 2012).

Le système relaxine-3/RXFP-3 est impliqué dans les comorbidités émotionnelles de la douleur, ainsi les travaux de Ryan et al, et ceux de Zhang et al ont montré que la relaxine-3 diminue le taux élevé d'anxiété (Ryan et al., 2013; Zhang et al., 2015). De plus la relaxine-3 serait impliquée dans la dépression, vu que son absence perturbe la maturation neuronale et la neurogenèse, phénomène observé avec la dépression (Kumar et al., 2017).

D'autre part les études menées au sein de l'équipe bordelaise sur un modèle de douleur inflammatoire persistante, ont montré que l'injection intracérébroventriculaire de l'agoniste de RXFP-3 mène à une augmentation du seuil de douleur chez des souris C57Bl6/J injectées de CFA, soulignant ainsi le potentiel analgésique du système relaxine-3/RXFP-3. L'effet global observé nous incite alors à vouloir approfondir nos études, afin d'élucider les régions cérébrales impliquées dans l'analgésie qui fait suite à l'ICV de l'agoniste de RXFP-3.

Par conséquent l'objectif de cette thèse est d'étudier la modulation de la douleur persistante par le système relaxine-3/RXFP-3 dans l'amygdale et l'ACC, régions cérébrales impliquées dans la composante émotionnelle de la douleur. De déterminer la nature des neurones relaxine-3 et RXFP-3, ainsi que leurs répartitions.

Dans un deuxième temps, et en se basant sur la répartition cérébrale du système relaxine/RXFP-1 qui est retrouvé dans des régions impliquées dans la douleur telles que l'amygdale, l'ACC et le thalamus, on étudiera de même la modulation de la douleur par le système relaxine/RXFP-1, la neuroanatomie et la neurochimie de ce système, ainsi que sa répartition cérébrale.

Matériels et Méthodes

1. Animaux

Les expériences ont été menées en respectant les directives européennes et ont été validées par le comité éthique local (Apafis # 21890).

Les expériences ont été menées sur des souris C57Bl6/J (Charles Rivers, France) males, âgées de 8 à 10 semaines. Les souris avaient un accès libre à l'eau et à la nourriture, étaient maintenues en cycle standard de jour et nuit (12h :12h) et étaient suivies régulièrement par des animaliers.

Après réception, les animaux étaient laissés le temps d'une semaine pour s'acclimater à l'animalerie. Les animaux qui passaient en chirurgie avaient un temps de récupération d'au moins 2 jours, puis ils étaient habitués pendant 3 jours (jour-1 habituation à l'expérimentateur, jours-2-3 habituation aux appareils de comportements). Par la suite le comportement douloureux basal était enregistré, et la patte postérieure est injectée par du CFA (ou du NaCl) pour induire le modèle douloureux (ou contrôle). Le lendemain de l'injection, le comportement basal post-CFA est enregistré. 2 jours après les modulateurs des récepteurs RXFP1 et RXFP-3 sont injectés, et le comportement douloureux enregistré. Afin de réduire le nombre d'animaux, les mêmes animaux sont injectés le lendemain par d'autres modulateurs. Enfin l'animal sacrifié.

Afin de répondre aux différents objectifs de la thèse, une frise chronologique ci-dessous résume les manip liées à l'évaluation de la modulation de la douleur par les systèmes rln3/RXFP-3 et rln/RXFP-1 :

Jour 1	Jours 2-3	Jours 4-5-6	Jour 7	Jour 8	Jour 9	Jour 10	Jour 11
Implantation de canules	Récupération	Habitude aux comportements	Baseline et injection intraplantaire de CFA/NaCl	Baseline post CFA	Injection intracérébrale + comportements	Injection intracérébrale + comportements	Perfusion

Table 1: Frise chronologique des manip

2. Implantation des guides canules

Afin d'étudier la modulation de la douleur par les peptides relaxines et leurs récepteurs cognitifs, on a eu recours à l'implantation des canules cérébrales (Fig. 17).

L'animal est anesthésié à l'isoflurane : dans un premier temps l'animal est placé dans une chambre d'induction avec 4% d'isoflurane, puis l'animal est placé sur le cadre stéréotaxique (RWD Desktop Digital Stereotaxic Instruments 68025, RWD) où l'anesthésie est maintenue à travers un masque avec de l'isoflurane à 1.5 %. L'animal est placé sur un tapis chauffant (FHC) afin de maintenir sa température à 37°C. Du gel oculaire est mis sur les yeux de l'animal afin d'éviter la déshydratation oculaire (Ocry-gel, Lab TVM, France). Par la suite, le crâne de l'animal est rasé et désinfecté par de la Bétadine rouge puis jaune. Puis l'animal est injecté en sous cutanée (SC) de 100 µl (0.03mg/ml) buprénorphine, (et une dose de 100 µl, 6 heures après la chirurgie). De plus l'animal est aussi injecté en SC sous le scalp de 20 µl de lidocaine 1%. Par la suite la peau est incisée et le crâne exposé et nettoyé, afin d'exposer bregma et lambda. Les guides canules (Bilaney, Allemagne) ont été placés par rapport au bregma selon les coordonnées suivantes ACC (AP : 0.8 mm, ML : +/-0.4 mm, DV :-2 mm), BLA (AP :-1.6 mm, ML : +/-3.2 mm, DV :-4.8 mm), ventricule latéral (AP : 0.7 mm, ML : +/-0.7 mm, DV :-2.85 mm). Après implantation, les guides canules sont fixés à l'aide du ciment dentaire (Phymep,France). Une fois le ciment dur, les guides canules sont bouchés par leurs dummies respectifs (Bilaney, Allemagne), et l'animal est ensuite mis dans une cage qui repose sur un tapis chauffant, le temps qu'il se réveille. Il est par la suite remis dans sa cage de départ et laissé le temps de récupérer tout en assurant leur suivi post – opération.

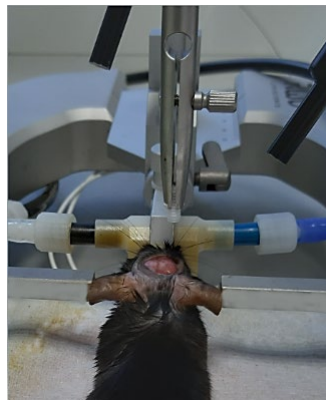


Figure 20: Implantation de guide canule

	Guides canules	Canules internes	Dummy
ACC	C315GS-5/SPC/1.5 mm	C315IS-5/SPC/ to fit 1.5 mm C235I with 0.5 mm projection	C315DCS-5/SPC to fit 1.5 mm With 0.5 mm projection
BLA	C315GS-5/SPC/4 mm	C315IS-5/SPC/ to fit 4 mm C235I with 0.8 mm projection	C315DCS-5/SPC to fit 4 mm With 0.8 mm projection
LV	C315GS-5/SPC/2 mm	C315IS-5/SPC/ to fit 2 mm C235I with 0.85 mm projection	C315DCS-5/SPC to fit 2 mm With 0.85 mm projection

Table 2: Détails des guides canules, canules internes et dummies

3. Validation histologique

Une fois les manips de comportement sont finies (cf. tableau 1) les souris sont perfusées (le protocole de perfusion et de récupération des tissus perfusés est expliqué ultérieurement dans le paragraphe 7.1.1) et leurs cerveaux récupérés pour valider l'emplacement de la canule. Les cerveaux sont coupés au cryostat (Leica) et les coupes de 25 µm sont incubées dans une solution de crésyl violet 0.1% (0.25g de crésyl violet est dilué dans 100 ml d'eau avec pH 4) pendant 1 minute. Les coupes sont ensuite rincées à l'eau distillée et déshydratées dans un premier temps à l'alcool 70% pour 10 secondes puis à l'alcool 50% pendant 10 secondes. Les coupes sont laissées à sécher et puis montées entre lame et lamelle.

4. Modèle de douleur inflammatoire persistante (CFA)

Afin de créer un modèle de douleur inflammatoire persistante, la patte postérieure controlatérale au guide canule, est injectée de l'adjuvant complet de Freund (CFA) (Sigma-Aldrich) qui est une suspension de mycobactérie inactivées (ce modèle est détaillé dans la partie 1.3 de l'introduction). L'inflammation est observée 24h après et est maintenue pour 1 à 2 semaines. En effet, l'animal est anesthésié par de l'isoflurane (induction 5%, maintien 1.5%), 20 µl de CFA sont injectés en intraplantaire pour induire la douleur (Fig. 18). Le groupe contrôle reçoit une injection de 20 µl de NaCl 0.9%. L'animal est mis dans sa cage qui repose sur un tapis chauffant le temps qu'il se réveille. Les seuils d'hypersensibilités mécanique et thermique ont été évalués 24h après.



Figure 21: Injection intraplanataire de CFA

5. Comportement douloureux.

5.1. Von Frey : Hypersensibilité mécanique

Le test de Von Frey (VF) (Fig. 19) permet de mesurer l'hypersensibilité mécanique de l'animal, en mesurant le seuil de retrait de la patte suite à des stimulations mécaniques par les filaments de Von Frey (Bioseb, France). L'animal est placé dans le dispositif d'expérimentation (Ugo Basile, Italie), qui est formé de cage en plexi glace reposant sur un plancher formé d'une grille métallique. Quand l'animal est posé sur ses 4 pattes, le filament de Von Frey est appliqué perpendiculairement pendant 2-3 secondes sur la planche de la patte postérieure. Cette même étape est répétée 5 fois pour le même filament avec un délai de 30 secondes entre chaque application, pour éviter tout risque de sensibilisation. Les filaments de VF utilisés avaient des grammages différents et on commençait par le filament de plus petit grammage et on montait crescendo. Le seuil est déterminé quand l'animal retirait sa patte à 3 applications sur 5. Le test VF est réalisé avant l'injection de CFA (NaCl) pour établir la baseline pré-CFA, puis 24 h après injection de CFA (NaCl) pour établir la baseline post-CFA, et enfin après injection des modulateurs des récepteurs RXFP-1 et 3 (à 30 minutes, 1h et 2h d'injection).

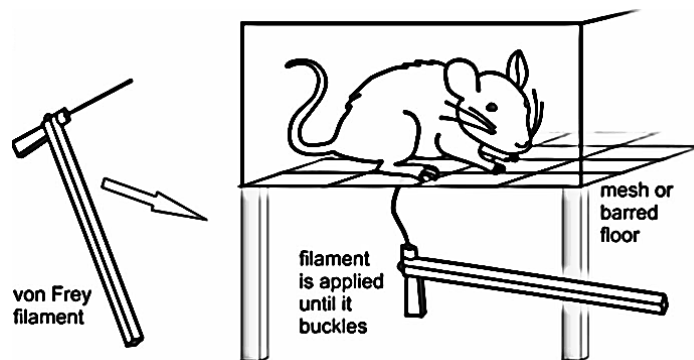


Figure 22: Test Von Frey (Deuis et al., 2017)

5.2. Plantar/Hargreaves : Hypersensibilité thermique

Le test plantar (Fig. 20) permet de mesurer l'hypersensibilité thermique de l'animal, en mesurant le seuil de retrait de la patte suite à des stimulations thermiques émises par un faisceau laser infrarouge. L'animal est placé dans le dispositif d'expérimentation Hargreaves (IITC Inc. Life Science, Tem Sega), qui est formé de cage en plexi glace reposant sur un plancher en verre transparent qui permet le passage de la lumière. Quand l'animal est posé sur ses 4 pattes, un faisceau laser infrarouge d'intensité IR 40 est appliqué sur la surface plantaire de l'animal et la durée de latence au retrait de la patte est mesurée. Le test est répété 3 fois, avec au moins 2 minutes de latence entre chaque application pour diminuer le risque de sensibilisation. Le seuil correspond à la moyenne des valeurs des 3 tests. De même ce test est réalisé avant l'injection de CFA (NaCl) pour établir la baseline pré-CFA, puis 24 h après injection de CFA (NaCl) pour établir la baseline post-CFA, et enfin après injection des modulateurs des récepteurs RXFP-1 et 3 (à 30 minutes, 1h et 2h d'injection).



Figure 23: Test Plantar

6. Approches pharmacologiques

6.1. Injections des modulateurs de RXFP-3

Afin d'étudier l'impact du système rln3/RXFP-3 sur la modulation de la douleur, les modulateurs de RXFP-3 sont injectés dans les régions cérébrales d'intérêt : ACC et BLA, à travers les canules internes (Bilaney, Allemagne) respectives pour chaque région (le détail des canules internes, guides canules et dummies figurent dans le tableau 2). Ainsi les animaux sont anesthésiés

à l'isoflurane (induction 4%, maintien 1.5%), et la canule interne est reliée par un tube en polyéthylène à une seringue Hamilton (Fisher Scientific, France) montée sur une pompe à perfusion (Ugo Basile, Italie), ce qui permet de délivrer, avec un débit spécifique, la dose voulue du modulateur (voir tableau 3). Pour RXFP-3 on a testé, l'agoniste A2 (Florey Institute, Australie) formé par les 2 chaînes peptidiques A et B, le nouvel agoniste A5 (Florey Institute, Australie) formé uniquement par la chaîne B, le mélange antagoniste R3 (B1-22) R (Florey Institute, Australie) + agoniste (A2 ou A5), et le liquide céphalo-rachidien artificiel (aCSF) qui représente le véhicule dans lequel est dissout A2, A5 et R3. L'aCSF est une solution de pH 7.4 et renferme (en mM) : NaCl 130.5, KCl 2.4, CaCl₂ 2.4, NaHCO₃ 19.5, MgSO₄ 1.3, KH₂PO₄ 1.2, HEPES 1.25, Glucose 10.

Les agonistes A2 (0.22 nmol) et A5 (0.46 nmol) sont administrés à raison de 200 nl d'une solution de 5 µg/µl avec un débit de 100nl/minute. Le mélange d'agoniste -antagoniste est administré à raison de 400 nl d'une solution de 5 µg/µl d'agoniste et de 10 µg/µl d'antagoniste (1.136 nmol) avec un débit de 100 nl/minute.

	A2	A5	A2/A5+R3	aCSF
Concentration	5 µg/µl	5 µg/µl	A2/A5 : 5 µg/µl R3 : 10 µg/µl	305-310 mOsM
Volume	200 nl	200 nl	400 nl	200 nl
Débit	100nl/ min	100nl/ min	100nl/ min	100nl/ min

Table 3: Concentration, volume et débit de chaque modulateur RXFP-3

6.2. Injections des modulateurs RXFP-1

Afin d'étudier l'impact du système rln/RXFP-1 sur la modulation de la douleur, les modulateurs de RXFP-1 sont injectés à travers les canules internes (Bilaney, Allemagne), dans le ventricule latéral (voir tableau 2). Ainsi les animaux sont anesthésiés à l'isoflurane (induction 4%, maintien 1.5%), et la canule interne est reliée par un tube en polyéthylène à une seringue Hamilton (Fisher Scientific, France) montée sur une pompe à perfusion (Ugo Basile, Italie), ce qui permet de

délivrer, avec un débit spécifique, la dose voulue du modulateur (voir tableau 4). Pour RXFP-1 on a testé, la relaxine humaine H2 (Florey Institute, Australie), l'agoniste B7.33 (Florey Institute, Australie) formé uniquement par la chaîne B, le mélange antagoniste H2B (Florey Institute, Australie) + agoniste (H2 ou B7.33), et le liquide céphalo-rachidien artificiel (aCSF).

H2 (0.84 nmol) et B7.33 (1.67 nmol) sont administrés à raison de 2,5 µl d'une solution de 2 µg/µl avec un débit de 1 µl/minute. Le mélange d'agoniste -antagoniste est administré à raison de 5 µl d'une solution 2 µg/µl d'agoniste et de 4 µg/µl d'antagoniste (1.67 nmol) avec un débit de 1 µl/minute (Tableau 4).

	H2	B7.33	H2/B7.33+H2B	aCSF
Concentration	2 µg/µl	2 µg/µl	H2/B7.33 : 2µg/µl H2B : 4 µg/µl	305-310 mOsM
Volume	2,5 µl	2,5 µl	2,5 µl	2,5 µl
Débit	1 µl/minute	1 µl/minute	1 µl/minute	1 µl/minute

Table 4: Concentration, volume et débit de chaque modulateur RXFP-1

7. Approche neuroatomique

7.1. Récupération des tissus

7.1.1. Tissus perfusés

Les souris sont divisées en 2 groupes : CFA et contrôle. Le groupe CFA est injecté de 20 µl de CFA, alors que le groupe contrôle est injecté de 20 µl NaCl 0.9%. Les injections sont administrées en intraplantaire sur animaux anesthésiés à l'isoflurane (4% induction, 1.5% maintien). Les souris sont perfusées en intracardiaque 4 jours après injection. La perfusion se déroule comme suit : l'animal est anesthésié par injection intrapéritonéale d'un mélange d'exagon (300mg/kg) et de lurocaine (30mg/kg). L'animal est premièrement perfusé par 20 ml de NaCl 0.9% chauffé à 37°C, puis par 40 ml de paraformaldéhyde à 4% (PFA) (la poudre de PFA est dissoute dans du tampon phosphate PBS (phosphate buffered saline) à 0.01M). Les cerveaux sont récupérés et mis dans du PFA 4% à pendant 4 heures à 4°C pour la post fixation, puis ils sont transférés dans une solution cryo-protectrice (PBS 0.1M + sucrose 12%) pendant 24h à 4°C. Par la suite les cerveaux sont

congelés à -80°C. Pour la congélation, les cerveaux sont placés dans des moules en plastiques et recouverts de tissu teck, les moules sont alors plongés dans de l'isopentane froid (-60°C).

7.1.2. Tissus frais

De même on a 2 groupes de souris (CFA et contrôle), les souris sont mises à mort 4 jours après injection de CFA/NaCl par surdosage d'anesthésique (isoflurane). Les souris sont décapitées rapidement et leurs cerveaux sont récupérés et congelés dans de l'isopentane (comme décrits dans le paragraphe 6.1.1).

7.2. Immunohistochimie

7.2.1. Double IHC : Relaxine3-Somatostatine

Les cerveaux perfusés sont coupés au cryostat (Leica), et les coupes de 25 µm sont déposées dans les puits d'une plaque de 12 puits contenant du PBS 0.1M. Ces coupes sont lavées 3x10 minutes dans du PBS 0.1M. Par la suite, les coupes sont incubées pendant 30 minutes à température ambiante et sous-agitation dans une solution renfermant du PBS 0.1M, du bovine sérum albumine (BSA) 1% (Sigma Aldrich) et du triton-X-100 à 0.3% (Acros organics, USA). Cette étape correspond à la saturation. Puis les coupes sont incubées pendant 24h à 4°C (sous agitation) avec l'anticorps primaire (Ac Iaires) anti-relaxine-3 fait chez la souris (Florey Institute, Australie) dilué au 1/10 dans une solution de PBS 0.1M, BSA 1%. Le lendemain, les coupes sont lavées 3x10 minutes dans du PBS 0.1M, et par la suite les coupes sont incubées pendant 2h à température ambiante (sous agitation) avec l'anticorps secondaire (Ac II aire) Alexa 488 anti-souris fait chez la chèvre dilué au 1/500 dans une solution de PBS 0.1M et BSA 1%. Les coupes sont ensuite lavées 3x10 minutes dans du PBS 0.1M puis incubées pendant 24h à 4°C (sous agitation) avec l'Ac Iaire anti-somatostatine fait chez le cochon-dinde dilué au 1/2000 dans une solution de PBS 0.1M, BSA 1%. 24h après les coupes sont lavées puis incubées pendant 2h à température ambiante (sous agitation) avec l'Ac IIaire Alexa 594 anti-cochon-dinde fait chez la chèvre diluée au 1/500 dans du PBS 0.1M, BSA 1%. Par la suite les coupes sont lavées puis montées sur lame.

7.2.2. Double IHC : Relaxine-3-Parvalbumine

Le même protocole est suivi comme dans le paragraphe 6.2.1, avec comme Ac Iaire anti-parvalbumine fait chez le poulet dilué au 1/2000 qui est révélé par l'Ac IIaire Alexa 594 anti-poulet fait la chèvre dilué au 1/500.

7.2.3. IHC relaxine

Les coupes sont lavées dans du PBS 0.1M (3x10 minutes) puis saturées pendant 30 minutes à température ambiante (sous-agitation) dans une solution de PBS 0.1M, BSA 1% et triton 1%. Par la suite les coupes sont incubées pendant 24h à 4°C (sous agitation) avec l'Ac Iaire anti-relaxine fait chez le lapin dilué au 1/200 dans du PBS 0.1M, BSA 1% et triton 1%. Le lendemain, les coupes sont lavées dans du PBS 0.1M (3x10 minutes), puis incubées pendant 2h à température ambiante (sous-agitation) avec l'Ac Iaire Alexa 488 anti-lapin fait chez la chèvre dilué au 1/500 dans du PBS 0.1M, BSA 1%. Les coupes sont ensuite lavées et montées sur lame. Les coupes sont stokées à -20°C le temps de lancer l'acquisition confocale.

Anticorps	Dilution	Références
<i>Anticorps primaires</i>		
Relaxine-3 (souris)	1/10	Florey Institute
Relaxine (lapin)	1/200	Biovision 3874
Somatostatine (cochon-dinde)	1/2000	Magendie Code IS-7/51
Parvalbumine (poulet)	1/2000	Biotechne NBP2-50036
<i>Anticorps secondaires</i>		
Alexa 488 anti-souris (chèvre)	1/500	Thermo Fisher Scientific A-11001
Alexa 488 anti-lapin (chèvre)	1/500	Thermo Fisher Scientific A-11008
Alexa 594 anti-cochon-dinde (chèvre)	1/500	Jackson Immuno Research 706-586-003
Alexa 594 anti-poulet (chèvre)	1/500	Thermo Fisher Scientific A-11042

Table 5: Détails des anticorps primaires et secondaires utilisées.

7.3. RNAscope

RNAscope est une technique d'hybridation in situ poussée qui permet de détecter l'ARNm voulu avec une meilleure spécificité et une meilleure sensibilité. Cette technique se déroule sur 3 jours en suivant les instructions du fabricant et en utilisant le RNAscope fluorescent Multiplex Assay (ACD, Newark, CA, USA) .

Au cours du premier jour, les cerveaux de tissus frais congelés sont coupés au cryostat et les coupes de tissus frais de 16 µm sont montées directement sur lame et sont incubées dans du PFA 4% froid (4°C) pendant 16 minutes. Puis elles ont déshydratées dans des bains d'alcool de 50, 70

et 100% à raison de 5 minutes par pourcentage. Par la suite les coupes sont transférées dans un autre bain d'éthanol à 100% et y restent la nuit à -20°C.

Le lendemain, les coupes sont laissées à température ambiante pendant 10 minutes pour sécher. Puis une barrière est dessinée tout au long des coupes à l'aide d'un stylo hydrophobe (ImmEdge Hydrophobic Barrier Pen, #310018), et les coupes sont laissées à température ambiante pendant 10 minutes, le temps pour que la barrière sèche. Puis les coupes ont été traitées par la protéase pretreat-4 (Cat#322340) pendant 20 minutes à température ambiante. Les coupes sont lavées dans de Wash buffer (Cat#310091) dilué au 1/50 dans du PBS 0.1M. Les coupes sont par la suite incubées avec un mélange de 3 sondes (détails du mélange de sonde dans le tableau 6) pendant 2h à 40°C. L'importance de RNAscope réside dans son amplification du signal qui se déroule en plusieurs étapes : les coupes sont incubées avec l'amplificateur AMP1 pour 30 minutes à 40°C, puis avec l'AMP2 pour 15 minutes à 40°C, ensuite AMP3 pour 30 minutes à 40°C et finalement AMP4 pour 15 minutes à 40°C. Par la suite les coupes sont incubées avec du DAPI (#320851) pour 20 secondes à température ambiante. Enfin les coupes sont montées avec du fluoromont (ThermoFisher Scientific) et gardées pendant 30 minutes à l'abri de la lumière et à température ambiante pour qu'elles sèchent. Les coupes sont conservées à -20°C le temps de réaliser les acquisitions confocales.

	Sondes	Références
Détermination de la neuropopulation exprimant RXFP-3	C1 : <i>RXFP-3</i> C2 : <i>Somatostatine</i> C3 : <i>Parvalbumine</i>	439381 404631-C2 421931-C3
Détermination de la neuropopulation exprimant RXFP-1	C1 : <i>RXFP-1</i> C2 : <i>Gad2</i> C3 : <i>Camk2a</i>	458001 415071-C2 445231-C3
Détermination de la neuropopulation relaxine +	C1 : <i>Gad2</i> C2 : <i>Relaxine</i> C3 : <i>Camk2a</i>	439371 539521-C2 445231-C3

Table 6: Mélange de sondes

7.4. Traçage des voies nerveuses

L'animal est anesthésié à l'isoflurane (4% induction et maintien à 1.5%). L'animal est placé sur le cadre stéréotaxique tout en reposant sur un tapis chauffant (FHC) afin de maintenir sa température à 37°C. Du gel oculaire est mis sur ses yeux afin d'éviter la déshydratation oculaire.

Par la suite, le crâne de l'animal est rasé et désinfecté par de la Bétadine rouge puis jaune. L'animal reçoit comme analgésiques : 100 µl (0.03mg/ml) buprénorphine en SC, (et une dose de 100 µl, 6 heures après la chirurgie) et 20 µl de lidocaine 1%. Par la suite la peau est incisée et le crâne exposé et nettoyé, afin d'exposer bregma et lambda. L'animal subit une injection stéréotaxique bilatérale de 200 nl d'un traceur rétrograde, le fluorogold (ref) pendant 5 minutes aux niveaux de l'ACC en 2 points et de la BLA en 2 points. Les animaux sont sacrifiés après **** , perfusés et leurs cerveaux récupérés.

7.5. Injection virale

Les souris 5-HT- cre adultes (B6.Cg-Tg (FeV-cre) 1Esd / J, Jackson Laboratory (Scott et al., 2005b) fournies par l'équipe de Cyril Henry, ont été injectées au niveau de la BLA (en suivant le même protocole décrit en 1.4) par l'associé adenovirus 5 (AAV-Flox-GFP) et perfusés 3 semaines après.

En effet les neurones 5-HT des souris 5-HT-cre ont la particularité d'exprimer la cre recombinase, puisque l'expression de cette dernière est sous le contrôle du promoteur FEV (de la famille des oncogènes ETS) présent chez les neurones 5-HT. Après injection du virus AAV-Flox-GFP, les neurones 5-HT de la BLA seront marqués par la GFP.

8. Analyses statistiques

Toutes les analyses statistiques ont été faites sur le logiciel Graphpad prism version 8.0.2. Les résultats sont exprimés en moyenne \pm SEM, et la différence est considérée comme significative quand $p < 0.05$.

Le test utilisé pour comparer les comportements douloureux chez les souris CFA vs souris contrôles, est le two-way Anova suivi du Turkey ou Sidak test. De même le 2-way Anova a été utilisé pour comparer les résultats de RNAscope et d'IHC entre souris CFA et contrôles.

Résultats

1. Signalisation relaxine-3/RXFP-3 et douleur inflammatoire persistante

1.1. Modulation pharmacologique de la douleur inflammatoire persistante par les modulateurs de RXFP-3

1.1.1. Après injection dans la BLA

Afin d'étudier l'effet du système relaxine-3/RXFP-3 après injection dans la BLA, les souris C57Bl6/J ont été équipées par des guides canules implantés au niveau de la BLA. L'effet a été étudié dans un modèle de douleur inflammatoire persistante (souris CFA), ainsi que dans un modèle contrôle (souris NaCl).

1.1.1.1. Hypersensibilité mécanique

Nous avons commencé par évaluer l'hypersensibilité mécanique des souris en ayant recours au test de Von Frey. Et on a eu recours à une Anova 2 voies suivie du test posthoc Tukey pour l'analyse statistique des résultats.

Le seuil de retrait de la patte postérieure (PWT paw withdrawal threshold) diminue significativement après injection de CFA (1.4 g à D-1 vs $0.373\text{g} \pm 0.027\text{ g}$ à D4, $p < 0.0001$), alors qu'il reste inchangé après injection de NaCl (1.4 g à D-1 vs 1.4 g à D4, $p > 0.05$) au cours de toutes les cohortes (Fig. 24 A et B).

Nos résultats montrent que l'injection de l'agoniste A2 chez les souris CFA ($n=9$), induit une augmentation significative du PWT à 30 minutes ($0.373\text{g} \pm 0.027\text{g}$ à D4 vs $1.089\text{g} \pm 0.059\text{g}$ à 30 minutes, $p < 0.0001$), qui se maintient à 60 minutes ($0.373\text{g} \pm 0.027\text{g}$ à D4 vs $0.689\text{g} \pm 0.106\text{g}$ à 60 minutes, $p < 0.0001$) et regagne les valeurs post injection de CFA après 120 minutes ($0.373\text{g} \pm 0.027\text{g}$ à D4 vs $0.418\text{g} \pm 0.043\text{g}$, $p > 0.05$) (Fig. 24 A). Par conséquent l'injection d'A2 au niveau de la BLA induit une analgésie mécanique transitoire. Par contre l'injection d'A2 chez les souris NaCl, ne modifie pas le PWT, qui reste inchangé (1.4g à D4 vs 1.4 à 30 minutes, $p > 0.05$).

D'autre part nos résultats montrent que l'injection du véhicule aCSF ($n=5$) ne modifie pas le seuil de retrait ni dans le modèle de douleur inflammatoire (0.4g à D4 vs $0.392\text{g} \pm 0.07\text{g}$ à 30 minutes, $p > 0.05$) ni dans le modèle contrôle (1.4g à D4 vs 1.4 à 30 minutes $p > 0.05$) (Fig. 24 B).

De plus nos résultats montrent que l'analgésie observée avec l'agoniste A2 est annulée par l'addition de l'antagoniste R3 ($n=9$). Ainsi l'injection d'A2 suivi de R3 ne modifie pas le PWT chez les souris CFA (0.4g à D4 vs 0.4g à 30 minutes, $p > 0.05$) (Fig. 24.C).

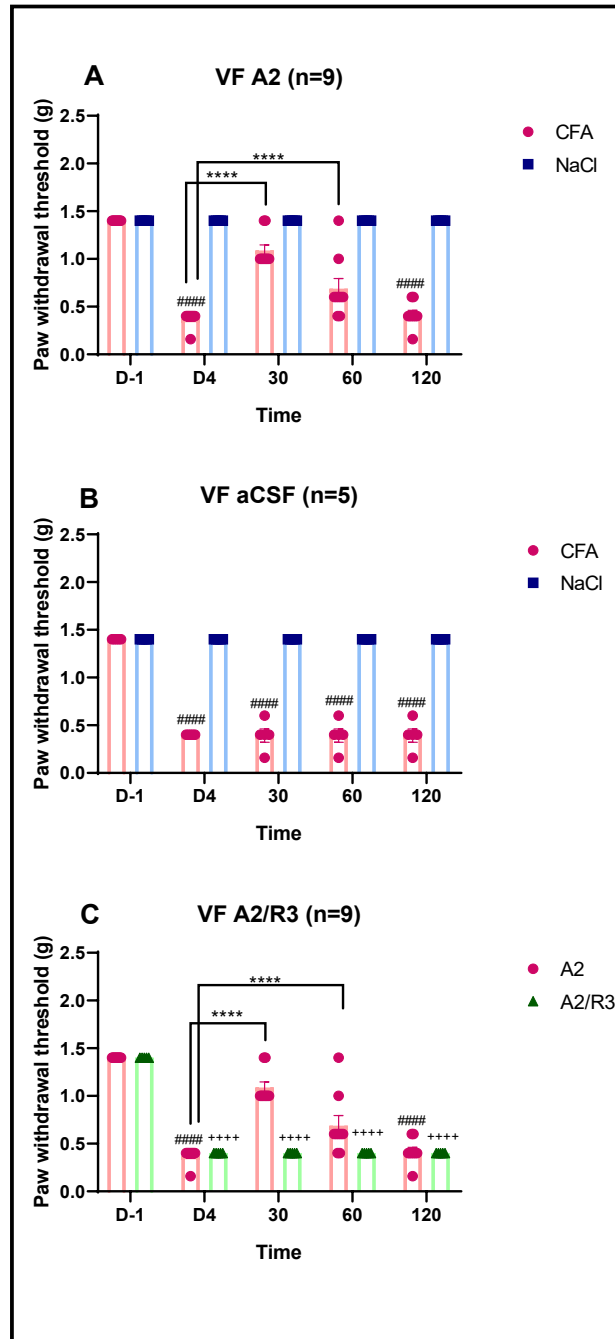


Figure 24 : Effets de modulateurs de RXFP-3 sur l'hypersensibilité mécanique.

Le seuil de retrait de la patte postérieure, en utilisant les filaments de Von Frey, est mesuré chez les souris CFA (rose) et NaCl (bleu), un jour avant injection intraplantaire (IP) de CFA ou NaCl (D-1), 4 jours après injection IP de CFA/NaCl (D4), puis après 30 minutes, 60 minutes et 120 minutes d'injection des modulateurs de RXFP-3.

(A) L'injection d'A2 induit une analgésie mécanique transitoire chez les souris CFA et pas d'effet chez les souris NaCl.

(B) L'injection d'aCSF n'induit aucune modification au PWT chez les souris CFA et NaCl

(C) L'injection de R3 (vert) annule l'analgésie observée avec l'A2 (rose) chez les souris CFA.

Les résultats sont exprimés en moyenne \pm SEM.

**** $p < 0.0001$ (comparaison du PWT par rapport au D4)

$p < 0.0001$ (comparaison par rapport à D-1)

++++ $p < 0.0001$ (comparaison par rapport à D-1 pour A2/R3)

1.1.1.2. Hypersensibilité thermique

Par la suite nous nous sommes intéressés à étudier l'effet du système relaxine-3/RFXP-3 sur l'hypersensibilité thermique en ayant recours au plantar test. De même on a eu recours à une Anova 2 voies suivie du test posthoc Tukey pour l'analyse statistique des résultats.

La latence de retrait de la patte postérieure (PWL paw withdrawal latency) diminue significativement chez les souris CFA ($4.42s \pm 0.0308s$ à D-1 vs $1.782s \pm 0.221s$ à D4, $p < 0.001$) et elle reste inchangée chez les souris NaCl ($6.968s \pm 0.534s$ à D-1 vs $7.290s \pm 0.447s$ à D4, $p > 0.05$) au cours de toutes les cohortes (Fig. 25 A et B).

Nos résultats montrent que l'injection de l'agoniste A2 chez les souris CFA ($n=6$) induit une augmentation significative de PWL à 30 minutes ($1.782s \pm 0.221s$ à D4 vs $3.704s \pm 0.760s$ à 30 minutes, $p < 0.01$) et à 60 minutes ($1.782s \pm 0.221s$ à D4 vs $3.837s \pm 0.948s$ à 60 minutes, $p < 0.01$) et qui retrouve les valeurs post injection de CFA à 120 minutes ($1.782s \pm 0.221s$ à D4 vs $1.895s \pm 0.070s$ à 120 minutes, $p > 0.05$) (Fig. 25 A). Par conséquent l'injection d'A2 induit une analgésie thermique transitoire chez les souris CFA. Par contre l'injection d'A2 chez les souris NaCl n'induit aucune modification significative de PWL ($7.290s \pm 0.447s$ à D4 vs $6.88s \pm 0.251s$ à 30 minutes, $p > 0.05$) (Fig. 25B).

D'autre part nos résultats montrent que l'injection d'aCSF n'induit aucune modification de PWL chez les souris CFA ($n=5$) ($1.938s \pm 0.310s$ à D4 vs $1.943s \pm 0.353s$ à 30 minutes, $p > 0.05$), ni chez les souris NaCl ($7.275s \pm 0.534s$ à D4 vs $6.28s \pm 0.055s$ à 30 minutes, $p > 0.05$).

De plus nos résultats montrent que l'analgésie observée avec l'agoniste A2 est annulée par l'addition de l'antagoniste R3 chez les souris CFA ($n=6$). Ainsi l'injection du mélange A2+R3 n'induit aucune modification de la PWL ($1.668s \pm 0.05s$ à D4 vs $1.572s \pm 0.120s$ à 30 minutes, $p > 0.05$) (Fig. 25C).

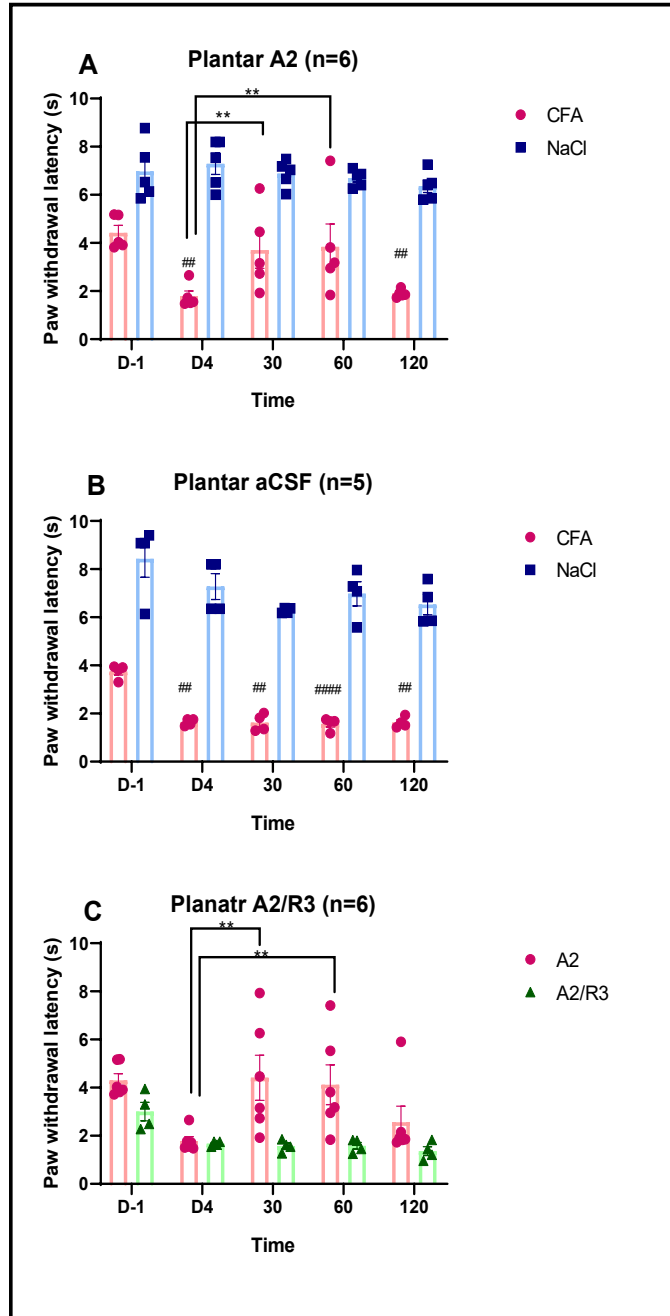


Figure 25 : Effets des modulateurs de RFXP-3 sur l'hypersensibilité thermique.

La latence de retrait de la patte postérieure, en utilisant laser IR, est mesurée chez les souris CFA (rose) et NaCl (bleu), un jour avant injection intraplantaire (IP) de CFA ou NaCl (D-1), 4 jours après injection IP de CFA/NaCl (D4), puis après 30 minutes, 60 minutes et 120 minutes d'injection des modulateurs de RFXP-3.

(A) L'injection d'A2 induit une analgésie thermique transitoire chez les souris CFA et pas d'effet chez les souris NaCl.

(B) L'injection d'aCSF n'induit aucune modification au PWL chez les souris CFA et NaCl

(C) L'injection de R3 (vert) annule l'analgésie observée avec l'A2 (rose) chez les souris CFA.

Les résultats sont exprimés en moyenne \pm SEM.

** $p < 0.01$ (comparaison du PWT par rapport au D4)

$p < 0.0001$ et ## $p < 0.01$ (comparaison par rapport à D-1)

1.1.1.3. Effet du nouvel peptide A5

Nos collaborateurs au Florey Institute en Australie, ont développé un nouvel agoniste de RXFP-3, formé uniquement de la chaîne B. Nous avons, comme pour l'agoniste A2, étudié la modulation de l'hypersensibilité mécanique et celle de l'hypersensibilité thermique par ce peptide. Les résultats obtenus miment, à quelques différences près, ceux obtenus avec A2.

Nos résultats montrent, comme pour A2, une analgésie mécanique transitoire. En effet l'injection de l'agoniste A5 chez les souris CFA (n=7) induit une augmentation significative de PWT à 30 minutes ($0.486\text{g} \pm 0.040\text{g}$ à D-4 vs $1.486\text{g} \pm 0.086\text{g}$ à 30 minutes, $p < 0.0001$), qui diminue à 60 minutes ($0.486\text{g} \pm 0.040\text{g}$ à D-4 vs $0.543\text{g} \pm 0.084$ à 60 minutes, $p > 0.05$) et retrouve les valeurs post injection de CFA à 120 minutes ($0.486\text{g} \pm 0.040\text{g}$ à D-4 vs $0.486\text{g} \pm 0.04\text{g}$, $p > 0.05$) (Fig. 26 A). Par contre l'injection d'A5 chez les souris NaCl n'induit aucune modification significative de PWT (1.4g à D-4 vs 1.4 à 30 minutes, $p > 0.05$) (Fig. 26A).

D'autre part nos résultats montrent que l'analgésie observée avec l'agoniste A5 est annulée par l'addition de l'antagoniste R3 chez les souris CFA (n=7). Ainsi l'injection du mélange A5+R3 n'induit aucune modification de la PWT ($0.457\text{g} \pm 0.037\text{g}$ à D-4 vs $0.457\text{g} \pm 0.037\text{g}$ à 30 minutes, $p > 0.05$) (Fig. 26C).

Par ailleurs nos résultats montrent que l'injection d'A5 induit une analgésie thermique. En effet l'injection de l'agoniste A5 chez les souris CFA (n=7) induit une augmentation significative de PWL à 30 minutes ($1.435\text{s} \pm 0.097\text{s}$ à D-4 vs $2.817\text{s} \pm 0.164\text{s}$ à 30 minutes, $p < 0.0001$), qui diminue à 60 minutes ($1.435\text{s} \pm 0.097\text{s}$ à D-4 vs $1.884\text{s} \pm 0.166\text{s}$ à 60 minutes, $p > 0.05$) et retrouve les valeurs post injection de CFA à 120 minutes ($1.435\text{s} \pm 0.097\text{s}$ à D-4 vs $1.534\text{s} \pm 0.047\text{s}$, $p > 0.05$) (Fig. 26 B). Par contre l'injection d'A5 chez les souris NaCl n'induit aucune modification significative de PWL ($3.662\text{s} \pm 0.238\text{s}$ à D-4 vs $3.220\text{s} \pm 0.129\text{s}$ à 30 minutes, $p > 0.05$) (Fig. 26B).

D'autre part nos résultats montrent que l'analgésie observée avec l'agoniste A5 est annulée par l'addition de l'antagoniste R3 chez les souris CFA (n=7). Ainsi l'injection du mélange A5+R3 n'induit aucune modification de la PWL ($1.502\text{s} \pm 0.132\text{s}$ à D-4 vs $1.557\text{s} \pm 0.062\text{s}$ à 30 minutes, $p > 0.05$) (Fig. 26D).

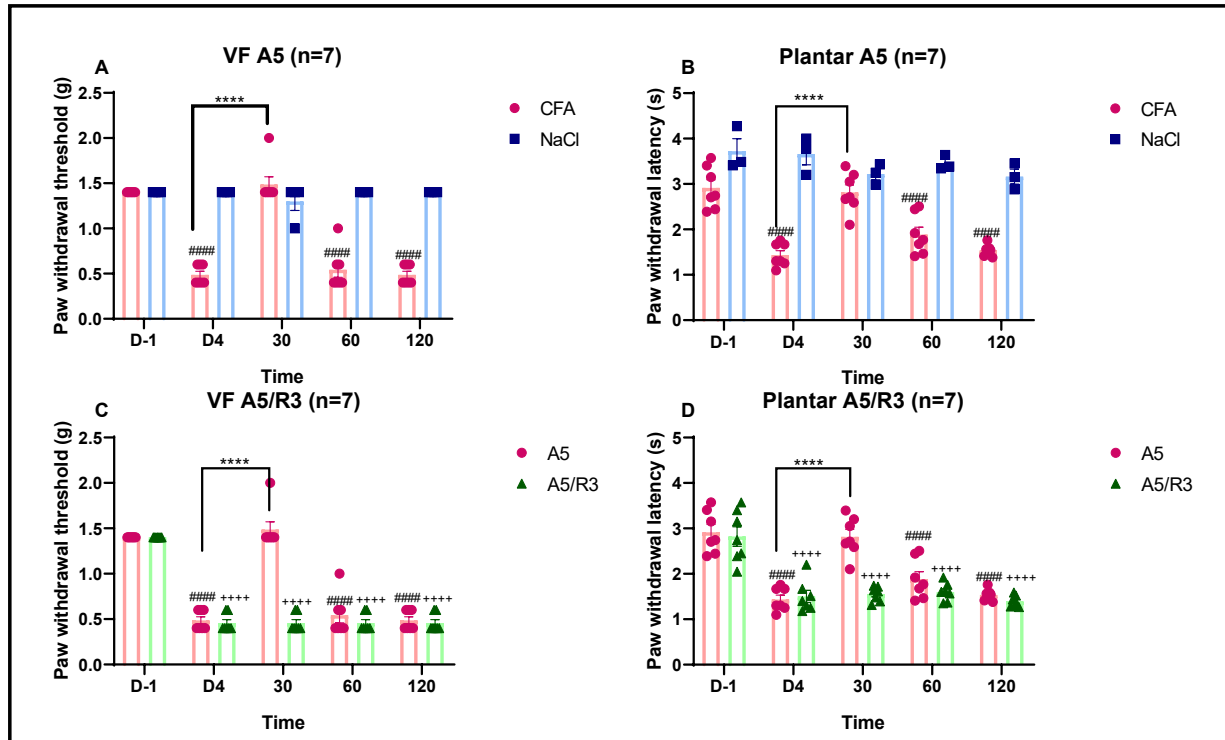


Figure 26 : Effets d'A5 sur l'hypersensibilité mécanique et thermique

Le seuil (A,B) et la latence (C, D) de retrait de la patte postérieure, ont été mesurés chez les souris CFA (rose) et NaCl (bleu), un jour avant injection intraplantaire (IP) de CFA ou NaCl (D-1), 4 jours après injection IP de CFA/NaCl (D4), puis après 30 minutes, 60 minutes et 120 minutes d'injection de modulateurs de RXFP-3.

(A) L'injection d'A5 induit une analgésie mécanique transitoire chez les souris CFA et pas d'effet chez les souris NaCl. (C) L'injection de R3 (vert) annule l'analgésie observée avec l'A5 (rose) chez les souris CFA. (B) L'injection d'A5 induit une analgésie thermique transitoire chez les souris CFA et pas d'effet chez les souris NaCl. (D) L'injection de R3 (vert) annule l'analgésie observée avec l'A5 (rose) chez les souris CFA.

Les résultats sont exprimés en moyenne \pm SEM.

**** $p < 0.0001$ (comparaison par rapport au D4), ##### $p < 0.0001$ et ++++ $p < 0.0001$ (comparaison par rapport à D-1)

Donc en conclusion, chez les souris CFA, l'injection locale d'A2 au niveau de la BLA, induit une analgésie mécanique et une analgésie thermique qu'on observe à 30 et 60 minutes. Alors que l'injection d'A5, induit une analgésie mécanique et une analgésie thermique à 30 minutes. Et dans les 2 cas les effets sont annulés par l'addition de l'antagoniste R3. Aucun effet n'a été observé chez les souris contrôle.

1.1.2. Après injection dans l'ACC

Afin d'étudier l'effet du système relaxine-3/RXFP-3 après injection dans l'ACC, les souris C57Bl6/J ont été équipés par des guides canules implantés au niveau de l'ACC. L'effet a été étudié dans un modèle de douleur inflammatoire persistante (souris CFA), ainsi que dans un modèle contrôle (souris NaCl).

1.1.2.1. Hypersensibilité mécanique

Nous avons commencé par évaluer l'hypersensibilité mécanique des souris en ayant recours au test de Von Frey. Et on a eu recours à une Anova 2 voies suivie du test posthoc Tukey pour l'analyse statistique des résultats.

Le seuil de retrait de la patte postérieure diminue significativement après injection de CFA ($1.52 \text{ g} \pm 0.120 \text{ g}$ à D-1 vs $0.440 \text{ g} \pm 0.040 \text{ g}$ à D4, $p < 0.0001$), alors qu'il reste inchangé après injection de NaCl ($1.52 \text{ g} \pm 0.120 \text{ g}$ à D-1 vs $1.32 \text{ g} \pm 0.08 \text{ g}$ à D4, $p > 0.05$) au cours de toutes les cohortes (Fig. 27 A,B,C).

Nos résultats montrent que l'injection d'A5 au niveau de l'ACC induit une augmentation significative du PWT à 30 minutes ($0.440 \text{ g} \pm 0.040 \text{ g}$ à D4 vs $1.520 \text{ g} \pm 0.120 \text{ g}$ à 30 minutes, $p < 0.0001$). Le PWT diminue et retrouve les valeurs de post injection de CFA à 60 minutes ($0.440 \text{ g} \pm 0.040 \text{ g}$ à D4 vs 0.4 g à 60 minutes, $p > 0.05$) et à 120 minutes ($0.440 \text{ g} \pm 0.040 \text{ g}$ à D4 vs 0.4 g à 120 minutes, $p > 0.05$). Par conséquent, l'injection d'A5 induit une analgésie mécanique transitoire chez les souris CFA. Par contre l'injection d'A5 chez les souris NaCl n'induit aucune modification du PWT ($1.32 \text{ g} \pm 0.08 \text{ g}$ à D4 vs 1.4 g à 30 minutes, $p > 0.05$) (Fig. 27 A)

D'autre par nos résultats montrent que l'injection d'aCSF ($n=5$) n'induit aucune modification chez les souris CFA ($0.440 \text{ g} \pm 0.040 \text{ g}$ à D4 vs $0.440 \text{ g} \pm 0.040 \text{ g}$ à 30 minutes, $p > 0.05$), ni chez les souris NaCl ($1.32 \text{ g} \pm 0.08 \text{ g}$ à D4 vs 1.4 g à 30 minutes, $p > 0.05$) (Fig. 27 B)

De plus nos résultats montrent que l'analgésie observée avec l'agoniste A5 est annulée par l'addition de l'antagoniste R3 ($n=5$), ainsi l'injection d'A5 suivi de R3 ne modifie pas le PWT chez les souris CFA ($0.440 \text{ g} \pm 0.040 \text{ g}$ à D4 vs $0.440 \text{ g} \pm 0.040 \text{ g}$ à 30 minutes, $p > 0.05$). De même l'injection du mélange A5+R3 n'induit aucune modification chez les souris NaCl ($1.32 \text{ g} \pm 0.08 \text{ g}$ à D4 vs $1.32 \text{ g} \pm 0.08 \text{ g}$ à 30 minutes, $p > 0.05$) (Fig. 27.C).

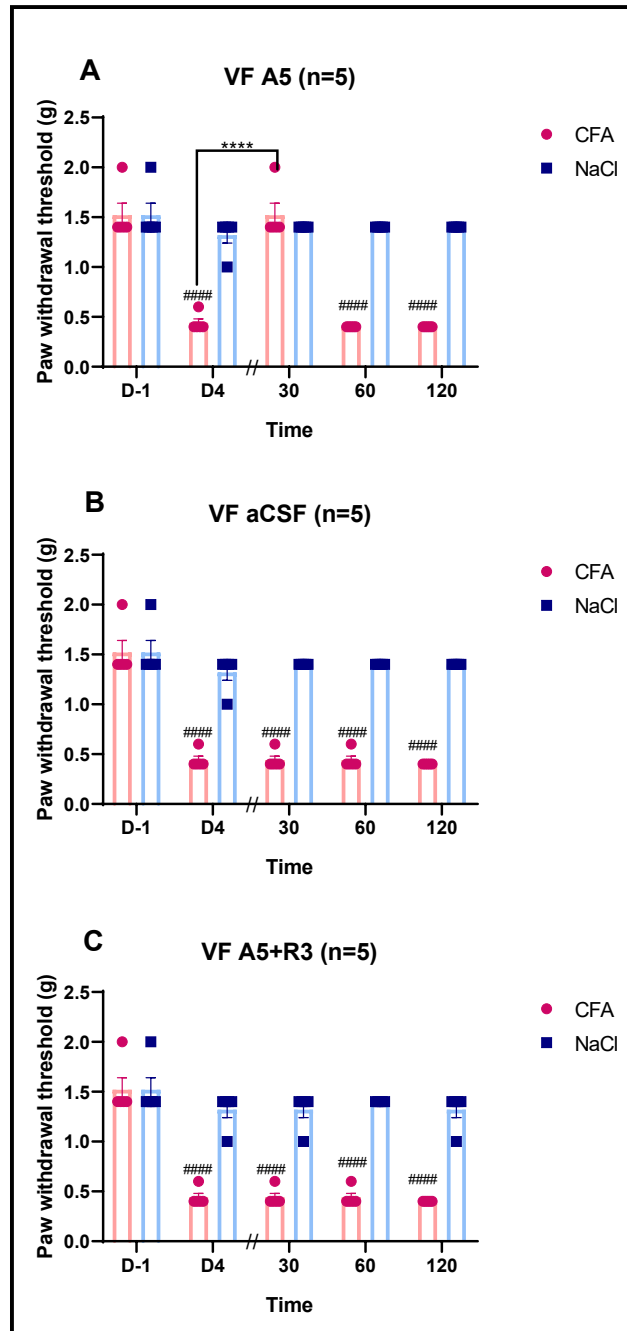


Figure 27 : Effets de modulateurs de RFXFP-3 sur l'hypersensibilité mécanique.

Le seuil de retrait de la patte postérieure, en utilisant les filaments de Von Frey, est mesuré chez les souris CFA (rose) et NaCl (bleu), un jour avant injection intraplantaire (IP) de CFA ou NaCl (D-1), 4 jours après injection IP de CFA/NaCl (D4), puis après 30 minutes, 60 minutes et 120 minutes d'injection des modulateurs de RFXFP-3.

(A) L'injection d'A5 induit une analgésie mécanique transitoire chez les souris CFA et pas d'effet chez les souris NaCl.

(B) L'injection d'aCSF n'induit aucune modification au PWT chez les souris CFA et NaCl

(C) L'injection de R3 annule l'analgésie observée avec l'A5 chez les souris CFA. Et aucun effet n'est observé chez les souris NaCl

Les résultats sont exprimés en moyenne \pm SEM.

**** $p < 0.0001$ (comparaison du PWT par rapport au D4)

$p < 0.0001$ (comparaison par rapport à D-1)

1.1.2.2. Hypersensibilité thermique

Par la suite nous nous sommes intéressés à étudier l'effet du système relaxine-3/RFXP-3 sur l'hypersensibilité thermique en ayant recours au plantar test. De même on a eu recours à une Anova 2 voies suivie du test posthoc Tukey pour l'analyse statistique des résultats.

La latence de retrait de la patte postérieure diminue significativement chez les souris CFA ($2.908s \pm 0.06s$ à D-1 vs $1.37s \pm 0.09s$ à D4, $p < 0.0001$) et elle reste inchangée chez les souris NaCl ($3.43s \pm 0.146s$ à D-1 vs $3.598s \pm 0.166s$ à D4, $p > 0.05$) au cours de toutes les cohortes (Fig. 28 A,B,C).

Nos résultats montrent que l'injection de l'agoniste A5 chez les souris CFA ($n=5$) n'induit aucune modification du PWL chez les souris CFA ($1.37s \pm 0.09s$ à D4 vs $1.442s \pm 0.202s$ à 30 minutes, $p > 0.05$), ni chez les souris NaCl ($3.598s \pm 0.166s$ à D4 vs $3.598s \pm 0.169s$ à 30 minutes, $p > 0.05$) (Fig. 28A).

D'autre part nos résultats montrent que l'injection d'aCSF n'induit aucune modification de PWL chez les souris CFA ($n=5$) ($1.37s \pm 0.09s$ à D4 vs $1.316s \pm 0.039s$ à 30 minutes, $p > 0.05$), ni chez les souris NaCl ($3.596s \pm 0.166s$ à D4 vs $3.304s \pm 0.07s$ à 30 minutes, $p > 0.05$) (Fig. 28B).

De plus nos résultats montrent que l'injection du mélange A5+R3 n'induit aucune modification du PWL chez les souris CFA ($n=5$) ($1.37s \pm 0.09s$ à D4 vs $1.488s \pm 0.094s$ à 30 minutes, $p > 0.05$). Cependant l'injection de ce mélange induit une diminution significative du PWL chez les souris NaCl à 30 minutes ($3.596s \pm 0.166s$ à D4 vs $2.17s \pm 0.067s$ à 30 minutes, $p < 0.0001$). Cette diminution se maintient à 60 minutes ($3.596s \pm 0.166s$ à D4 vs $2.424s \pm 0.208s$ à 60 minutes, $p < 0.0001$) pour revenir aux valeurs initiales ($3.596s \pm 0.166s$ à D4 vs $3.246s \pm 0.054s$ à 120 minutes, $p > 0.05$) (Fig. 28 C).

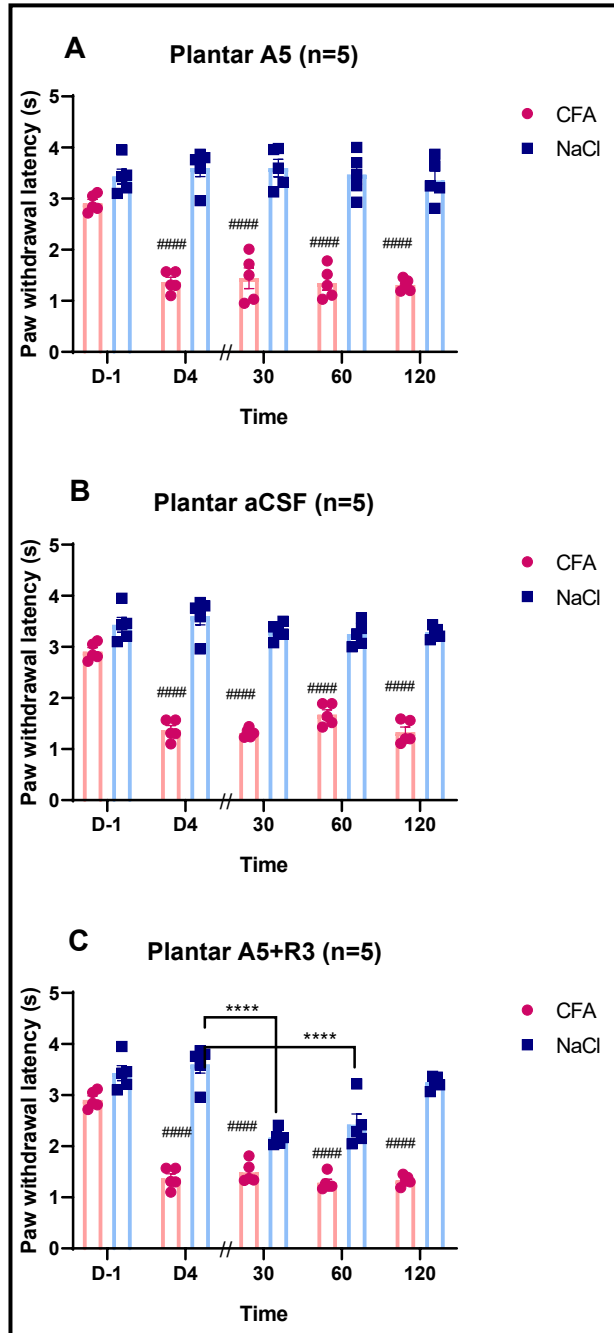


Figure 28 : Effets de modulateurs de RXFP-3 sur l'hypersensibilité thermique.

La latence de retrait de la patte postérieure, en utilisant un laser IR, est mesuré chez les souris CFA (rose) et NaCl (bleu), un jour avant injection intraplantaire (IP) de CFA ou NaCl (D-1), 4 jours après injection IP de CFA/NaCl (D4), puis après 30 minutes, 60 minutes et 120 minutes d'injection des modulateurs de RXFP-3.

- (A) L'injection d'A5 n'induit aucune modification du PWL chez les souris CFA ni chez les souris NaCl
- (B) L'injection d'aCSF n'induit aucune modification du PWL chez les souris CFA et NaCl
- (C) L'injection du mélange A5+R3 n'induit aucune modification du PWL chez les souris CFA. Mais induit une diminution du PWL chez les souris NaCl.

Les résultats sont exprimés en moyenne \pm SEM.

**** $p < 0.0001$ (comparaison du PWT par rapport au D4)

$p < 0.0001$ (comparaison par rapport à D-1)

En conclusion, l'injection locale d'A5 au niveau de l'ACC, induit une analgésie mécanique transitoire chez les souris CFA, alors que l'injection du mélange A5+R3 induit une proalgie thermique transitoire chez les souris NaCl.

1.2. Distribution et caractérisation neuronale du système relaxine-3/RXFP-3

Nos résultats comportementaux ont mis en évidence que le système relaxine-3/RXFP-3 joue un rôle dans la modulation de la douleur dans un modèle de douleur inflammatoire persistante. Par conséquent nous nous sommes penchés sur la détermination de la nature des neurones de ce système, leurs interactions ainsi que leurs distributions cérébrales.

1.2.1. Caractérisation des neurones relaxine-3

Afin de déterminer le phénotype des neurones à relaxine-3 aux niveaux des régions cérébrales d'intérêt notamment la BLA et l'ACC, on a eu recours à l'immunohistochimie.

Nos résultats montrent qu'au niveau de la BLA, on a plus de contact entre les neurones relaxine-3 et les neurones SOM (Fig.29 a) qu'entre les neurones relaxine-3 et les neurones PV (Fig.29 d et e) aux niveaux de souris naïves. Le contact entre les neurones relaxine-3 et SOM a été aussi mis en évidence par l'IHC réalisé sur les souris 5-HT-Cre (Fig.29 b et c).

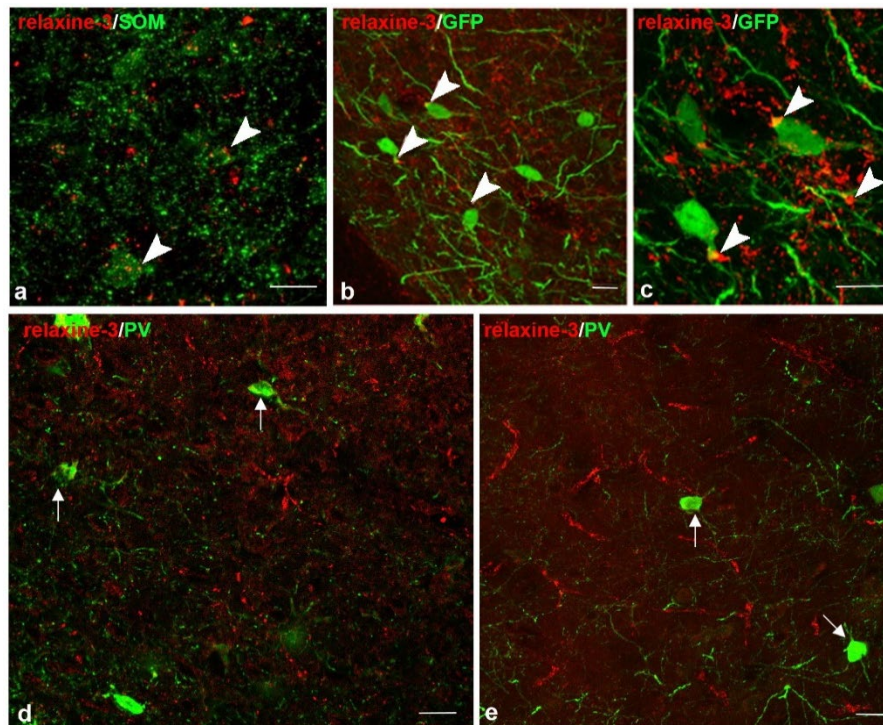


Figure 29: IHC des neurones relaxine-3 au niveau de la BLA. Contact entre neurones relaxine-3 et neurones SOM chez souris naïves (a). Contact neurones relaxine-3 et neurones SOM chez les souris 5-HT-Cre (b et c). Contact neurones relaxine-3 et neurones PV chez souris naïves (c et d). Les pointes des flèches indiquent les neurones en contact. Barre 10 μ m

De même la double IHC anti-relaxine-3 et anti-SOM montrent qu'il existe un contact entre les neurones relaxine-3 et les neurones SOM au niveau de l'ACC (Fig.30). Les 2 neurones visualisés en IHC sont aussi illustrés sous forme d'une reconstruction 3D (Fig.31).

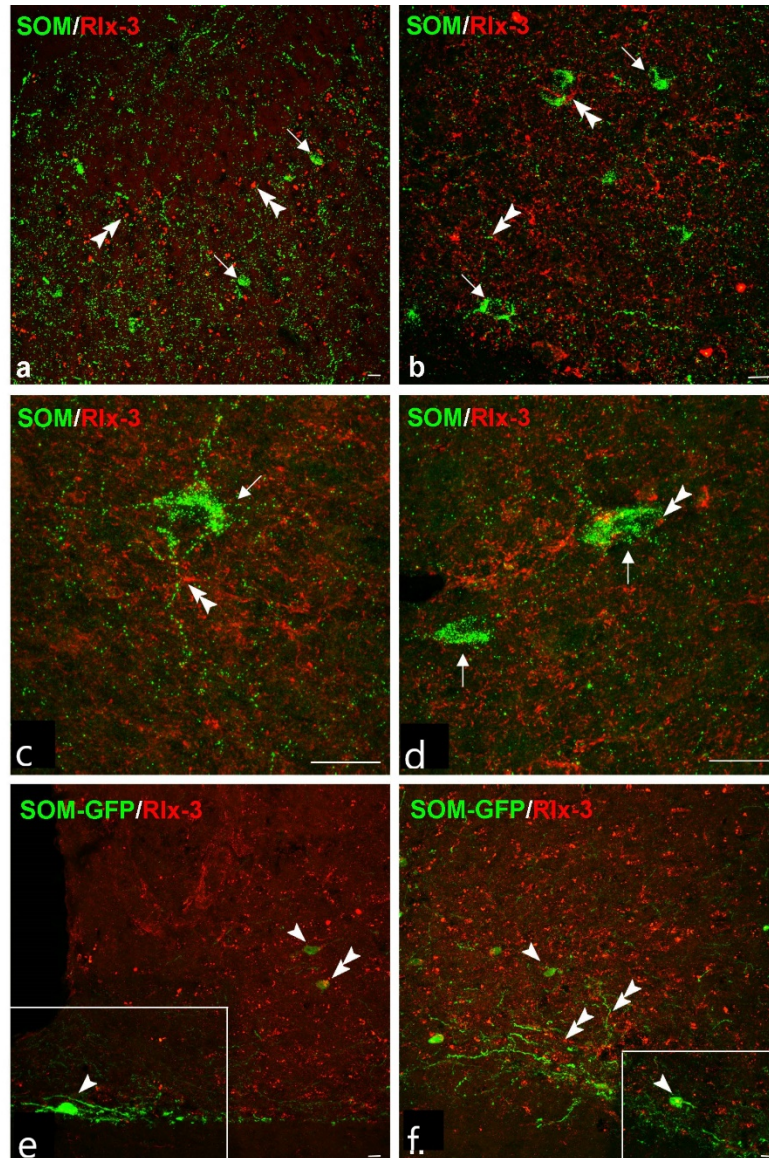


Figure 30 : IHC des neurones relaxine-3 au niveau de l'ACC. On observe un contact entre un neurone relaxine-3 et neurone SOM à différents grossissements (a, c, e). On observe un contact entre un autre neurone relaxine-3 et neurone SOM (b, d, f).
Barre 10µm

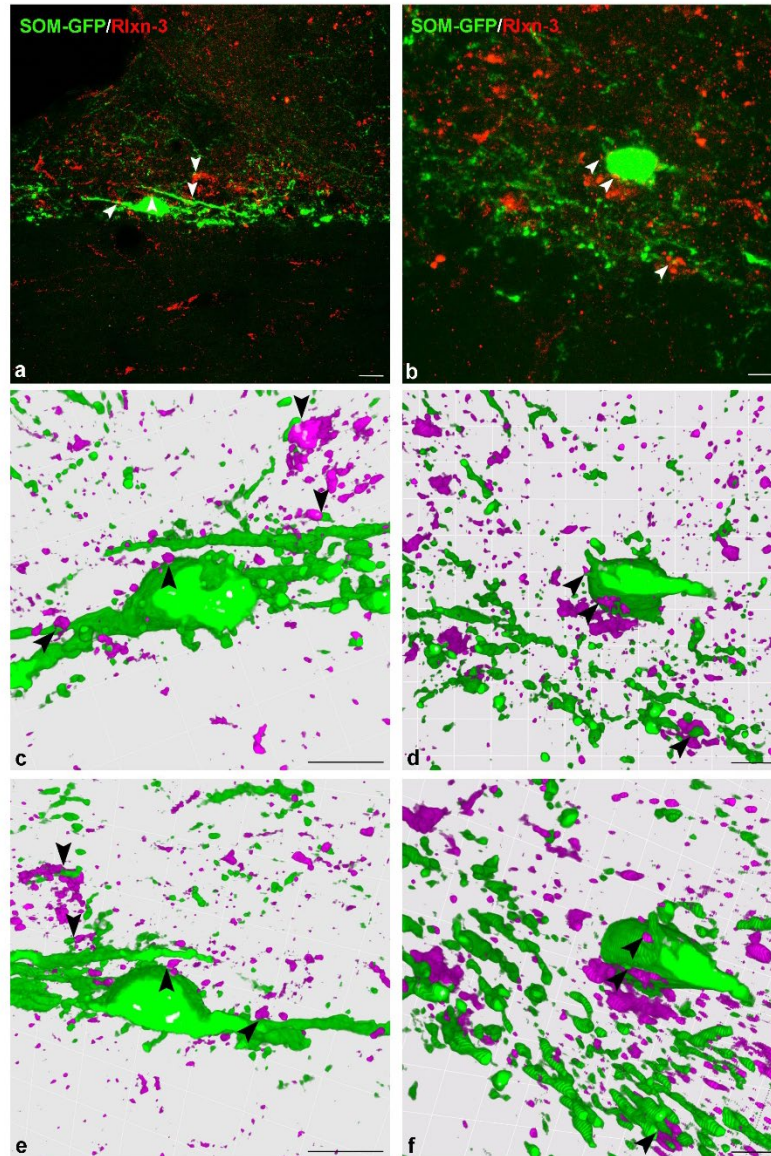


Figure 31 : Reconstruction 3D des neurones relaxine-3 de l'ACC

1.2.2. Distribution et caractérisation des neurones RXFP-3

Afin de déterminer le phénotype neurochimique des neurones exprimant l'ARNm (acide ribonucléique messager) de RXFP-3 dans les régions cérébrales d'intérêt (ACC et amygdale), nous avons eu recours au RNAscope.

Nos résultats montrent que la majorité des neurones exprimant l'ARNm RXFP-3 chez les souris CFA (n=10) aussi bien que chez les souris NaCl (n=10) sont majoritairement des neurones somatostatine (SOM) aux niveaux de l'ACC (Fig. 34) ($90\% \pm 10\%$ CFA, $80\% \pm 7.37\%$ NaCl), de l'amygdale basolatérale (BLA, Fig. 35) ($83.33\% \pm 7.397\%$ CFA, $83.33\% \pm 11.386\%$ NaCl), de

l'amygdale médiane (BMA, Fig. 36) ($62.659\% \pm 4.563\%$ CFA, $38.89\% \pm 18.088\%$ NaCl) et de l'amygdale centrale (CeA, Fig. 37) ($62.879\% \pm 6.452\%$ CFA, $600556\% \pm 15.821\%$ NaCl). Minoritairement les neurones exprimant l'ARNm RXFP-3 chez les souris CFA et NaCl sont des neurones parvalbumine (PV) aux niveaux de l'ACC (0% CFA, 0% NaCl), la BLA ($2.778\% \pm 2.778\%$ CFA, 0% NaCl), la BMA ($0.794\% \pm 0.794\%$ CFA, 0% NaCl) et la CeA (0% CFA, $15.556\% \pm 11.440\%$ NaCl). D'autre part nos résultats montrent qu'un faible pourcentage des neurones exprimant l'ARNm RXFP-3 co-expriment la SOM et la PV aux niveaux de l'ACC (0% CFA, $10\% \pm 5.66\%$ NaCl) et de la BLA ($2.778\% \pm 2.778\%$ CFA, $16.67\% \pm 11.386\%$ NaCl) (Fig.32)

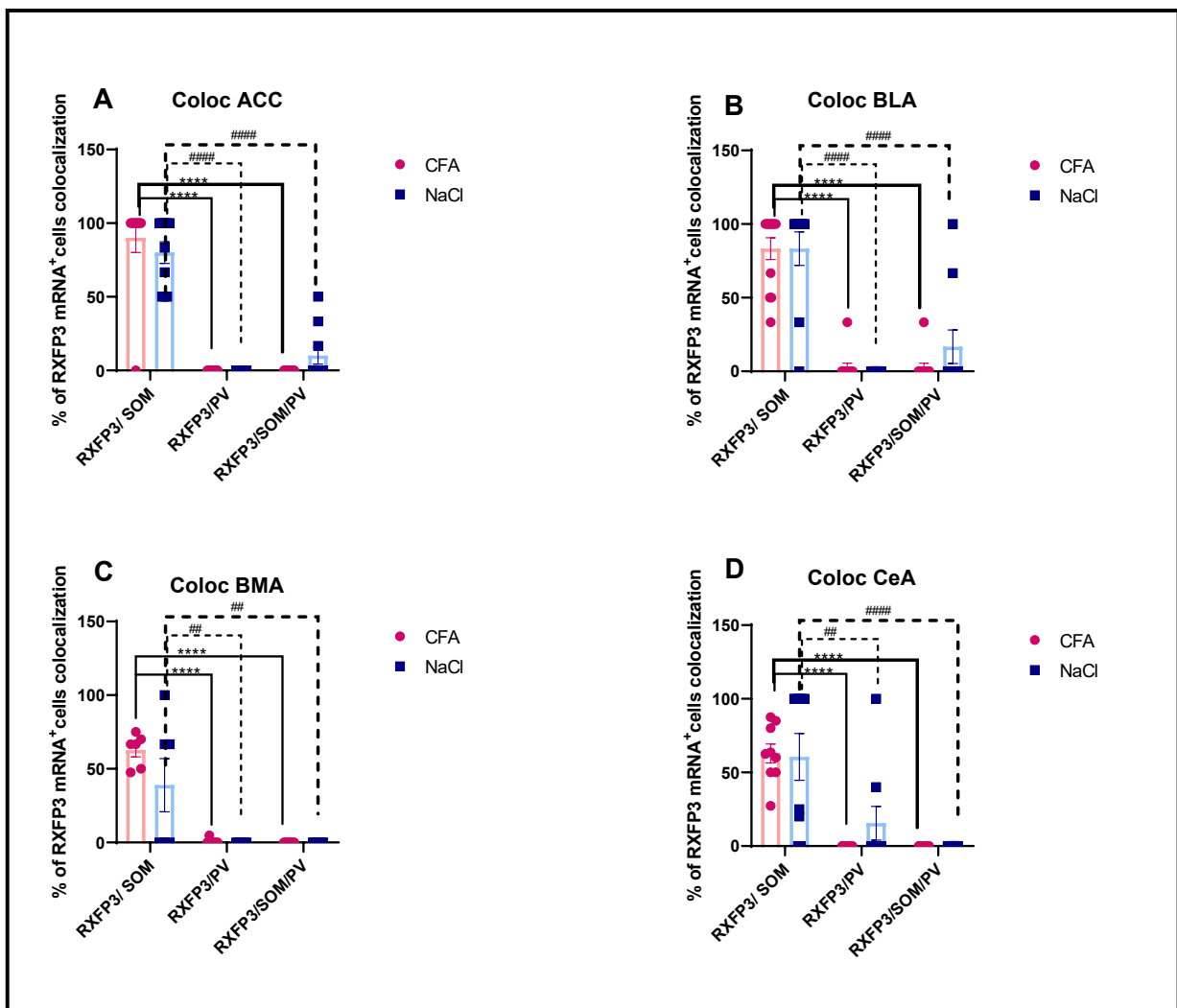


Figure 32: Co-localisation des neurones exprimant RXFP-3
 Les résultats sont exprimés en moyenne \pm SEM, avec **** $p < 0.0001$, ** $p < 0.01$, ##### $p < 0.0001$, ## $p < 0.01$

Dans un deuxième temps nous nous sommes intéressés à la distribution des neurones RXFP-3 aux niveaux des régions d'intérêt ainsi que de leurs répartitions entre souris CFA (n=10) et NaCl (n=10). De ce fait nous avons eu recours au RNAscope et nous avons mesuré l'intensité de marquage de RXFP-3. Nos résultats montrent que l'intensité de marquage est significativement supérieure chez les souris CFA au niveau de l'ACC ($145.906 \mu\text{m}^2 \pm 50.616 \mu\text{m}^2$ CFA vs $38.897 \mu\text{m}^2 \pm 12.959 \mu\text{m}^2$ NaCl, $p < 0.01$). Au niveau de l'amygdale, il y a une augmentation non significative de cette intensité chez les souris CFA aux niveaux de la BMA ($106.911 \mu\text{m}^2 \pm 35.286 \mu\text{m}^2$ CFA vs $15.045 \mu\text{m}^2 \pm 5.664 \mu\text{m}^2$ NaCl, $p > 0.05$) et de la CeA ($89.734 \mu\text{m}^2 \pm 18.449 \mu\text{m}^2$ CFA vs $57.063 \mu\text{m}^2 \pm 115.084 \mu\text{m}^2$ NaCl, $p > 0.05$), cependant nous avons observé une diminution non significative de cette intensité au niveau de la BLA ($36.565 \mu\text{m}^2 \pm 7.637 \mu\text{m}^2$ CFA vs $99.039 \mu\text{m}^2 \pm 20.631 \mu\text{m}^2$ NaCl, $p > 0.05$) (Fig.33)

De plus nos résultats montrent qu'il n'existe pas de différence d'intensité de marquage entre les différentes régions cérébrales au sein d'un même groupe de souris sauf chez les souris CFA où on observe une différence significative d'intensité entre l'ACC et la BLA ($145.906 \mu\text{m}^2 \pm 50.616 \mu\text{m}^2$ ACC vs $36.565 \mu\text{m}^2 \pm 7.637 \mu\text{m}^2$ BLA, $p < 0.01$) (Fig. 33).

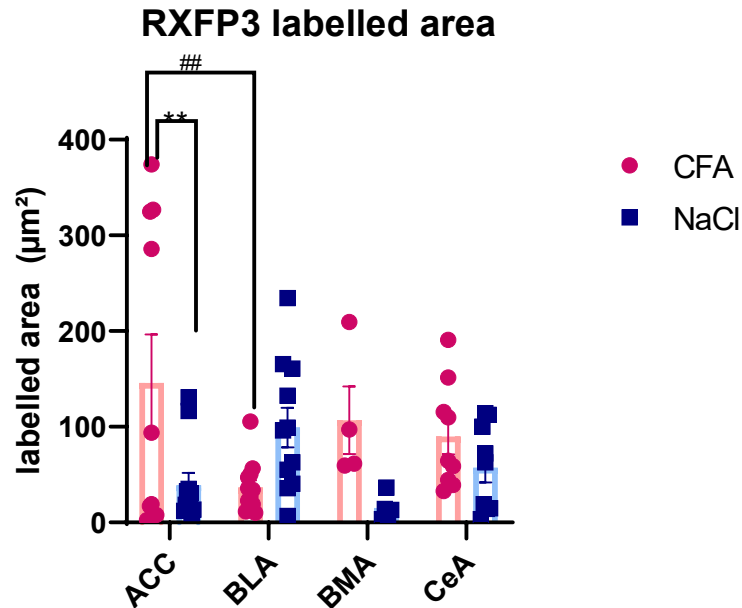


Figure 33: Intensité de marquage de RXFP-3.
Les résultats sont exprimés en moyenne \pm SEM avec ** $p < 0.01$, ## $p < 0.01$

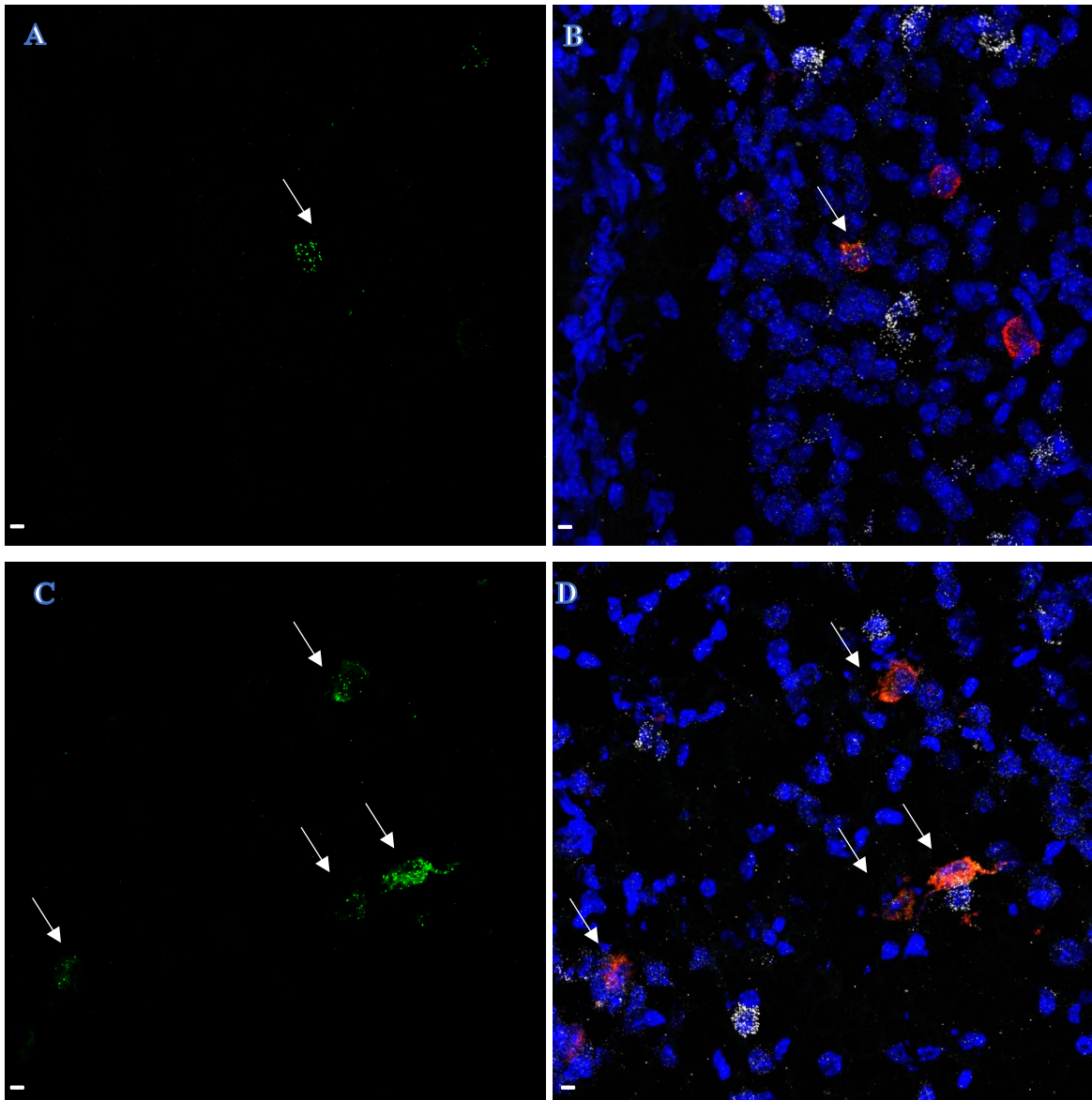


Figure 34: Phénotype neurochimique des neurones RXFP-3 au niveau de l'ACC chez les souris NaCl (A et B) et les souris CFA (C et D). ARNm Rxfp-3 (vert), ARNm Som (rouge), ARNm PV (blanc). L'intensité de marquage est significativement supérieure dans le cas de souris CFA. Les neurones exprimant l'ARNm de RXFP-3 sont majoritairement des neurones SOM. Les pointes des flèches indiquent les neurones marqués. Barres= 20 μ m

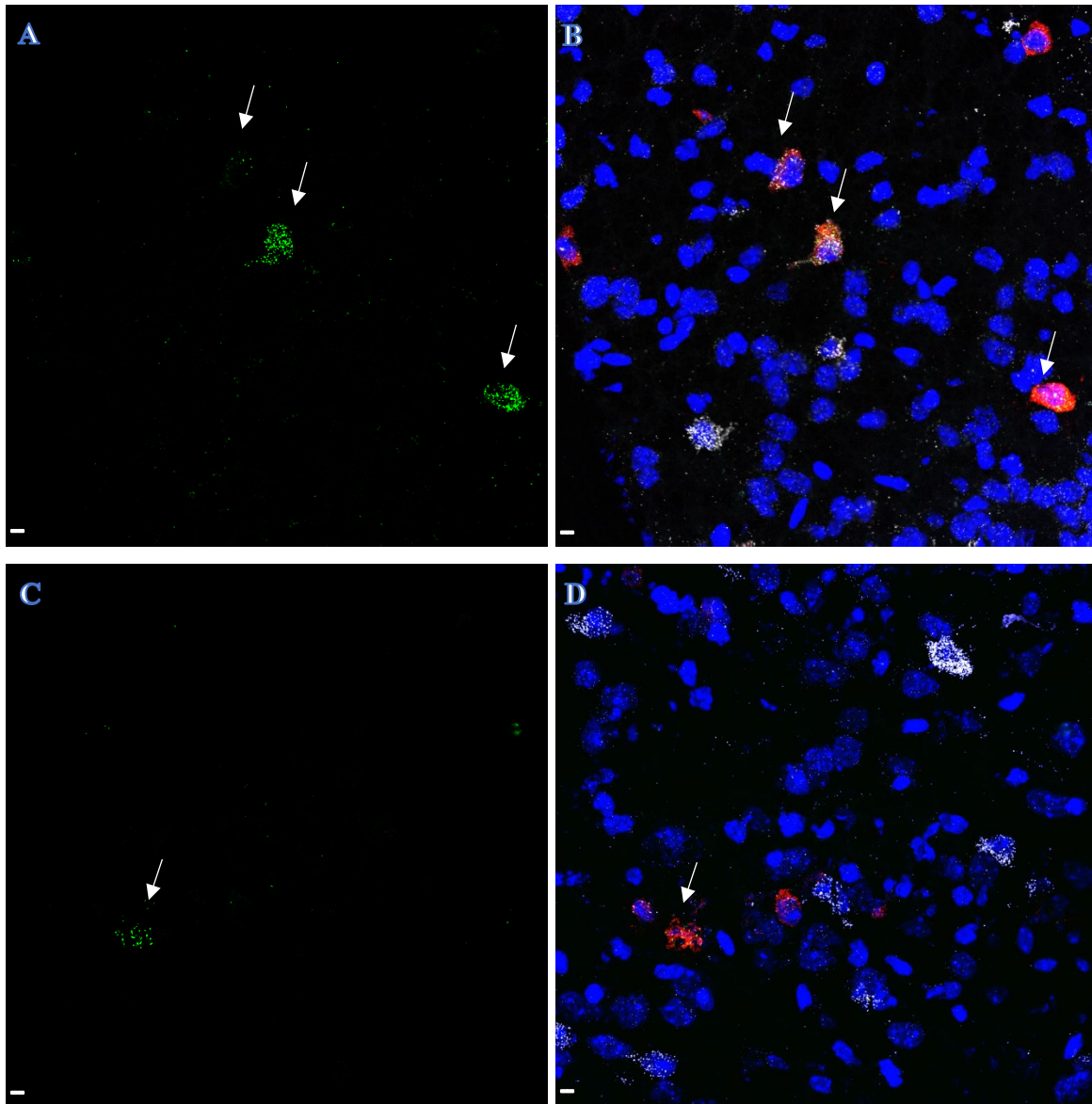


Figure 35: Phénotype neurochimique des neurones RXFP-3 au niveau de la BLA chez les souris NaCl (A et B) et les souris CFA (C et D). ARNm Rxfp-3 (vert), ARNm Som (rouge), ARNm PV (blanc). L'intensité de marquage a une tendance d'être supérieure dans le cas de souris NaCl. Les neurones exprimant l'ARNm de RXFP-3 sont majoritairement des neurones SOM. Les pointes des flèches indiquent les neurones marqués. Barres = 20 μ m

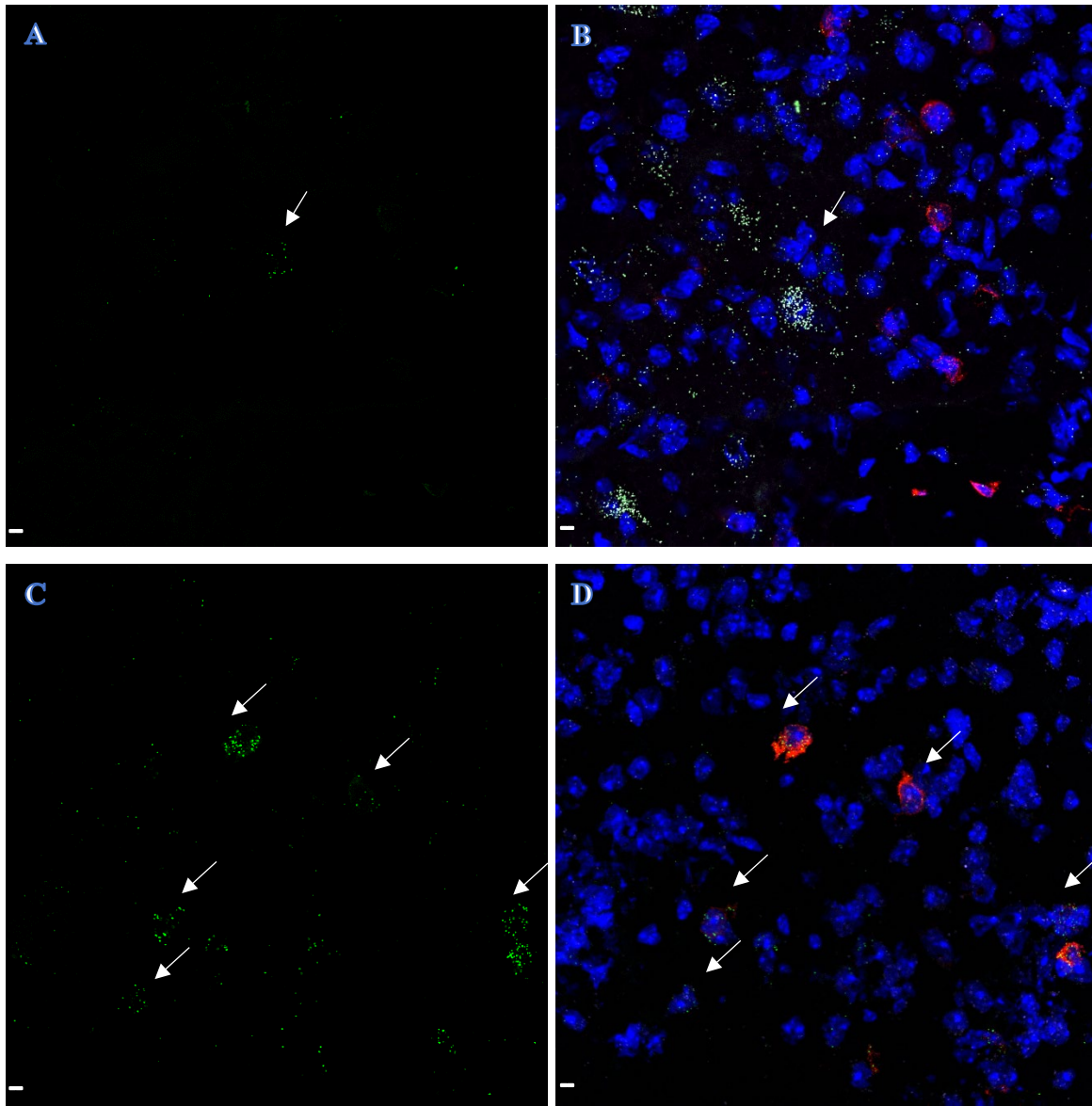


Figure 36 : Phénotype neurochimique des neurones RXFP-3 au niveau de la BMA chez les souris NaCl (A et B) et les souris CFA (C et D). ARNm Rfxp-3 (vert), ARNm Som (rouge), ARNm PV (blanc). L'intensité de marquage a une tendance d'être supérieure dans le cas de souris CFA. Les neurones exprimant l'ARNm de RXFP-3 sont majoritairement des neurones SOM. Les pointes des flèches indiquent les neurones marqués. Barres= 20 μ m

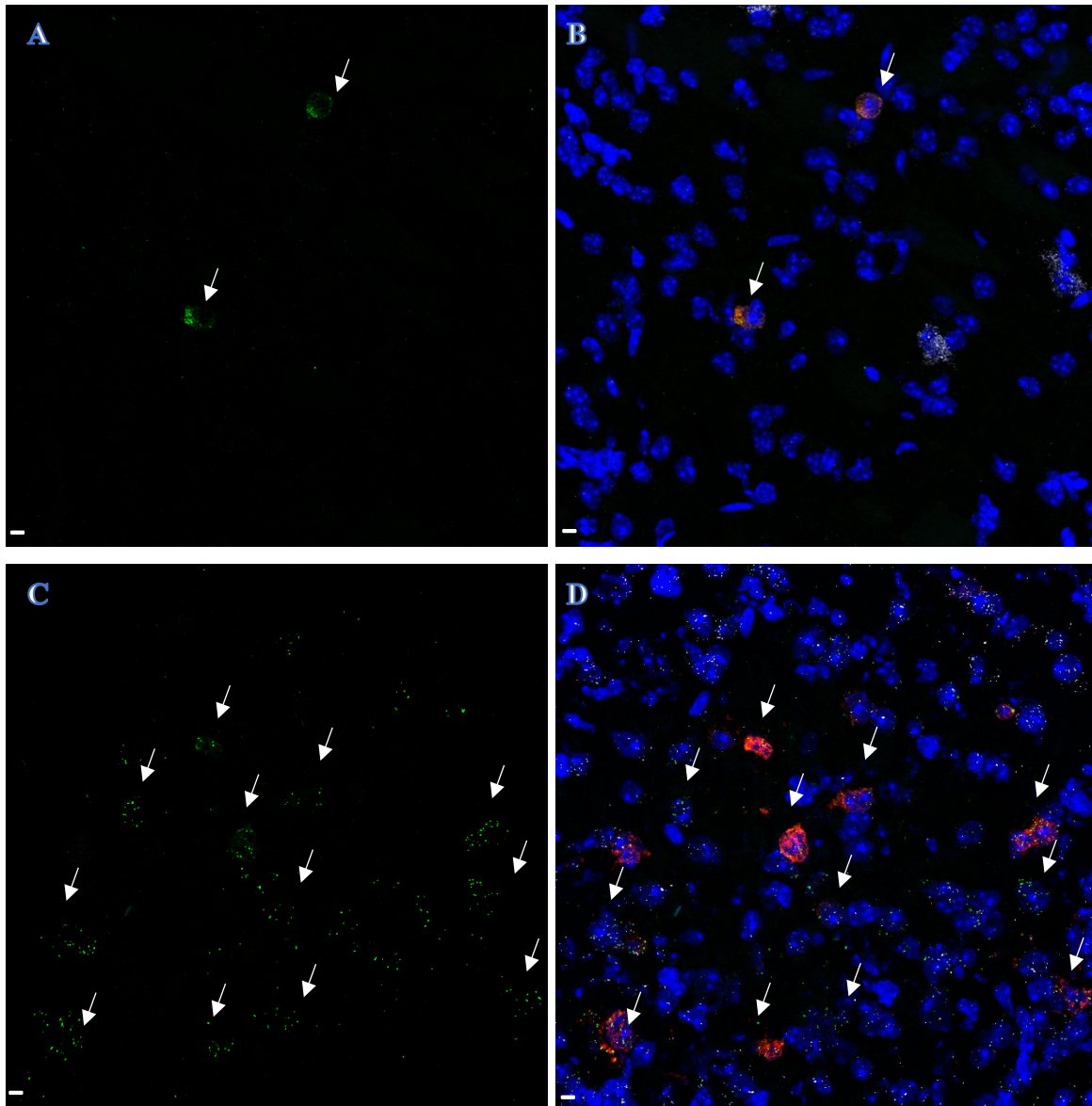


Figure 37: Phénotype neurochimique des neurones RXFP-3 au niveau de la CeA chez les souris NaCl (A et B) et les souris CFA (C et D). ARNm Rxfp-3 (vert), ARNm Som (rouge), ARNm PV (blanc). L'intensité de marquage a une tendance d'être supérieure dans le cas de souris CFA. Les neurones exprimant l'ARNm de RXFP-3 sont majoritairement des neurones SOM. Les pointes des flèches indiquent les neurones marqués. Barres = 20 μ m

2. Signalisation relaxine/RXFP-1 et douleur inflammatoire persistante.

Dans un deuxième temps nous nous sommes intéressés à la modulation de la douleur par le système relaxine/RXFP-1.

La famille relaxine renferme, en plus de la relaxine-3, la relaxine qui est surtout connue pour ses propriétés antifibrotiques. Il existe très peu d'études s'attardant sur le rôle de la relaxine au niveau du système nerveux. Cependant les données de l'Allen Brain Atlas mettent en évidence la présence de RXFP-1 aux niveaux des régions cérébrales impliquées dans la douleur telles que l'ACC, le CLA et le SUB. Par conséquent nous avons décidé d'étudier l'implication du système relaxin/RXFP-1 dans la modulation de la douleur inflammatoire persistante. De ce fait on a implanté des canules au niveau du ventricule latéral de souris C57Bl6/J (CFA et contrôle), et on a testé leurs hypersensibilités mécanique et thermique (Von Frey et Plantar respectivement) après injection intracérébrale des modulateurs de RXFP-1.

D'autre part nous nous sommes attardés sur la neuroanatomie du système relaxine/RXFP-1, plus particulièrement sur le phénotype neurochimique et la distribution des neurones de ce système en ayant recours à l'ISH et l'IHC.

Les résultats de cette étude sont décrits dans l'article ci-dessous, qui fait l'objet d'une soumission au journal Pain reports (Abboud et al en soumission).

Research Article

Analgesic effect of relaxin receptor (RXFP1) activation on persistent inflammatory pain in mice: behavioral and neurochemical data

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Abstract

Introduction: The relaxin peptide signaling system is involved in diverse physiological processes but its possible roles in the brain, including nociception, are largely unexplored.

Objective: In light of abundant expression of relaxin receptor (RXFP1) mRNA/protein in brain regions involved in pain processing, we investigated the effects of central RXFP1 activation on nociceptive behavior in a mouse model of inflammatory pain; and examined the neurochemical phenotype and connectivity of relaxin and RXFP1 mRNA positive neurons.

Methods: Mice were injected with Complete Freund's Adjuvant (CFA) into the hind paw. The RXFP1 agonist peptides, H2-relaxin or B7-33 ± the RXFP1 antagonist, B-R13/17K-H2, were injected into the lateral cerebral ventricle and mechanical and thermal sensitivity were assessed at 30-120 min. Relaxin and RXFP1 mRNA was examined using multiplex, fluorescent *in situ* hybridization in excitatory and inhibitory neurons. Relaxin-containing neurons were detected using immunohistochemistry and their projections assessed using fluorogold retrograde tract-tracing.

Results: Both H2-relaxin and B7-33 produced a strong, but transient, reduction in mechanical and thermal sensitivity of the CFA-injected hind paw only, at 30 min post-injection. Notably, co-injection of B-R13/17K-H2, blocked mechanical, but not thermal analgesia. In claustrum, cingulate cortex and subiculum, RXFP1 mRNA was expressed in CaMKII mRNA-positive, presumed excitatory neurons. Relaxin-immunoreactivity was detected in neurons in forebrain and midbrain areas involved in pain processing and sending projections to the RXFP1-rich, claustrum and cingulate cortex. No changes were noticed in CFA mice.

Conclusion: Our studies identify a previously unexplored peptidergic system that can control pain processing in the brain, and produce analgesia. (250/250 words)

Keywords: Chronic pain, G-protein-coupled receptor, relaxin, RXFP1

1. Introduction

Twenty percent of the world population suffers from chronic pain, which is often associated with emotional comorbidities such as anxiety and depression.^{3,39,66} Unfortunately chronic pain and its comorbidities are poorly managed clinically, with 40% of patients not receiving adequate treatment.^{7,8,69,70} In the brain, changes in the excitation/inhibition balance alter the way neurons or microcircuits integrate and respond to chronic nociceptive information.³⁸ This maladaptive plasticity is controlled by synaptic mechanisms⁴¹ and intrinsic properties of neurons.²³ Neuronal plasticity is also shaped by regulatory molecules, among which neuropeptides act by binding to G-protein-coupled receptors. Various neuropeptide/receptor systems are highly versatile in function and play key roles in pathophysiological functions,^{20,31} especially chronic pain.^{21,74}

How neuropeptides control pain largely depends on their site of release and target cells. In the spinal cord, for instance, neuropeptide Y¹⁵ and substance P⁷⁴ modulate pain at primary afferent fiber terminals. Neuropeptides and their receptors are widely distributed in the brain, where they modulate the sensory discriminative and emotional components of pain (e.g., in the primary somatosensory cortex and amygdala, respectively).^{67,73} Neuropeptides are also involved in the control of descending pain pathways (e.g., periaqueductal grey matter).^{40,58} We recently demonstrated the ability of the cholecystokinin/CCK2 receptor system within the amygdala, to inhibit spinal neuron excitability and produce analgesia in an inflammatory pain condition.⁵⁵ The role of neuropeptides as pain modulators has attracted increasing interest, since their action can be quite specific, and with fewer side-effects than targeting classical transmitter systems.³¹⁻³³

Human relaxin-2 (H2-relaxin) is a 6 kDa heterodimeric peptide of 53 amino acids consisting of two chains (A and B) linked by two disulphide bonds.⁴ RXFP1 is the cognate G-protein-coupled receptor for relaxin^{27,36} and was discovered in 2002. RXFP1 is characterized by a leucine-rich repeat (LRR) domain and a low density lipoprotein A (LDL_A) module,^{27,36} which are required for activation. Relaxin/RXFP1 signaling is involved in collagen metabolism⁵ and in anti-fibrotic and vasodilatory actions in various animal models of disease.⁵⁶ The therapeutic potential of relaxin has expanded in recent years to include the regulation of cardiovascular and renal function,⁵⁰ and the treatment of heart failure.⁵

The role of the relaxin/RXFP1 system in the brain has been the subject of very few studies, although it has been implicated in the regulation of thirst and osmotic homeostasis,^{49,59} and in emotional memory.⁴³ Anatomical mapping studies^{42-44,53} have revealed that RXFP1 is expressed in brain regions involved in pain processing, such as claustrum (CLA), anterior cingulate cortex (ACC) and subiculum (SUB), but to our knowledge, the role of relaxin/RXFP1 signaling has not been previously investigated in a pain context.

Therefore, the present study aimed to characterize the possible effects of the relaxin/RXFP1 signaling system on pain processing in the brain. We employed a mouse model of inflammatory persistent pain¹ to investigate any analgesic action of centrally administered relaxin peptides which selectively activate RXFP1. We also conducted a neuroanatomical characterization of the relaxin/RXFP1 system in brain areas involved in pain processing.

2. Materials and methods

2.1 Mice

Male C57BL/6J mice (*Charles Rivers, France*), 8-10 weeks old, were caged in groups of 4-5, under a standard 12:12 light/dark cycle, with free access to food, water and enrichment devices. All experimental procedures followed the ethical guidelines of the International Association for the Study of Pain and were approved by the French Ministry of Agriculture (Agreement #21890).

2.2 Cannula implantation

Mice were anesthetized by inhalation of isoflurane at 4% in an induction chamber. The anesthesia was then maintained at 2% using a mask. Mice were placed in stereotaxic frame (*RWD Desktop Digital Stereotaxic Instruments San Diego, CA, USA*). The skull was exposed and cleaned with Betadine. To alleviate pain, 100 µl of buprenorphine (*0.03 mg/ml*) and 20 µl of 1 % lidocaine were injected subcutaneously. Guide cannulae (*Bilaney, Düsseldorf, Germany*) were positioned just above the lateral ventricle (*AP: 0.7 mm, ML:-0.7 mm, DV:-2.85 mm*) to perform the injection of relaxin analogs, and were fixed in place by dental cement (*Phymep, Paris, France*).

2.3 Inflammatory pain model

At 4 days post-surgery recovery, 20 μ l of Complete Freund's Adjuvant (CFA) (*Sigma-Aldrich, St Louis, MO, USA*) was injected into the hind paw of 'inflamed CFA' mice, whereas 20 μ l of NaCl was injected into the hind paw of 'sham' mice.

2.4 Intracerebroventricular infusion of relaxin peptides

Four days after CFA/NaCl injections, mice were anesthetized with isoflurane and relaxin analogs were injected intracerebroventricularly. RXFP1 agonists (H2-relaxin⁴³ and B7-33³⁴) and antagonist (B-R13/17K-H2³⁵, referred to as B-R/K in the figures) were delivered using an internal cannula that was inserted into the guide cannula. The internal cannula was connected by polyethylene tubing to a Hamilton syringe (*Fisher Scientific, Illkirch, France*) mounted on an infusion pump (*Ugo Basile, Gemonio, Italy*). Mice were divided into 10 groups (see **Table 1**). RXFP1 activation was tested with a single injection of either 2.5 μ l of H2-relaxin-2 (H2) at 2 μ g/ μ l or 2.5 μ l of B7.33 at 2 μ g/ μ l. Control mice received a single injection of 2.5 μ l artificial cerebrospinal fluid (aCSF). The specificity of RXFP1 activation was assessed with a single injection of 5 μ l of H2 (2 μ g/ μ l) + RXFP1 antagonist, B-R13/17K-H2 (4 μ g/ μ l) or B7-33 (2 μ g/ μ l) + B-R13/17K-H2 (4 μ g/ μ l).

Table 1. Summary of groups of mice receiving intracerebroventricular infusion of relaxin peptides

Relaxin analogs (nmol)/aCSF vehicle	CFA	NaCl
H2-relaxin (0.84)	n = 6	n = 6
B7.33 (1.67)	n = 9	n = 5
aCSF	n = 9	n = 5
H2-relaxin (0.84) + B-R13/17K-H2 (1.67)	n = 5	n = 5
B7.33 (1.67) + B-R13/17K-H2 (1.67)	n = 5	n = 5

2.5 Pain assessment

Mechanical response thresholds were monitored in ‘CFA’ and ‘sham’ mice one day before their hind paw injections (D–1; reference value for each mouse). Changes in mechanical sensitivity were evaluated on day 4 after CFA/NaCl (D4). Mice were placed in the testing cage (*Ugo Basile*) one hour before the icv injection for habituation. The withdrawal threshold of the injected and uninjected hind paw/leg was determined using Von Frey hairs (*Bioseb, France*) before, and at 30 minutes, 1 hour and 2 hours after relaxin analog(s) infusion. Von Frey filaments were applied 5 successive times (with a 30-second delay in between) on the plantar surface of the hind paw of the mouse standing on its four paws. The paw withdrawal threshold was established when the mouse responded positively (withdrawal of the hind paw) to 3 out of 5 applications.

Thermal sensitivity was evaluated with the Hargreaves test. Mice were placed in the Hargreaves cages (*IITC Inc. Life Science, Tem Sega, Pessac, France*) and thermal sensitivity was tested at D–1, and D4 before and at 30 minutes, 1 hour and 2 hours after icv injection of relaxin peptides. An infrared laser beam (IR 40) was applied 3 successive times (with at least 2 minutes delay in between) on the plantar surface of the hind paw of the mouse standing on its four paws, until the mouse withdrew its hind paw. The average time to withdrawal of the 3 tests was recorded as the paw withdrawal latency.

2.6 Multiplex *in situ* hybridization histochemistry (RNAscope™)

Multiplex *in situ* hybridization (*Advanced Cell Diagnostics, Hayward, CA, USA*) was conducted according to the manufacturer's instructions with slight modifications. Fresh frozen mouse brains were cut on a cryostat (*Leica, Wetzlar, Germany*). The 16 µm coronal sections were mounted on Super Frost Plus slides (*ThermoFisher Scientific, Illkirch, France*), fixed in 4% paraformaldehyde (PFA) for 16 minutes, then dehydrated in increasing concentrations of ethanol (50, 70 and 100%) and left overnight in 100% ethanol at -20°C.

The next day, sections were dried for 10 minutes at room temperature, and a hydrophobic barrier was drawn around sections using a hydrophobic pen (*Advanced Cell Diagnostics, Hayward, CA, USA*). Sections were incubated with protease pretreatment for 20 minutes at room temperature, then washed twice in wash buffer diluted in PBS (wash buffer/PBS). Sections were then incubated with probe mix 1 [complementary to RXFP1 (catalog#: 458001), CaMKII (catalog#: 445231-C3), and GAD (catalog#: 415071-C2) mRNAs] or probe mix 2 [complementary to relaxin (catalog#: 539521-C2), CaMKII (catalog#: 445231-C3), and GAD65 (catalog#: 439371) mRNAs] for 2 hours at 40°C. Sections were washed in wash buffer/PBS and the signal amplification was achieved by incubations in solutions provided by the manufacturer, AMP1 (30 minutes at 40°C), AMP2 (15 minutes at 40°C), AMP 3 (30 minutes at 40°C) and finally AMP4 (AltA for mix 1 and AltB for mix 2) (15 minutes at 40°C). Sections were then incubated in DAPI for 20 minutes at room temperature, covered in fluorescent mounting medium, coverslipped and left in the dark for 30 minutes at room temperature before being stored at 4°C.

2.7 Neuronal tract-tracing

In studies to map some of the projections of brain relaxin neurons, 3 mice were injected, following the aforementioned surgical procedure, with 200 nl of fluorogold into the basolateral amygdala (BLA) at two positions (*AP: -1.8 mm, ML: -3.4 mm, DV: -3.4mm and -3.8 mm*). A separate group of 3 mice were injected with 200 nl of fluorogold in the anterior cingulate cortex (ACC) at 2 positions (*AP: 0.8 mm, ML: -0.35 mm, DV: -1.8 mm and -2.2 mm*). Mice were then kept single-housed for 2 weeks before brain fixation by intra-cardiac perfusion with 4% PFA in 0.1 M PB)¹¹, and immunohistochemical staining for relaxin-immunoreactivity, as described below.

2.8 Immunohistochemistry

Perfusion-fixed mouse brains were cut on a cryostat. Coronal sections (25 μ m) were incubated in 1 \times PBS, and 1% bovine serum albumin (BSA) (*Sigma-Aldrich*) for 30 minutes at room temperature. Rabbit anti-rat relaxin primary antibody (1:200) (*Biovision, Milpitas, CA, USA*) was diluted in PBS + BSA (1%) + Triton (0.3%) (*Sigma-Aldrich*) and sections were incubated in this solution for 24 hours at 4°C. Sections were then washed in 1 \times PBS and incubated for 2 hours at room temperature with Alexa Fluor 568, anti-rabbit secondary antibody (1:500 in 1x PBS + BSA 1%) (*Thermo Fisher Scientific*). The specificity of the anti-relaxin antibody has been validated by western blot (see supplier website at <https://www.biovision.com>). Control studies involving omission of the primary antibody resulted in a loss of specific staining. The distribution of relaxin-immunoreactivity observed in mouse brain matched that reported for rat relaxin peptide and mRNA.^{26,42-44}

Sections processed for immunohistochemistry or multiplex *in situ* hybridization were observed in a Nanozoomer 2.0HT (*Hamamatsu, Hamamatsu City, Japan*) and under a SPE confocal microscope (*Leica*).

2.9 Statistical analysis

All statistical analyses were done using Graph Pad Prism. Behavioral analysis was conducted using a 2-way ANOVA, followed by a Tukey test. *In situ* hybridization (RNAscope) data analysis was conducted using a 2-way ANOVA, followed by a Sidak test. Data are presented as mean \pm SEM, and differences were considered significant when $p < 0.05$.

3. Results

3.1 Analgesic effects of RXFP1 activation

We analysed nociceptive behavioral responses to mechanical and heat stimuli using the von Frey test and Hargreaves test in mice (Fig. 1). We studied the effects of RXFP1 activation in

control mice (NaCl injection in the hind paw) and in mice with persistent inflammatory pain produced by CFA injection in the hind paw.

Injection of CFA produced a subsequent significant decrease in PWT in mice (Fig. 1A-a; $F_{(1,12)} = 270.63$; $p < 0.0001$). Mechanical sensitization was obtained in the CFA-injected paw on day 4 post-injection (PWT = $1.467 \text{ g} \pm 0.067 \text{ g}$ at D-1 vs $0.42 \text{ g} \pm 0.02 \text{ g}$ at D4, $p < 0.0001$). No changes in PWT were detected in NaCl-injected mice (Fig. 1A-a; $p > 0.05$), or in the uninjected paw of CFA mice (Fig. S1A-a; $F_{(1,12)} = 1.503$; $p > 0.05$). Icv injection of B7.33 induced a significant PWT increase 30 min after the injection (Fig. 1A-a; PWT = $1.467 \text{ g} \pm 0.067 \text{ g}$ at 30 min, $p < 0.0001$ vs D4, $p > 0.05$ vs D-1) that was absent 1 hour after injection. Similar results were obtained after the injection of H2-relaxin (H2) (Fig. 1B-a; $F_{(1,10)} = 164.3$; $p < 0.0001$), which also increased PWT at 30 min after icv injection (Fig. 1B-a; PWT = $1.200 \text{ g} \pm 0.089 \text{ g}$ at 30 min, $p < 0.0001$ vs D4, $p > 0.05$ vs D-1). The PWT increase was prevented by co-injection of the RXFP1 antagonist (H2B) with the respective RXFP1 agonists (B7.33 or H2; Fig. 1A-b; PWT = 0.4 g at 30 min, Fig. 1B-b; PWT = $0.44 \text{ g} \pm 0.04 \text{ g}$ at 30 min, $p > 0.05$ vs D4, $p < 0.0001$ vs D-1). No effect of the agonists or the antagonist, were detected in sham, NaCl-injected mice (Fig. 1A-a, b and 1B-a, b), or after von Frey stimulation of the uninjected paw in the CFA mice (Fig. S1A-a/b and S1B-a, b). Icv injection of aCSF did not significantly alter PWT under control or inflammatory pain conditions (Fig. 1A, B-c and S1A, B-c).

PWL was decreased after stimulation of the CFA-injected paw (Fig. 1A-d; $F_{(1,8)} = 136.9$; $p < 0.0001$; PWL = $3.34 \text{ s} \pm 0.152 \text{ s}$ at D-1 vs $1.37 \text{ s} \pm 0.089 \text{ s}$ at D4, $p < 0.0001$), but not in NaCl-injected mice (Fig. 1A-d; $F_{(1,8)} = 136.9$; $p > 0.05$), or after stimulation of the uninjected paw (Fig. S1A-d; $F_{(1,8)} = 1.062$; $p > 0.05$). Icv injection of B7.33 (Fig. 1A-d) and H2-relaxin (Fig. 1B-d) increased PWL 30 min after the injection (Fig. 1A-d; PWL = $2.614 \text{ s} \pm 0.074 \text{ s}$ at 30 min, $p < 0.001$ vs D4, $p > 0.05$ vs D-1) (Fig. 1B-d; PWL = $3.838 \text{ s} \pm 0.398 \text{ s}$ at 30 min, $p < 0.01$ vs D4, $p > 0.05$ vs D-1). This effect was absent 1 hour after injection of B7.33 and 2 hours after H2-relaxin injection (Fig. 1B-d; PWL = $2.214 \text{ s} \pm 0.120 \text{ s}$ at 60 min, $p < 0.0001$ vs D4, $p > 0.05$ vs D-1). Notably, the B7.33-induced effect was not prevented by co-injection of the RXFP1 antagonist, H2B (Fig. 1A-e; PWL = $2.428 \text{ s} \pm 0.253 \text{ s}$ at 30 min, $p < 0.0001$ vs D4, $p > 0.05$ vs D-1). Similarly, the H2-induced PWL increase was not affected by the co-injection of the RXFP1 antagonist (Fig. 1B-e; PWL = $3.342 \text{ s} \pm 0.189 \text{ s}$ at 30 min, $p < 0.0001$ vs D4, $p > 0.05$ vs D-1). Icv injection of

RXFP1 agonists or antagonist did not modify PWL in sham, NaCl-injected mice (Fig. 1A-d/e and 1B-d/e), or after thermal stimulation of the uninjected paw in CFA mice (Fig. S1A-d/e and S1B-d/e). The CFA-induced PWL decrease was not altered by icv aCSF injection under control or inflammatory pain conditions (Fig. 1A/B-f and S1A/B-f).

These data indicate that RXFP1 activation produced both mechanical and thermal analgesia under inflammatory conditions.

3.2 Distribution of RXFP1 mRNA in mouse brain

The mouse anterior cingulate cortex (ACC), claustrum (CLA) and subiculum (SUB), regions strongly implicated in processing pain and emotions^{25,47,48,57,65}, contain a high density of *Rxfp1* mRNA (see ref⁵³; Allen Brain Atlas (<http://mouse.brain-map.org/experiment/show/70562124>)). Thus, multiplex fluorescence ISH identified intense labeling in these areas, particularly in the ACC and CLA (Fig. 2A), while the labeling intensity was much weaker in the surrounding areas. Multiplex fluorescence ISH also identified key aspects of the neurochemical phenotype of *Rxfp1* mRNA-expressing neurons. Presumed excitatory and inhibitory neurons were identified by their expression of CaMKII or GAD65 mRNA, respectively. CaMKII mRNA-positive and GAD-65 mRNA-positive neurons were identified as two clearly separate populations, with only a very small number of neurons exhibiting colocalization of CaMKII and GAD65 mRNA. In the brain areas investigated, virtually all RXFP1 mRNA-positive neurons contained CaMKII mRNA (Fig. 2C), indicating the receptor is almost exclusively expressed in excitatory neurons. No significant differences in the relative colocalization or the levels of RXFP1 mRNA in these areas were observed between sham and CFA mice (Fig. 2B, C).

3.3 Distribution of relaxin immunoreactivity in mouse brain

In studies aimed at identifying the possible sources of endogenous relaxin that would activate RXFP1 in these receptor-rich areas, we first performed immunohistochemical staining for relaxin peptide immunoreactivity throughout the normal mouse brain (Fig. 3). We identified relaxin-containing areas widely distributed throughout the forebrain (Fig. 3Aa-e, Ba-h), and to a lesser extent in the hindbrain (Fig. 3Af, Bi-l). Notably, the midbrain did not contain prominent populations of relaxin-immunoreactive neurons. The highest density of relaxin-labeled cells was

found in the cortex (e.g. cingulate, claustrum, piriform, somatosensory), and the hypothalamus (medial preoptic area, lateral hypothalamus). In the hindbrain, cerebellar Purkinje cells exhibited clear and homogenous labeling. All positive cells were characterized by diffuse cytosolic labelling, restricted to the cell body or proximal processes, with little immunoreactivity in distal elements, consistent with earlier reports on the rat brain from our laboratory.⁴³

We then employed fluorogold retrograde tracing to identify the relaxin neuron populations that send projections to the RXFP1-rich, ACC and CLA. We focused on these two pain processing brain areas because they exhibited the highest density of RXFP1 transcripts but other areas will also require future investigations. Fluorogold was detected in neurons in various areas of the telencephalon and diencephalon that contained relaxin-immunoreactivity. A fluorogold injection into the ACC (Fig. 4a, c, d, g, h, k, l, o, p)x-y) resulted in fluorogold accumulation in relaxin-positive neurons in the CLA, basolateral amygdala (BLA), primary somatosensory cortex (SST), and the ACC itself. Fluorogold injection into the CLA (Fig. 4b, e, f, i, j, m, n, q, r) resulted in colocalization of fluorogold and relaxin-immunoreactivity in BLA, ACC and the CLA itself, in addition to the posterior complex of the thalamus. These data indicate that ACC, CLA and BLA contain relaxin neurons that project to both ACC and CLA.

3.4 Neurochemical phenotype of relaxin mRNA-containing neurons in mouse brain

In studies aimed at identifying the neurochemical phenotype of relaxin-expressing neurons in the ACC, CLA and BLA that project to these RXFP1-expressing brain nuclei involved in pain processing, we first attempted double immunohistochemical-labeling experiments to colocalize relaxin and phenotypical markers of excitatory and inhibitory neurons. However, unfortunately these experiments were unsuccessful since relaxin labelling could not be visualized when antisera were used in combination, or sequentially.

Therefore, we next employed multiplex fluorescent ISH, as described above, to detect the possible co-expression of relaxin mRNA with GAD65 and CaMKII mRNA. Although some differences were observed in the areas examined, the majority of relaxin mRNA-positive neurons were identified as GAD65 mRNA-containing neurons (Fig. 5A). Thus, the proportion of relaxin mRNA-positive cells was $76.6 \pm 4.96\%$ in the CLA, $70.8 \pm 11.37\%$ in the ACC, and $78.4 \pm 4.82\%$

in the BLA (Fig. 5B). In contrast, a minority of relaxin mRNA-positive cells were identified as excitatory neurons (i.e., the proportion of relaxin mRNA-positive cells was $9.2 \pm 1.07\%$ in the CLA, $29 \pm 6.82\%$ in the ACC, and $8.2 \pm 1.77\%$ in the BLA). No significant differences in relaxin mRNA expression were observed between sham and CFA mice in the level of colocalization (Fig. 5B) or the levels of relaxin mRNA in various brain areas (Fig. 5C).

4. Discussion

The present study represents the first to demonstrate the function of the relaxin/RXFP1 system in pain processing in the brain. Relaxin was originally identified as a hormone of reproduction and pregnancy,³⁰ but was subsequently found not to be mandatory for pregnancy in humans.²⁷ Relaxin is now well recognized for its vasodilatory action on blood vessels,^{13,27} its positive chronotropic and ionotropic effects on the heart^{37,71} and its powerful antifibrotic actions,⁵⁶ among others.⁵ Despite these pleiotropic effects described with various organs⁴, and some early anatomical studies⁴²⁻⁴⁴, very few studies have been performed in recent years to explore the role of the relaxin/RXFP1 system *within* the central nervous system. Circulating relaxin is known to activate RXFP1 present in the subfornical organ and the organum vasculosum of the lamina terminalis, outside the blood-brain barrier, to cause a reduction in plasma osmolality.⁶⁰ Circulating and centrally administered relaxin also increases water consumption in rats, via actions at these sites.⁵⁹ In addition to the circumventricular organs and hypothalamic nuclei accessible to circulating peptides like relaxin, relaxin was also shown to alter the activity of the BLA following local administration in the rat, which impaired fear memory consolidation.⁴³

Thus, in light of the presence of RXFP1 mRNA and relaxin binding sites in areas involved in pain processing,^{9,44,53} in the current study we tested the effect of intracerebroventricular administration of relaxin peptide analogs on nociceptive behaviors in mice. While this strategy lacked anatomical specificity, it allowed testing for a global effect of RXFP1 activation in the brain. Our results demonstrated an analgesic effect of centrally injected RXFP1 agonist peptides on two different sensory modalities, mechanical and thermal sensitivity. The results obtained after icv injection of H2-relaxin and B7-33 were very similar, indicating the analgesic effect is mediated by RXFP1. Indeed, in cell-based systems, H2-relaxin can act as a biased ligand at the relaxin-3

receptor, RXFP3,^{4,68} and therefore its effects *in vivo* cannot be unequivocally attributed to RXFP1 activation.

In contrast, however, B7-33 is a single-chain analog of the H2-relaxin B-chain, which retains binding to RXFP1.^{34,54} B7-33 has lower binding affinity than H2-relaxin at RXFP1, but has similar potency to H2-relaxin in activation of the pERK pathway in RXFP1-expressing HEK cells and rodent myofibroblasts.³⁴ Functionally, the small B7-33 peptide agonist efficiently prevented or reversed organ fibrosis and dysfunction in rodent models of heart or lung disease.^{34,45,54} B7-33 also conferred cardioprotection and attenuated cardiomyocyte death after cardiac infarction.^{14,24} In the current study, the concentration of H2-relaxin used was based on that reported as effective in rat previous reports.⁴³ The concentration of B7-33 was twice that of H2-relaxin to take into account the lower receptor affinity of the single-chain agonist.³⁴

Relaxin-induced analgesic effects are transitory after a rapid onset, but are not likely to be due to RXFP1 desensitization; since prolonged exposure of RXFP1 to H2-relaxin does not trigger β -arrestin-coupling and receptor internalization, and results in sustained signaling (up to 6 hours) *in vitro*.^{4,10,29} The short duration of the analgesic effects observed (between 30 and 60 min) more likely indicates that the ligand is degraded quite rapidly, or possibly that the intracellular signaling cascade is rapidly terminated *in vivo*.

In previous studies, the peptide, B-R13/17K-H2 (H2B), was fully characterized as an RXFP1 antagonist in cells which express RXFP1, rat renal myofibroblasts and MCF-7 cancer cells.³⁵ Notably, in the current study, the thermal analgesia produced by H2-relaxin and B7-33 was not reversed by the antagonist. One interpretation is that any action that is not reversed by a higher molar amount of RXFP1 antagonist, is caused by the relaxin agonist analogs interacting with other transducing systems, independent of RXFP1.²⁸ The glucocorticoid receptor is a potential target of H2-relaxin,^{16,17} but its involvement in activation of gene expression is not consistent with the rapid effect of H2-relaxin and B7-33 on thermal sensitivity. Ligand-directed signaling bias at the relaxin-3 receptor, RXFP3, is a further possibility, since H2-relaxin can activate RXFP3 and has potency and efficacy at the MAP kinase and AP-1 transduction pathways in cell lines.⁶⁸ However, such biased signaling has not been observed with B7-33, which is a more selective RXFP1 agonist.^{5,54}

Alternatively, it is possible that B-R13/17K-H2 preferentially inhibits RXFP1 transduction in a specific population of those neurons activated by the RXFP1 agonists, or at selective signaling pathways, as observed with RXFP3. This hypothesis implies that different cell populations or different signaling pathways, modulate mechanical and thermal pain in the brain. In this regard, different circuits convey noxious mechanical^{18,46,52} and thermal^{6,22} information. However, while the existence of specific thermal and mechanical transduction systems and circuits are well described in the periphery and spinal cord,^{19,51} such a distinction remains elusive in the brain. Therefore, further studies are warranted to investigate the complex mechanisms associated with brain RXFP1 signaling and modulation of mechanical and thermal pain.

Since the injection of relaxin analogs into the lateral cerebral ventricle does not provide information on their precise sites of action, in initial efforts to identify these loci, we studied the distribution of RXFP1 mRNA expression in a limited number of brain areas known to play a role in pain transmission. The pain responses tested in our study rely on spinal reflexes, and therefore, we focused on brain areas potentially involved in descending pain pathways whose modulatory effects on spinal circuits could be altered by RXFP1 activation.

RXFP1 mRNA expression has been demonstrated in the brain of several species.⁴² It has been well characterized in rat brain^{43,44} and some data is available in the mouse (see ref 46; Allen Brain Atlas). A comparison of previous studies suggests RXFP1 mRNA expression is more restricted in mouse brain than in rat brain. In particular, the BLA displays a much higher density of RXFP1 mRNA in the rat than in mouse. Thus, we chose to investigate three forebrain regions involved in pain processing, namely the ACC, CLA and SUB.^{2,65} The relative abundance of RXFP1 mRNA in the ACC and CLA and the ability of RXFP1 activation to produce analgesia, is in agreement with the prominent role of these areas in modulating descending pain pathways. Indeed, the ACC exerts a facilitating action on pain transmission.^{12,57,63} The broad and unique cortical connections of the CLA suggest it serves as a central network hub, coordinating activity within cortical circuitry.⁷⁵ The CLA displays a strong connectivity with sensory modalities and a significant preference for peripheral sensory information.²⁵ In all the brain areas examined, RXFP1 mRNA was almost exclusively expressed in CaMKII mRNA-positive, presumed excitatory neurons, thus identifying a novel signaling system to control modulatory pain outputs from these regions.

The detailed cellular and regional distribution of relaxin, the preferred, cognate ligand for RXFP1, remains largely unexplored in the brain. Therefore, we conducted a broad mapping of relaxin-immunoreactivity throughout the mouse brain using immunohistochemistry. Although not exhaustive, our data are in agreement with early published reports,^{42,43} and identify relaxin-immunoreactive neurons in several brain areas of importance for pain processing. We identified many cortical and sub-cortical regions, hypothalamic nuclei, and midbrain structures that contain a moderate to high number of relaxin-immunoreactive cells. The presence of relaxin-immunoreactivity in the cell body, with only rare positive neural processes, indicate that relaxin may serve as a paracrine/autocrine neuromodulator. However binding sites also exist in rat brain areas devoid of relaxin immunoreactivity.⁴² Moreover, our study has determined that several neuronal populations immunoreactive for relaxin, project to distant RXFP1-containing brain areas. Thus, relaxin might be transported along the axon and released in the target sites, as proposed earlier.⁴³ Interestingly, the ACC and CLA have strong ipsilateral reciprocal connections⁷² that potentially contain relaxin. The BLA also sends relaxin-immunoreactive neural outputs to ACC and CLA. In these different regions connected to ACC and CLA, relaxin neurons are mostly inhibitory, based on their expression of GAD65 mRNA, consistent with a recent analysis of the transcriptome of neurons in the mouse visual cortex.⁶⁴

The effects of RXFP1 activation on paw withdrawal thresholds were observed when the mice tested were experiencing persistent pain, but not under control conditions. Our study demonstrated that this restriction of RXFP1 action to CFA-induced persistent pain-like states does not depend on changes in the relative expression of relaxin or *Rxfp1* transcripts. Instead, it could be due to alterations in RXFP1 signaling under persistent pain conditions, including possible RXFP1 oligomerization.^{61,62} Another possibility is that specific neuronal systems must be physiologically challenged to become sensitive to RXFP1 activation, as observed for other neuropeptides that play a particular role in pathophysiological conditions.³¹

In the current study, we centrally administered exogenous relaxin analogs to demonstrate the analgesic action of RXFP1 activation in adult mice. These data do not preclude a possible role for endogenous relaxin signaling in nociception that warrants further assessment in future studies. Additional studies will be also required to identify the brain areas involved in the analgesic effects,

and the underlying signaling mechanisms associated with relaxin/RXFP1 system control of mechanical and thermal pain.

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Author contributions

ALG and **ML** conception of the study. **CA**, **LB**, **AD** and **ML** design and performance of experiments. **MAH** production and provision of relaxin analogs. **CA** and **ML** writing of original manuscript. **MAH**, **WH** and **ALG** writing, critical review and editing of final manuscript. **ALG**, **WH** and **ML** funding acquisition and supervision. All authors read and approved the final manuscript.

Abbreviations

ACC: anterior cingulate cortex

aCSF: artificial cerebro spinal fluid

AMP: amplifier

AMY: amygdala

ARC: arcuate nucleus

AON: anterior olfactory nucleus

BLA: basolateral amygdala

CaMKII: calcium/calmodulin-dependent protein kinase II

CFA: complete Freund's adjuvant
CLA: claustrum
ENTl: entorhinal area, lateral part
FN: facial nucleus
GAD: glutamic acid decarboxylase
H2: human relaxin-2
HIP: hippocampal region
Icv: intracerebroventricular
kDa: kilodalton
LDL_A: low density lipoprotein A
LH: lateral habenula
LRR: leucine rich repeat
LS: lateral septal nucleus
MD: mediodorsal nucleus of the thalamus
MEA: medial amygdalar nucleus
MH: medial habenula
MPO: medial preoptic area
mRNA: messenger ribonucleic acid
NaCl: sodium chloride
NDB: nucleus of the diagonal band of Broca
PAG: periaqueductal grey
PBS: phosphate-buffered saline
Pc: Purkinje cells
PERI: perirhinal area
PFA: paraformaldehyde
PIR: piriform cortex
PRN: pontine reticular nucleus
PWL: paw withdrawal latency
PWT: paw withdrawal threshold
RN: red nucleus
RT: reticular nucleus of the thalamus

SEM: standard error of the mean

SSp: primary somatosensory cortex

SST: primary somatosensory cortex

SUB: subiculum

TT: taenia tecta

Figure Legends

Figure 1. Assessment of mechanical sensitivity (paw withdrawal threshold, von Frey test, VF) and thermal sensitivity (paw withdrawal latency, Hargreaves test) of the CFA-injected hind paw after icv injection of the RXFP1 agonists, (A) B7-33 or (B) H2-relaxin (left panels), co-injection of B7-33 or H2-relaxin with the RXFP1 antagonist, B-R13/17K-H2 (B-R/K) (central panels), or control aCSF injection (right panels).

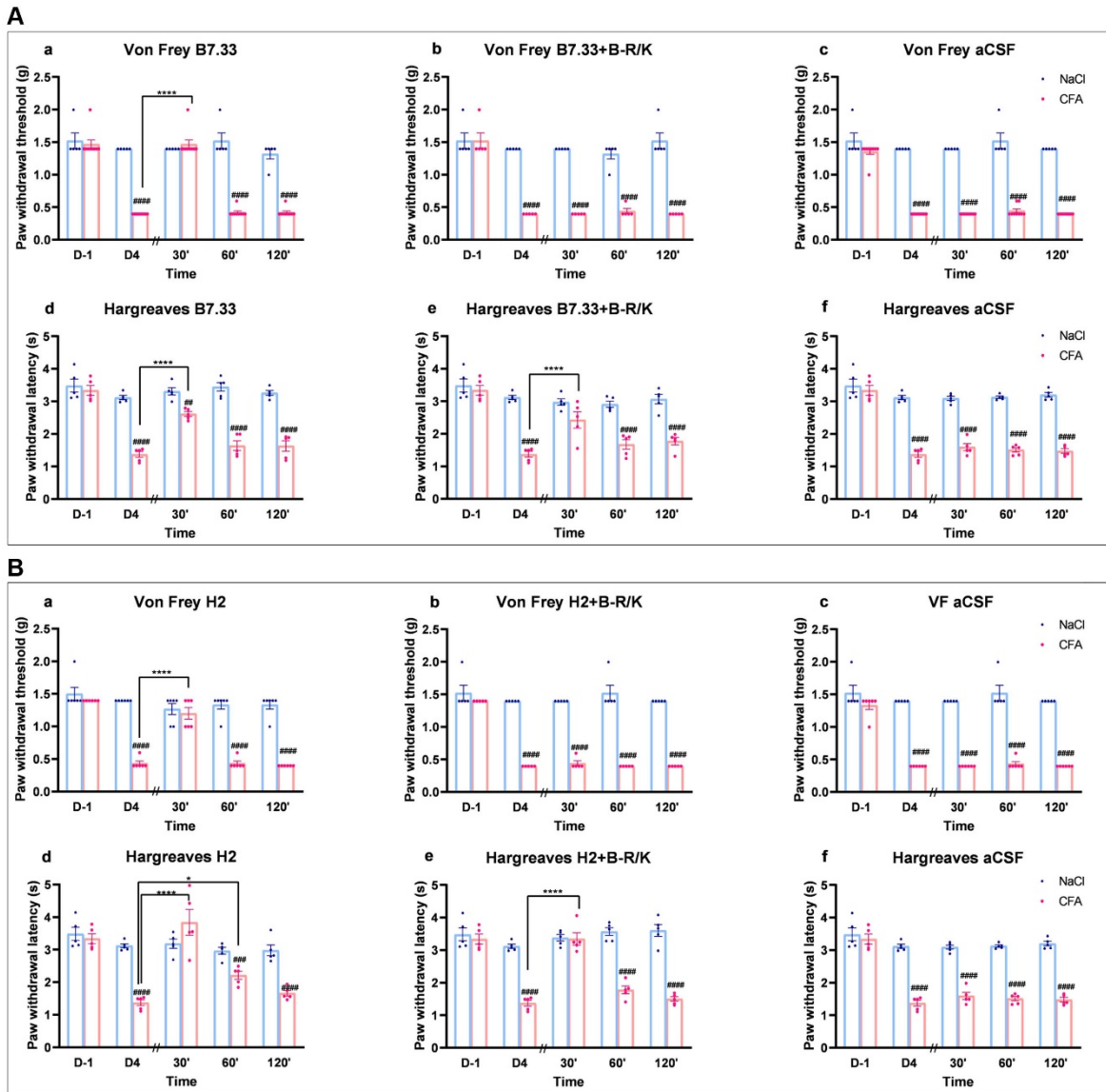
Figure 2. Multiplex fluorescent ISH detection of RXFP1, GAD65 and CaMKII mRNA in forebrain of sham mice. **A.** Detection of RXFP1 mRNA (green; a, e, i), GAD65 mRNA (red; b, f, j), CaMKII mRNA (white; e, g, k) and DAPI staining (blue; d, h, l) in the ACC (a-d), CLA (e-h) and SUB (i-l). RXFP1 mRNA was frequently colocalized with CaMKII mRNA (arrows), while colocalization with GAD65 mRNA was scarce (arrowheads). Rare cells display colocalization of all three transcripts (double arrowheads). Bar (a-l) = 20 μm . **B.** Quantification of neurons displaying colocalization of RXFP1 mRNA with GAD65 mRNA or CaMKII mRNA (5 sections in each area, n = 4 mice under sham (blue) and CFA (red) conditions). **** p < 0.0001 vs sham RXFP1/GAD65; ##### p < 0.0001 vs CFA RXFP1/GAD65. **C.** Quantification of areas labeled using multiplex fluorescent ISH for RXFP1 mRNA in CLA, ACC and SUB of sham (blue) and CFA (red) mice.

Figure 3. Relaxin immunoreactivity in the forebrain of sham mice. **A.** Low magnification images were acquired using a Nanozoomer. Bars = 1 mm. **B.** Confocal micrographs illustrating relaxin immunoreactivity (arrowheads) in the anterior olfactory nucleus (AON), piriform cortex (PIR), anterior cingulate cortex (ACC), claustrum (CLA), lateral hypothalamus (LH), arcuate nucleus (ARC), medial habenula (MH), medial amygdalar nucleus (MEA), periaqueductal grey (PAG), Purkinje cells (Pc), pontine reticular nucleus (PRN), and facial nucleus (FN). Bars = 20 μm .

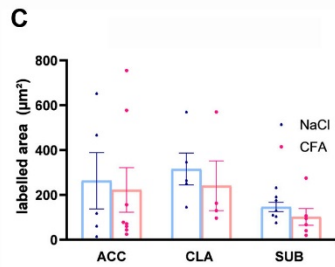
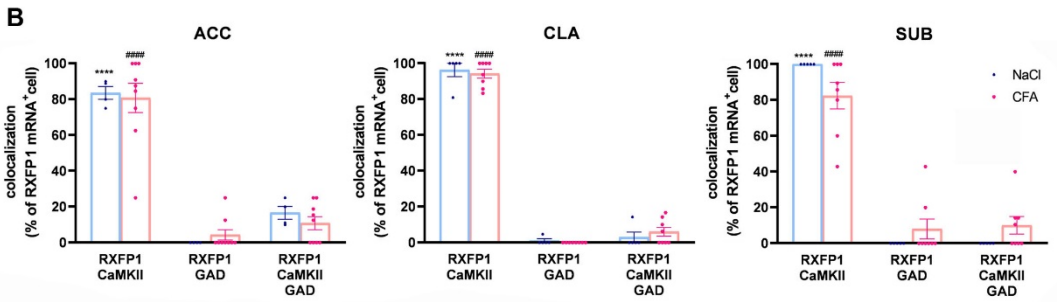
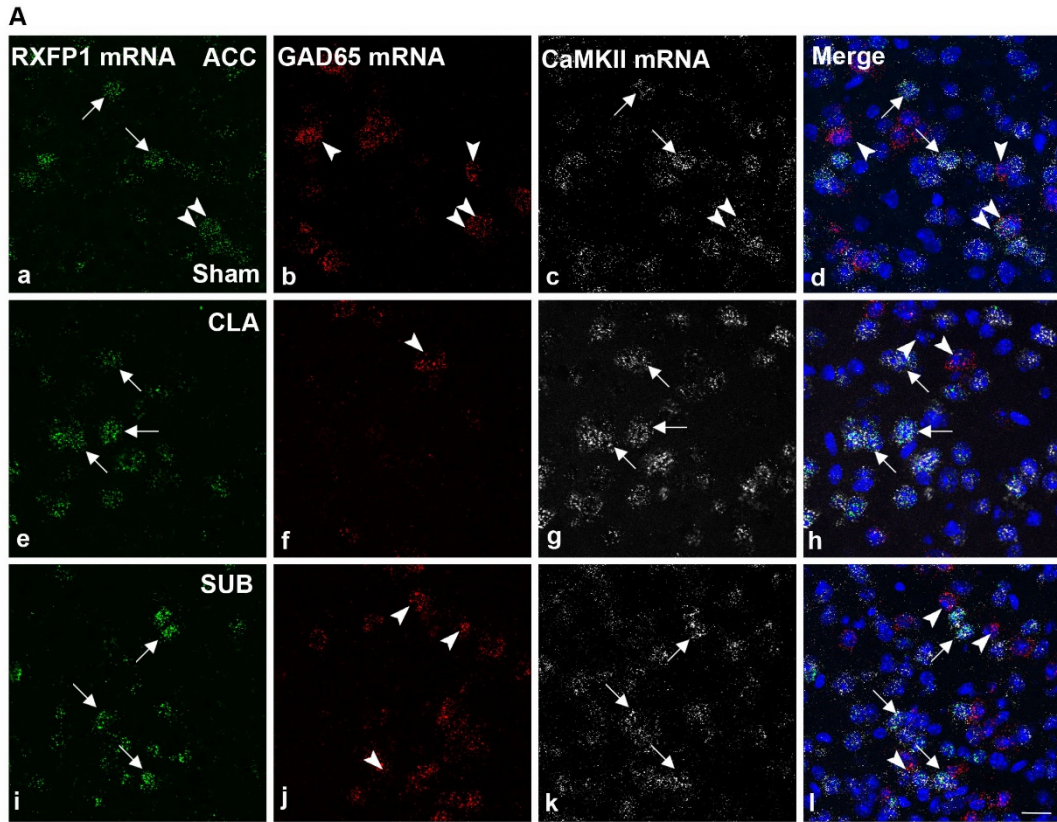
Figure 4. Fluorogold retrograde tracing (blue) of relaxin-immunoreactive neurons (red) in various forebrain areas that project to the ACC (left panel) or CLA (right panel) of sham mice. Examples of injection sites, visualized one day after fluorogold injection, are shown in a and b. Results of retrograde labelling were observed after 2 weeks (c-q). Arrows indicate neurons retrogradely labelled with fluorogold and immunopositive for relaxin. Arrowheads indicate neurons that are single-labelled for one marker only. Bars = 20 μ m.

Figure 5. Multiplex fluorescent ISH detection of relaxin (Rln) mRNA, GAD65 mRNA and CaMKII mRNA in the forebrain of sham mice. **A.** Multiple detection of Rln mRNA (green; a, e, i), GAD65 mRNA (red; b, f, j), CaMKII (white; e, g, k) and DAPI staining (blue; d, h, l) in the ACC (a-d), CLA (e-h) and BLA (i-l). Rln mRNA was often colocalized with GAD65 mRNA (arrowheads), while colocalization with CaMKII mRNA was scarce (arrows). Bar (a-l) = 20 μ m. **B.** Quantification of neurons displaying colocalization of Rln mRNA with GAD65 mRNA or CaMKII mRNA (5 sections in each area, n = 4 mice under sham (blue) and CFA (red) conditions). ****p < 0.0001 vs sham Rln/CaMKII; #####p < 0.0001 vs CFA Rln/CaMKII). **C.** Quantification of areas labeled using multiplex fluorescent ISH for Rln mRNA in various forebrain regions of sham (blue) and CFA (red) mice.

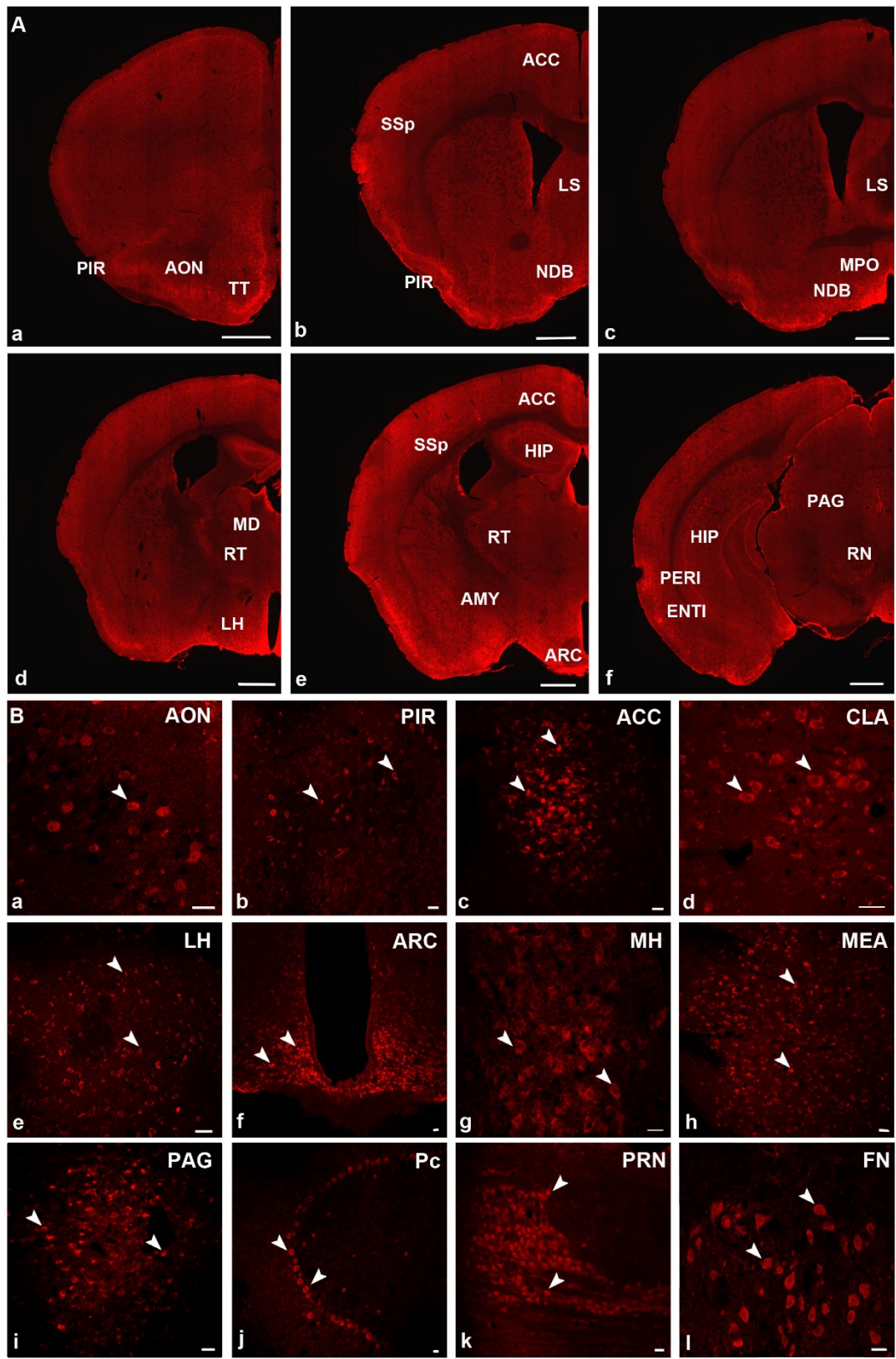
Figure S1. Assessment of mechanical sensitivity (paw withdrawal threshold, von Frey test, VF) and thermal sensitivity (paw withdrawal latency, Hargreaves test) of the uninjected hind paw after icv injection of the RXFP1 agonists (**A**) B7-33 or (**B**) H2-relaxin (left panels), co-injection of B7-33 and H2-relaxin with the RXFP1 antagonist, B-R13/17K-H2 (B-R/K) (centre panels), or control aCSF injection (right panels).



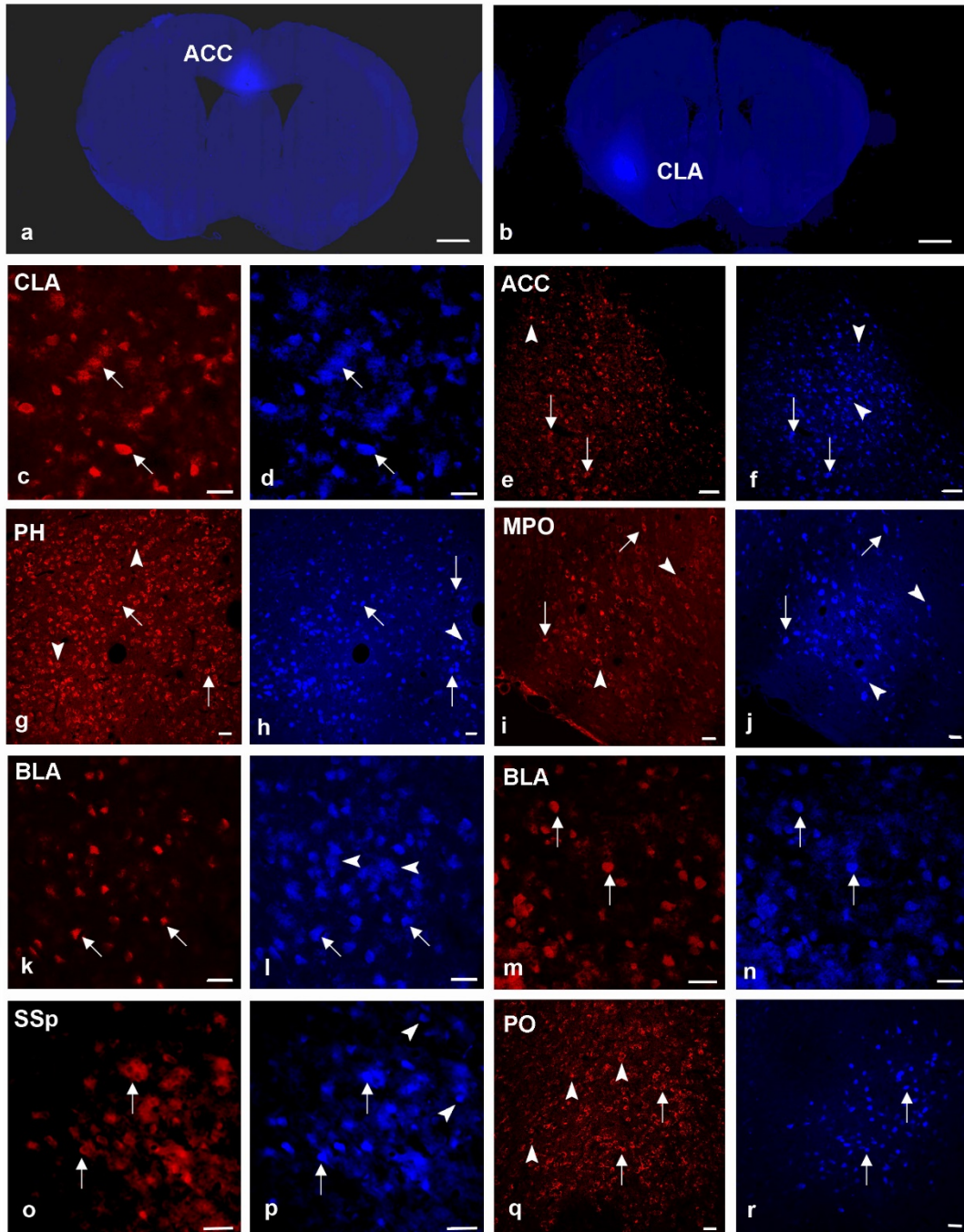
(Fig. 1, Abboud et al.)



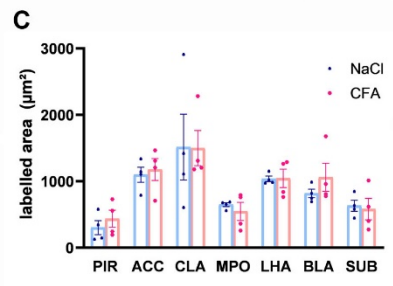
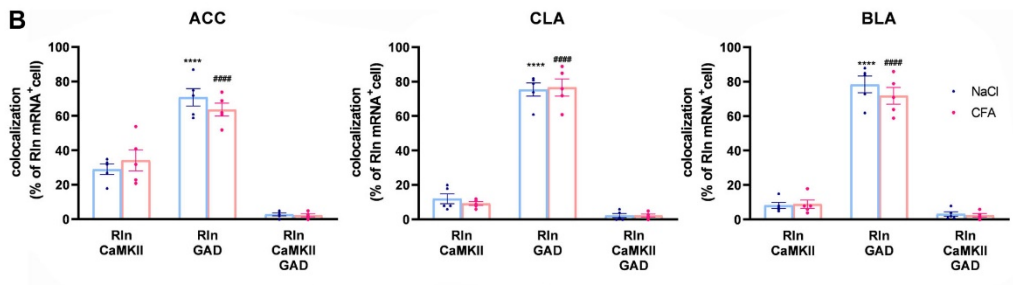
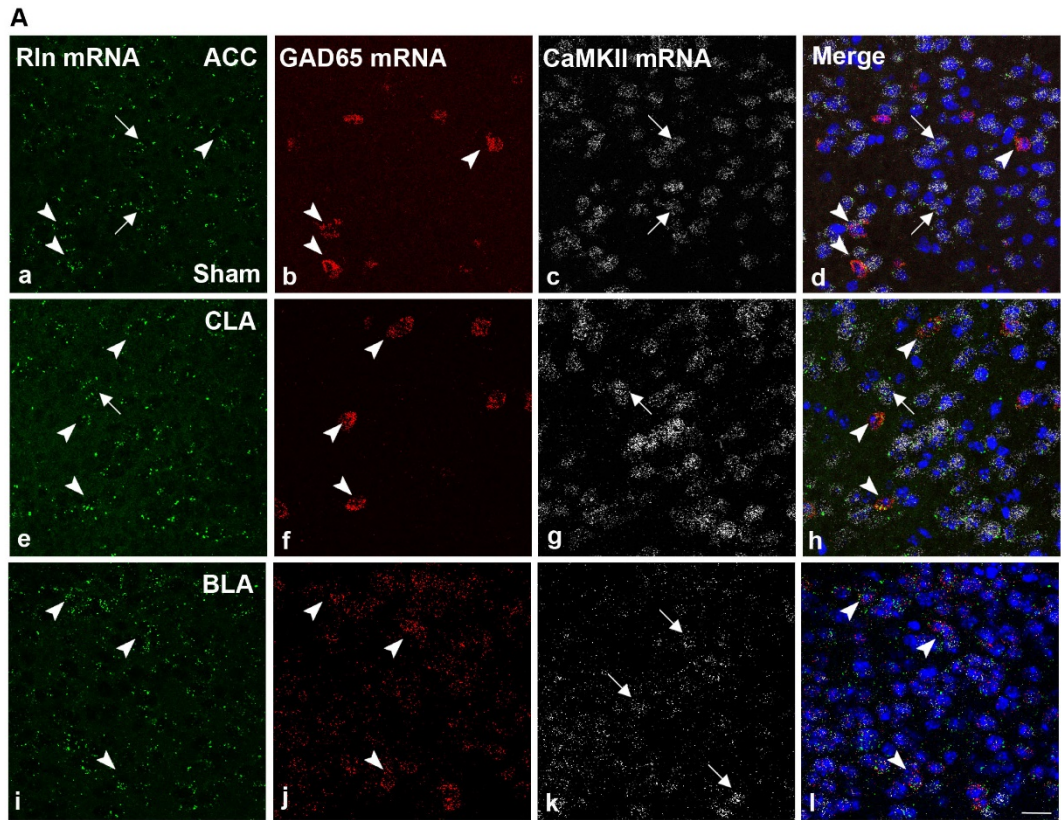
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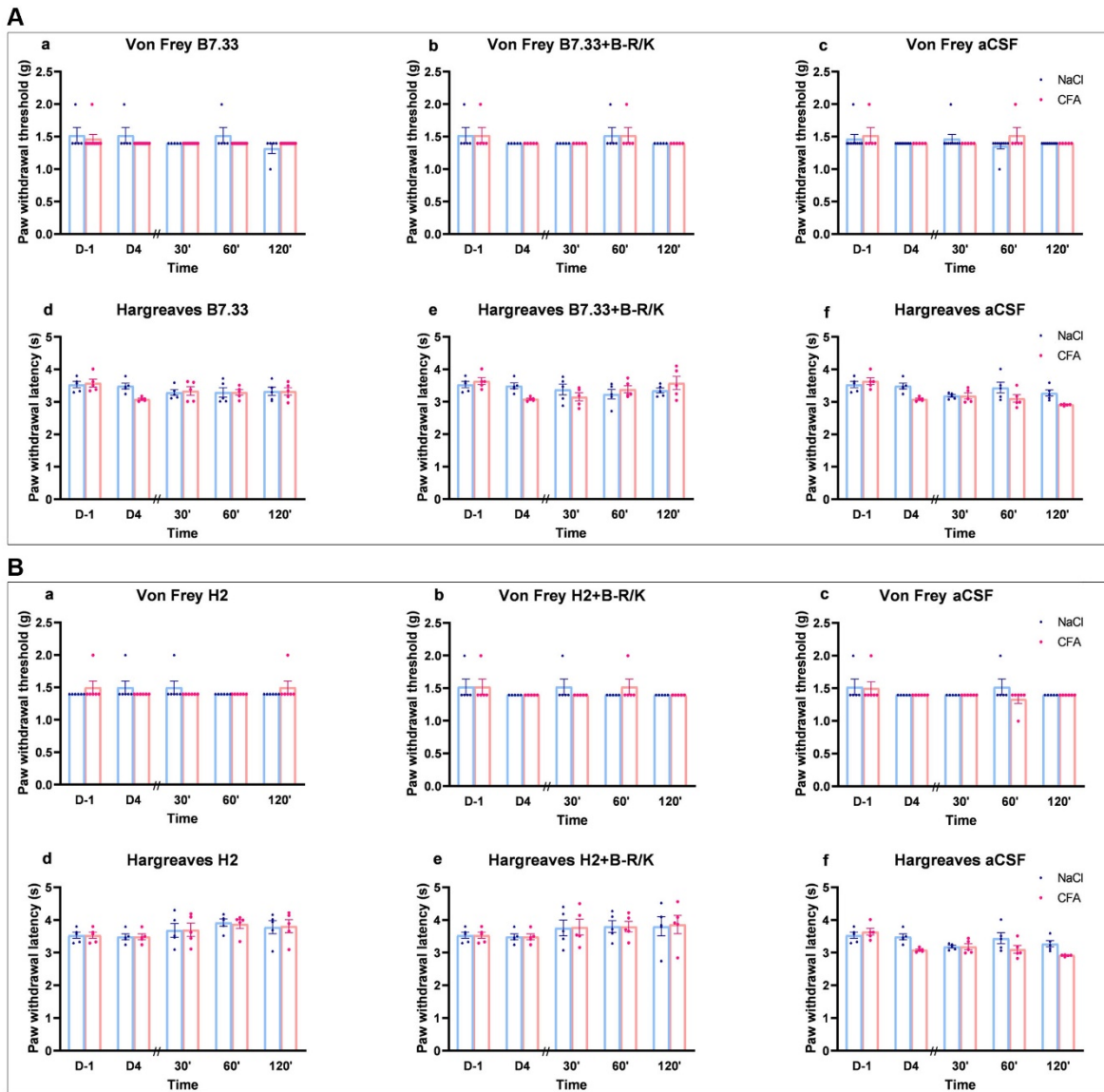
(Fig. 3, Abboud et al.)



(Fig. 4, Abboud et al.)



(Fig. 5, Abboud et al.)



(Fig. S1, Abboud et al.,)

References

- [1] Abboud C, Duveau A, Bouali-Benazzouz R, Masse K, Mattar J, Brochoire L, Fossat P, Boue-Grabot E, Hleihel W, Landry M. Animal models of pain: diversity and benefits. *Journal of neuroscience methods* 2020;108997.
- [2] Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. *European journal of pain* 2005;9(4):463-484.
- [3] Attal N, Lanteri-Minet M, Laurent B, Fermanian J, Bouhassira D. The specific disease burden of neuropathic pain: results of a French nationwide survey. *Pain* 2011;152(12):2836-2843.
- [4] Bathgate RA, Halls ML, van der Westhuizen ET, Callander GE, Kocan M, Summers RJ. Relaxin family peptides and their receptors. *Physiol Rev* 2013;93(1):405-480.
- [5] Bathgate RAD, Kocan M, Scott DJ, Hossain MA, Good SV, Yegorov S, Bogerd J, Gooley PR. The relaxin receptor as a therapeutic target - perspectives from evolution and drug targeting. *Pharmacol Ther* 2018;187:114-132.
- [6] Bokiniec P, Zampieri N, Lewin GR, Poulet JF. The neural circuits of thermal perception. *Curr Opin Neurobiol* 2018;52:98-106.
- [7] Bouhassira D, Lanteri-Minet M, Attal N, Laurent B, Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. *Pain* 2008;136(3):380-387.
- [8] Breivik H, Eisenberg E, O'Brien T. The individual and societal burden of chronic pain in Europe: the case for strategic prioritisation and action to improve knowledge and availability of appropriate care. *BMC Public Health* 2013;13:1229.
- [9] Burazin TC, Johnson KJ, Ma S, Bathgate RA, Tregear GW, Gundlach AL. Localization of LGR7 (relaxin receptor) mRNA and protein in rat forebrain: correlation with relaxin binding site distribution. *Ann N Y Acad Sci* 2005;1041:205-210.
- [10] Callander GE, Thomas WG, Bathgate RA. Prolonged RXFP1 and RXFP2 signaling can be explained by poor internalization and a lack of beta-arrestin recruitment. *Am J Physiol Cell Physiol* 2009;296(5):C1058-1066.
- [11] Catapano LA, Magavi SS, Macklis JD. Neuroanatomical tracing of neuronal projections with Fluoro-Gold. *Methods in molecular biology* 2008;438:353-359.
- [12] Chen T, Taniguchi W, Chen QY, Tozaki-Saitoh H, Song Q, Liu RH, Koga K, Matsuda T, Kaito-Sugimura Y, Wang J, Li ZH, Lu YC, Inoue K, Tsuda M, Li YQ, Nakatsuka T, Zhuo M. Top-down descending facilitation of spinal sensory excitatory transmission from the anterior cingulate cortex. *Nat Commun* 2018;9(1):1886.
- [13] Conrad KP, Shroff SG. Effects of relaxin on arterial dilation, remodeling, and mechanical properties. *Curr Hypertens Rep* 2011;13(6):409-420.
- [14] Devarakonda T, Mauro AG, Guzman G, Hovsepian S, Cain C, Das A, Praveen P, Hossain MA, Salloum FN. B7-33, a Functionally Selective Relaxin Receptor 1 Agonist, Attenuates Myocardial Infarction-Related Adverse Cardiac Remodeling in Mice. *J Am Heart Assoc* 2020;9(8):e015748.
- [15] Diaz-delCastillo M, Woldbye DPD, Heegaard AM. Neuropeptide Y and its Involvement in Chronic Pain. *Neuroscience* 2018;387:162-169.

- [16] Dschietzig T, Bartsch C, Baumann G, Stangl K. RXFP1-inactive relaxin activates human glucocorticoid receptor: further investigations into the relaxin-GR pathway. *Regul Pept* 2009;154(1-3):77-84.
- [17] Dschietzig T, Bartsch C, Wessler S, Baumann G, Stangl K. Autoregulation of human relaxin-2 gene expression critically involves relaxin and glucocorticoid receptor binding to glucocorticoid response half-sites in the relaxin-2 promoter. *Regul Pept* 2009;155(1-3):163-173.
- [18] Duan B, Cheng L, Bourane S, Britz O, Padilla C, Garcia-Campmany L, Krashes M, Knowlton W, Velasquez T, Ren X, Ross S, Lowell BB, Wang Y, Goulding M, Ma Q. Identification of spinal circuits transmitting and gating mechanical pain. *Cell* 2014;159(6):1417-1432.
- [19] Dubin AE, Patapoutian A. Nociceptors: the sensors of the pain pathway. *J Clin Invest* 2010;120(11):3760-3772.
- [20] Eiden LE, Gundlach AL, Grinevich V, Lee MR, Mecawi AS, Chen D, Buijs RM, Hernandez VS, Fajardo-Dolci G, Zhang L. Regulatory peptides and systems biology: A new era of translational and reverse-translational neuroendocrinology. *J Neuroendocrinol* 2020;32(5):e12844.
- [21] Eliava M, Melchior M, Knobloch-Bollmann HS, Wahis J, da Silva Gouveia M, Tang Y, Ciobanu AC, Triana del Rio R, Roth LC, Althammer F, Chavant V, Goumon Y, Gruber T, Petit-Demouliere N, Busnelli M, Chini B, Tan LL, Mitre M, Froemke RC, Chao MV, Giese G, Sprengel R, Kuner R, Poisbeau P, Seeburg PH, Stoop R, Charlet A, Grinevich V. A New Population of Parvocellular Oxytocin Neurons Controlling Magnocellular Neuron Activity and Inflammatory Pain Processing. *Neuron* 2016;89(6):1291-1304.
- [22] Filingeri D. Neurophysiology of Skin Thermal Sensations. *Compr Physiol* 2016;6(3):1429.
- [23] Fossat P, Dobremez E, Bouali-Benazzouz R, Favereaux A, Bertrand SS, Kilk K, Leger C, Cazalets JR, Langel U, Landry M, Nagy F. Knockdown of L calcium channel subtypes: differential effects in neuropathic pain. *J Neurosci* 2010;30(3):1073-1085.
- [24] Gertz ZM, Cain C, Kraskauskas D, Devarakonda T, Mauro AG, Thompson J, Samidurai A, Chen Q, Gordon SW, Lesnefsky EJ, Das A, Salloum FN. Remote Ischemic Pre-Conditioning Attenuates Adverse Cardiac Remodeling and Mortality Following Doxorubicin Administration in Mice. *JACC CardioOncol* 2019;1(2):221-234.
- [25] Goll Y, Atlan G, Citri A. Attention: the claustrum. *Trends Neurosci* 2015;38(8):486-495.
- [26] Gunnarsen JM, Crawford RJ, Tregear GW. Expression of the relaxin gene in rat tissues. *Mol Cell Endocrinol* 1995;110(1-2):55-64.
- [27] Halls ML, Bathgate RA, Sutton SW, Dschietzig TB, Summers RJ. International Union of Basic and Clinical Pharmacology. XCV. Recent advances in the understanding of the pharmacology and biological roles of relaxin family peptide receptors 1-4, the receptors for relaxin family peptides. *Pharmacological reviews* 2015;67(2):389-440.
- [28] Halls ML, Hewitson TD, Moore XL, Du XJ, Bathgate RA, Summers RJ. Relaxin activates multiple cAMP signaling pathway profiles in different target cells. *Ann N Y Acad Sci* 2009;1160:108-111.
- [29] Hartley BJ, Scott DJ, Callander GE, Wilkinson TN, Ganella DE, Kong CK, Layfield S, Ferraro T, Petrie EJ, Bathgate RA. Resolving the unconventional mechanisms underlying RXFP1 and RXFP2 receptor function. *Ann N Y Acad Sci* 2009;1160:67-73.

- [30] Hisaw FL. Experimental relaxation of the pubic ligament of the Guinea-Pig. *Proc Soc Exp Biol Med* 1926;23:661-663.
- [31] Hokfelt T, Barde S, Xu ZD, Kuteeva E, Ruegg J, Le Maitre E, Risling M, Kehr J, Ihnatko R, Theodorsson E, Palkovits M, Deakin W, Bagdy G, Juhasz G, Prud'homme HJ, Mechawar N, Diaz-Heijtz R, Ogren SO. Neuropeptide and Small Transmitter Coexistence: Fundamental Studies and Relevance to Mental Illness. *Front Neural Circuits* 2018;12:106.
- [32] Hokfelt T, Bartfai T, Bloom F. Neuropeptides: opportunities for drug discovery. *Lancet Neurol* 2003;2(8):463-472.
- [33] Holmes A, Heilig M, Rupniak NM, Steckler T, Griebel G. Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. *Trends Pharmacol Sci* 2003;24(11):580-588.
- [34] Hossain MA, Kocan M, Yao ST, Royce SG, Nair VB, Siwek C, Patil NA, Harrison IP, Rosengren KJ, Selemidis S, Summers RJ, Wade JD, Bathgate RAD, Samuel CS. A single-chain derivative of the relaxin hormone is a functionally selective agonist of the G protein-coupled receptor, RXFP1. *Chem Sci* 2016;7(6):3805-3819.
- [35] Hossain MA, Samuel CS, Binder C, Hewitson TD, Tregear GW, Wade JD, Bathgate RA. The chemically synthesized human relaxin-2 analog, B-R13/17K H2, is an RXFP1 antagonist. *Amino Acids* 2010;39(2):409-416.
- [36] Hsu SY, Nakabayashi K, Nishi S, Kumagai J, Kudo M, Sherwood OD, Hsueh AJ. Activation of orphan receptors by the hormone relaxin. *Science* 2002;295(5555):671-674.
- [37] Kakouris H, Eddie LW, Summers RJ. Cardiac effects of relaxin in rats. *Lancet* 1992;339(8801):1076-1078.
- [38] Kuner R, Flor H. Structural plasticity and reorganisation in chronic pain. *Nat Rev Neurosci* 2016;18(1):20-30.
- [39] Liu MG, Chen J. Preclinical research on pain comorbidity with affective disorders and cognitive deficits: Challenges and perspectives. *Prog Neurobiol* 2014;116:13-32.
- [40] Lueptow LM, Fakira AK, Bobeck EN. The Contribution of the Descending Pain Modulatory Pathway in Opioid Tolerance. *Front Neurosci* 2018;12:886.
- [41] Luo C, Kuner T, Kuner R. Synaptic plasticity in pathological pain. *Trends Neurosci* 2014;37(6):343-355.
- [42] Ma S, Gundlach AL. Relaxin-family peptide and receptor systems in brain: insights from recent anatomical and functional studies. *Adv Exp Med Biol* 2007;612:119-137.
- [43] Ma S, Roozendaal B, Burazin TC, Tregear GW, McGaugh JL, Gundlach AL. Relaxin receptor activation in the basolateral amygdala impairs memory consolidation. *Eur J Neurosci* 2005;22(8):2117-2122.
- [44] Ma S, Shen PJ, Burazin TC, Tregear GW, Gundlach AL. Comparative localization of leucine-rich repeat-containing G-protein-coupled receptor-7 (RXFP1) mRNA and [³³P]-relaxin binding sites in rat brain: restricted somatic co-expression a clue to relaxin action? *Neuroscience* 2006;141(1):329-344.
- [45] Marshall SA, O'Sullivan K, Ng HH, Bathgate RAD, Parry LJ, Hossain MA, Leo CH. B7-33 replicates the vasoprotective functions of human relaxin-2 (serelaxin). *Eur J Pharmacol* 2017;807:190-197.

- [46] Moehring F, Halder P, Seal RP, Stucky CL. Uncovering the Cells and Circuits of Touch in Normal and Pathological Settings. *Neuron* 2018;100(2):349-360.
- [47] Nakamura H, Katayama Y, Kawakami Y. Hippocampal CA1/subiculum-prefrontal cortical pathways induce plastic changes of nociceptive responses in cingulate and prelimbic areas. *BMC Neurosci* 2010;11:100.
- [48] O'Mara SM, Commins S, Anderson M, Gigg J. The subiculum: a review of form, physiology and function. *Progress in neurobiology* 2001;64(2):129-155.
- [49] Omi EC, Zhao S, Shanks RD, Sherwood OD. Evidence that systemic relaxin promotes moderate water consumption during late pregnancy in rats. *J Endocrinol* 1997;153(1):33-40.
- [50] Parikh A, Patel D, McTiernan CF, Xiang W, Haney J, Yang L, Lin B, Kaplan AD, Bett GC, Rasmusson RL, Shroff SG, Schwartzman D, Salama G. Relaxin suppresses atrial fibrillation by reversing fibrosis and myocyte hypertrophy and increasing conduction velocity and sodium current in spontaneously hypertensive rat hearts. *Circ Res* 2013;113(3):313-321.
- [51] Peirs C, Seal RP. Neural circuits for pain: Recent advances and current views. *Science* 2016;354(6312):578-584.
- [52] Peirs C, Williams SP, Zhao X, Walsh CE, Gedeon JY, Cagle NE, Goldring AC, Hioki H, Liu Z, Marell PS, Seal RP. Dorsal Horn Circuits for Persistent Mechanical Pain. *Neuron* 2015;87(4):797-812.
- [53] Piccenna L, Shen PJ, Ma S, Burazin TC, Gossen JA, Mosselman S, Bathgate RA, Gundlach AL. Localization of LGR7 gene expression in adult mouse brain using LGR7 knock-out/LacZ knock-in mice: correlation with LGR7 mRNA distribution. *Ann N Y Acad Sci* 2005;1041:197-204.
- [54] Praveen P, Kocan M, Valkovic A, Bathgate R, Hossain MA. Single chain peptide agonists of relaxin receptors. *Mol Cell Endocrinol* 2019;487:34-39.
- [55] Roca-Lapirot O, Fossat P, Ma S, Egron K, Trigilio G, Lopez-Gonzalez MJ, Covita J, Bouali-Benazzouz R, Favereaux A, Gundlach AL, Landry M. Acquisition of analgesic properties by the cholecystinin/CCK2 receptor system within the amygdala in a persistent inflammatory pain condition. *Pain* 2019;160(2):345-357.
- [56] Samuel CS, Royce SG, Hewitson TD, Denton KM, Cooney TE, Bennett RG. Anti-fibrotic actions of relaxin. *Br J Pharmacol* 2017;174(10):962-976.
- [57] Sellmeijer J, Mathis V, Hugel S, Li XH, Song Q, Chen QY, Barthas F, Lutz PE, Karatas M, Luthi A, Veinante P, Aertsen A, Barrot M, Zhuo M, Yalcin I. Hyperactivity of anterior cingulate cortex areas 24a/24b drives chronic pain-induced anxiodepressive-like consequences. *J Neurosci* 2018;38(12):3102-3115.
- [58] Solway B, Bose SC, Corder G, Donahue RR, Taylor BK. Tonic inhibition of chronic pain by neuropeptide Y. *Proc Natl Acad Sci U S A* 2011;108(17):7224-7229.
- [59] Summerlee AJ, Hornsby DJ, Ramsey DG. The dipsogenic effects of rat relaxin: The effect of photoperiod and the potential role of relaxin on drinking in pregnancy. *Endocrinology* 1998;139(5):2322-2328.
- [60] Sunn N, Egli M, Burazin TC, Burns P, Colvill L, Davern P, Denton DA, Oldfield BJ, Weisinger RS, Rauch M, Schmid HA, McKinley MJ. Circulating relaxin acts on subfornical organ neurons to stimulate water drinking in the rat. *Proc Natl Acad Sci U S A* 2002;99(3):1701-1706.

- [61] Svendsen AM, Vrecl M, Ellis TM, Heding A, Kristensen JB, Wade JD, Bathgate RA, De Meyts P, Nohr J. Cooperative binding of insulin-like Peptide 3 to a dimeric relaxin family peptide receptor 2. *Endocrinology* 2008;149(3):1113-1120.
- [62] Svendsen AM, Zalesko A, Konig J, Vrecl M, Heding A, Kristensen JB, Wade JD, Bathgate RA, De Meyts P, Nohr J. Negative cooperativity in H2 relaxin binding to a dimeric relaxin family peptide receptor 1. *Mol Cell Endocrinol* 2008;296(1-2):10-17.
- [63] Tan LL, Pelzer P, Heintz C, Tang W, Gangadharan V, Flor H, Sprengel R, Kuner T, Kuner R. A pathway from midcingulate cortex to posterior insula gates nociceptive hypersensitivity. *Nat Neurosci* 2017;20(11):1591-1601.
- [64] Tasic B, Menon V, Nguyen TN, Kim TK, Jarsky T, Yao Z, Levi B, Gray LT, Sorensen SA, Dolbeare T, Bertagnoli D, Goldy J, Shapovalova N, Parry S, Lee C, Smith K, Bernard A, Madisen L, Sunkin SM, Hawrylycz M, Koch C, Zeng H. Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. *Nat Neurosci* 2016;19(2):335-346.
- [65] Todd AJ, Wang F. Central Nervous System Pain Pathways In: JN Wood, editor. *The Oxford Handbook of the Neurobiology of Pain: Oxford Handbooks Online*, 2018.
- [66] Treede RD, Rief W, Barke A, Aziz Q, Bennett MI, Benoliel R, Cohen M, Evers S, Finnerup NB, First MB, Giamberardino MA, Kaasa S, Kosek E, Lavand'homme P, Nicholas M, Perrot S, Scholz J, Schug S, Smith BH, Svensson P, Vlaeyen JW, Wang SJ. A classification of chronic pain for ICD-11. *Pain* 2015;156(6):1003-1007.
- [67] van den Pol AN. Neuropeptide transmission in brain circuits. *Neuron* 2012;76(1):98-115.
- [68] van der Westhuizen ET, Christopoulos A, Sexton PM, Wade JD, Summers RJ. H2 relaxin is a biased ligand relative to H3 relaxin at the relaxin family peptide receptor 3 (RXFP3). *Mol Pharmacol* 2010;77(5):759-772.
- [69] van Hecke O, Austin SK, Khan RA, Smith BH, Torrance N. Neuropathic pain in the general population: a systematic review of epidemiological studies. *Pain* 2014;155(4):654-662.
- [70] van Hecke O, Torrance N, Smith BH. Chronic pain epidemiology and its clinical relevance. *Br J Anaesth* 2013;111(1):13-18.
- [71] Wade JD, Layden SS, Lambert PF, Kakouris H, Tregear GW. Primate relaxin: synthesis of gorilla and rhesus monkey relaxins. *J Protein Chem* 1994;13(3):315-321.
- [72] Wang Q, Ng L, Harris JA, Feng D, Li Y, Royall JJ, Oh SW, Bernard A, Sunkin SM, Koch C, Zeng H. Organization of the connections between claustrum and cortex in the mouse. *J Comp Neurol* 2017;525(6):1317-1346.
- [73] Yam MF, Loh YC, Tan CS, Khadijah Adam S, Abdul Manan N, Basir R. General Pathways of Pain Sensation and the Major Neurotransmitters Involved in Pain Regulation. *Int J Mol Sci* 2018;19(8).
- [74] Zieglgansberger W. Substance P and pain chronicity. *Cell Tissue Res* 2019;375(1):227-241.
- [75] Zingg B, Hintiryan H, Gou L, Song MY, Bay M, Bienkowski MS, Foster NN, Yamashita S, Bowman I, Toga AW, Dong HW. Neural networks of the mouse neocortex. *Cell* 2014;156(5):1096-1111.

Discussion

L'implication des peptides de la famille relaxine, ainsi que de leurs récepteurs dans la modulation de la douleur n'a pas encore fait l'objet de travaux de recherche. C'est pourquoi cette thèse s'est penchée sur ce sujet, et nos résultats mettent en évidence pour la première fois la modulation de la douleur par les systèmes relaxine-3/RXFP-3 et relaxine/RXFP-1, proposant ainsi de la matière pour réflexion sur des pistes thérapeutiques éventuelles.

1. Qualités des outils pharmacologiques

Afin d'étudier l'effet des différents peptides de la famille relaxine, nous avons eu recours dans le cas du système relaxine-3/RXFP-3 aux agonistes A2 et peptide 5, et l'antagoniste R3 (B1-22) R (Tableau 7), et dans le cas du système relaxine/RXFP-1 aux agonistes H2 et B7.33, et l'antagoniste H2B (Tableau 8).

1.1. Qualité des modulateurs de RXFP-3

La relaxine-3 humaine (H3) est un neuropeptide formé de 2 chaînes A et B, qui peut se fixer au niveau cérébral sur soit RXFP-1, soit avec plus d'affinité sur RXFP-3 qui est le récepteur apparenté de la relaxine-3. Notre étude s'intéresse à l'implication du système relaxine-3/RXFP-3 dans la modulation de la douleur, par conséquent et pour éliminer le risque d'effet biaisé, on a eu recours à des peptides RXFP-3 sélectifs.

En effet, la chaîne B est nécessaire pour la fixation de H3 sur RXFP-3 et l'activation de ce dernier. Les acides aminés [Arg (B^{8,12,16}), Ile (B¹⁵) et Phe (B²⁰)] qui sont nécessaires pour la liaison H3/RXFP-3, et les acides aminés Arg (B²⁶) et Trp (B²⁷) qui sont nécessaires pour l'activation, se trouvent sur cette chaîne. Tandis que la fixation sur RXFP-1 nécessite les 2 chaînes A et B (Bathgate et al., 2013). Cependant il est important de signaler que la chaîne B seule, avait une très faible affinité vis-à-vis de RXFP-3, en raison de l'incapacité de la chaîne B à former l'hélice α , conformation assurée par la chaîne A au niveau de H3 (Liu et al., 2003). Par conséquent on a utilisé le peptide A2 développé en 2012 (Shabanpoor et al., 2012). Cet analogue de H3 a été obtenu par élimination du pont disulfure présent au niveau de la chaîne A et par délétion des résidus présents au niveau N terminal de la chaîne A. Le peptide A2 est un agoniste complet de RXFP-3, en effet en comparant H3 et A2, l'étude de Shabanpoor montre que l'affinité de liaison à RXFP-3 d'A2 est similaire à celle de H3 (pki de 7.87 ± 0.12 pour A2 vs 7.78 ± 0.06 pour H3), alors que l'affinité vis-

à-vis de RXFP-1 d'A2 est nettement inférieure à celle de H3 ($pK_i < 5$ pour A2 vs 8.6 ± 0.01 pour H3). De plus l'étude a montré qu'A2 est un activateur puissant de RXFP-3 (pEC_{50} de 8.43 ± 0.09 pour A2 vs 9.0 ± 0.07 pour H3), la puissance d'activation a été mesurée en fonction de la capacité des peptides à inhiber l'accumulation d'AMPc induite par le forskolin (pEC_{50}). Cependant A2 n'active en aucun cas RXFP-1. Par la suite l'étude montre qu'A2 est un activateur (concentration dépendant tout comme H3) puissant de la phosphorylation d'ERK1/2 (pEC_{50} de 10.4 ± 0.11 pour A2 vs 9.83 ± 0.14 pour H3, $p < 0.05$). Finalement l'étude montre qu'A2 augmente la prise alimentaire après avoir été injecté en intracérébrale chez des rats (Shabanpoor et al., 2012).

Avec le développement des processus chimiques et notamment le développement de la technique de « hydrocarbon stapling » qui confère au peptide une structure en hélice α (Walensky and Bird, 2014), Hojo et al ont développé en 2016 le peptide 5 (Hojo et al., 2016). Le peptide 5 est formé uniquement de la chaîne B, qui à l'aide de l'hydrocarbon stapling (au niveau B13 et B17) a une structure en hélice α . Le peptide 5 est, tout comme l'A2, un agoniste complet de RXFP-3 pour l'inhibition de l'accumulation d'AMPc induite par le forskolin (pEC_{50} de 9.08 ± 0.07 pour peptide 5 vs 8.48 ± 0.06 pour H3), et un agoniste complet de l'activation de la voie ERK1/2 (pEC_{50} de 9.83 ± 0.10 pour peptide 5 vs 9.92 ± 0.10 pour H3). Le peptide 5 tout comme A2, induit significativement la prise alimentaire chez les rats (Hojo et al., 2016).

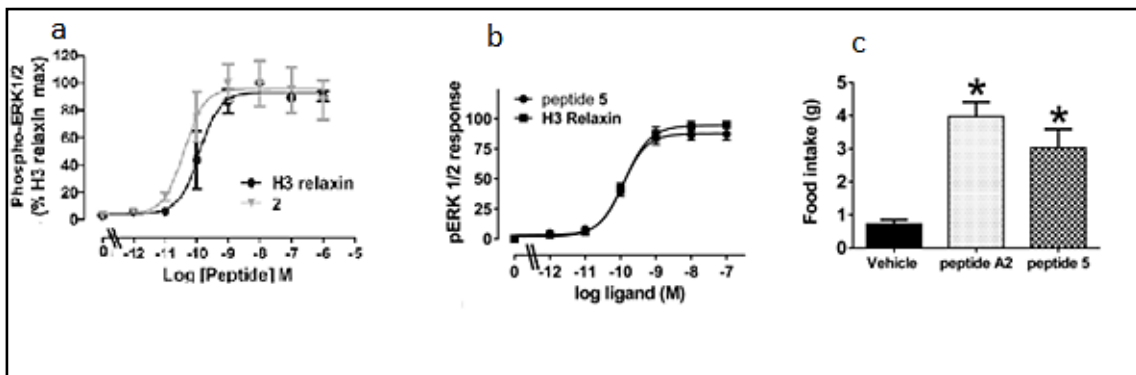


Figure 38: Représentations graphiques de (a) puissance d'activation de la phosphorylation d'ERK1/2 pour H3 et A2, (b) puissance d'activation de la phosphorylation d'ERK1/2 pour H3 et A5, (c) effet sur la prise alimentaire d'A2, et A5 (Hojo et al., 2016).

Par conséquent A2 et peptide 5 sont des agonistes complets et spécifiques de RXFP-3, sans qu'il y ait de différence significative entre les 2 de points de vue d'affinité et d'activation, ce qui est en concordance avec nos résultats qui ne montrent aucune différence entre les effets d'A2 et

du peptide 5 sur la modulation de la douleur. De plus A2 et le peptide 5 sont des agonistes sélectifs de RXFP-3, donc l'analgésie mécanique et thermique observées chez les souris CFA après injection des agonistes au niveau de la BLA et l'analgésie mécanique observée chez les souris CFA après injection des agonistes dans l'ACC sont le plus probablement dus à l'interaction de ces peptides avec RXFP-3.

D'autre part et dans un souci de sélectivité, on a choisi comme antagoniste spécifique de RXFP-3, le peptide R3(B1-22)R. Ce peptide a été développé en 2011 par Haugaard-Kedstrom et al, il s'agit d'un peptide formé uniquement de la chaîne B avec remplacement de la séquence GGSRW par l'arginine, et la mutation de Cys (B¹⁴) en Ser (Haugaard-Kedström et al., 2011). Ce peptide présente une forte affinité vis-à-vis de RXFP-3 (pKi de 7.69±0.18) , qui est due à l'arginine qui interagit significativement avec RXFP-3 et par conséquent compense la perte d'affinité due à la linéarité de la structure (absence d'hélice) (Haugaard-Kedström et al., 2011, 2018; Wong et al., 2018). R3(B1-22)R n'active pas RXFP-3, donc pas d'inhibition d'accumulation d'AMPc. De même R3(B1-22)R n'induit pas la prise alimentaire chez les rats (Haugaard-Kedström et al., 2011).

Les analgésies observées chez les souris CFA avec les agonistes sont annulées par l'ajout de l'antagoniste, ce qui souligne encore plus la sélectivité des peptides vis-à-vis de RXFP-3.

Cependant, nos résultats montrent que l'ajout de l'antagoniste au niveau de l'ACC chez les souris NaCl induit une proalgie. Notre résultat suggère alors un rôle analgésique éventuel de la relaxine-3 endogène au niveau de l'ACC.

Peptide	Séquence	Affinité RXFP-3 (pKi)	Puissance d'activation (pEC50)
H3	DVLAGLSSSCCKWGCSKSEISLC RAAPYGVRLCGREFIRAVIFTCGGSRW	7.78 ±0.06	9.0 ± 0.07
A2	CKWGASKSEISLC RAAPYGVRLCGREFIRAVIFTCGGSRW	7.87±0.12	8.43 ± 0.09
A5	Ac-SGRsFIRsVIFTSGGSRW	6.56±0.05	9.08 ± 0.07
R3(B1-22)R	RAAPYGVRLSGREFIRAVIFTSR	7.69 ±0.18	Pas d'activité

Table 7 : Séquence, affinité de liaison à RXFP-3 et puissance d'activation par rapport à l'inhibition de l'AMPc induite par le forskolin pour H3, A2, A5 et R3(B1-22)R.

1.2. Qualité des modulateurs de RXFP-1

La relaxine humaine H2 peut se fixer sur RXFP-1 (récepteur apparenté) et avec moins d'affinité sur RXFP-3 (Bathgate et al., 2013), de ce fait les effets observés avec H2 ne sont pas exclusivement attribués à RXFP-1. Afin d'étudier l'effet relatif à RXFP-1, on a utilisé l'agoniste B7.33. B7.33 développé en 2016 par Hossain et al, est un agoniste sélectif mais de faible affinité vis-à-vis de RXFP-1 (pki de 5.54 ± 0.13 pour B7.33 vs 8.96 ± 0.03 pour H2). Cette diminution d'affinité est due à la structure de B7.33 qui est formée uniquement de la chaîne B qui est tronquée du côté N-terminal et à laquelle on ajoute des résidus chargés positivement de la chaîne C de la pro-H2. En effet la liaison à RXFP-1 nécessite la fixation de la chaîne B sur le site de liaison de haute affinité (présent au niveau du domaine LRR) et la fixation de la chaîne A sur un site de fixation secondaire et de faible affinité (présent du domaine transmembranaire). La faible affinité de B7.33 est due donc à l'absence de la chaîne A. En ce qui concerne la puissance d'activation de B7.33, elle dépend du type cellulaire exprimant le RXFP-1, ainsi B7.33 est un agoniste complet de l'activation de l'AMPc et de la phosphorylation d'ERK1/2 aux niveaux des cellules exprimant nativement RXFP-1 telles que les myofibroblastes rénaux de rats et les cellules cancéreuses MCF-7 (Hossain et al., 2016). Nos résultats montrent que l'injection ICV de H2 et celle de B7.33 ont toutes les deux induit une analgésie mécanique et thermique, ce qui sous-entend que l'effet observé avec H2 est dû à l'activation de RXFP-1.

L'antagoniste B-R13/17K-H2 (H2B) est un antagoniste sélectif de RXFP-1 développé en 2010 par Hossain et al (Hossain et al., 2010). H2B est formé par les chaînes A et B tronquée avec mutation des Arg en position 13 et 17 en Lys. H2B est un antagoniste complet de RXFP-1 aux niveaux des cellules qui expriment nativement RXFP-1. Nos résultats montrent que l'analgésie mécanique observée avec les agonistes de RXFP-1 aux niveaux des souris CFA est annulée par l'ajout de l'antagoniste, cependant l'analgésie thermique ne l'est pas. Par conséquent on peut dire que l'effet sur l'analgésie mécanique passe par RXFP-1, cependant en ce qui concerne l'analgésie thermique on peut soupçonner que cet effet passe par RXFP-3, vu que H2 peut activer RXFP-3, mais vu que l'analgésie est aussi maintenue après injection de B7.33 qui est RXFP-1 sélectif, on a donc abandonné cette hypothèse (Bathgate et al., 2013; Praveen et al., 2019). Une autre explication serait que l'effet thermique serait dû à une population cellulaire différente, au niveau de laquelle RXFP-1 induit une voie de signalisation différente, vu que l'activation de RXFP-1 est cellule dépendante.

Peptide	Séquence	Affinité RXFP-1 (pKi)
H2	ZLYSALANKCCHVGCTKRSLARFC DSWMEEVIKLCGRELVRAQIAICGMSTWS	8.96±0.03
B7.33	VIKLSGRELVRAQIAISGMSTWSKRSL	5.54±0.13
B-R13/17K-H2 (H2B)	ZLYSALANKCCVVGCTKRSLARFC DSWMEEVIKLCGKELVKAQIAICGMSTWS	6.29±0.28

Table 8 : Séquence des peptides de RXFP-1

2. Mécanismes impliqués

2.1. Mécanismes impliqués dans la modulation de la douleur inflammatoire persistante par le système relaxine-3/RXFP-3

Le système relaxine-3/RXFP-3 a fait l'objet de plusieurs études ayant pour cible ses différentes fonctions au sein du système nerveux central (Olucha-Bordonau et al., 2018). Ce système serait impliqué dans l'éveil (Sutton et al., 2009), la prise alimentaire (McGowan et al., 2005), le stress et l'anxiété (Ryan et al., 2013) et la mémoire (Albert-Gascó et al., 2017). Néanmoins l'implication de ce système dans la modulation de la douleur n'a pas encore été étudiée. Cependant des études ont soulevé l'effet du système relaxine-3/RXFP-3 sur les comorbidités émotionnelles de la douleur, ainsi Ryan et al ont montré que l'activation de RXFP-3 diminue le taux d'anxiété et de dépression chez les rats (Ryan et al., 2013), l'effet anxiolytique a été mis en évidence aussi par Zhang et al qui ont montré que l'activation de RXFP-3 mène aussi à une diminution du taux d'anxiété aux niveaux de souris anxieuses (Zhang et al., 2015). De plus les études préliminaires au sein de l'équipe bordelaise montrent que l'injection intracérébrale de l'agoniste A2 induit une analgésie mécanique chez les souris CFA. Par conséquent l'objectif de cette thèse est de mettre en évidence le rôle de ce système dans la douleur, plus particulièrement après injection dans la BLA et l'ACC.

Dans un premier temps nous avons testé l'effet des analogues de la relaxine-3, injectés au niveau de la BLA. Notre choix s'est porté sur cette région puisque les études de Smith ont montré que la

BLA était une zone de très forte densité de sites de liaison (Smith et al., 2010). De plus, les résultats préliminaires de RNAscope menées sur des souris C57Bl6/J naïves au sein de l'équipe bordelaise montrent que 12% des neurones de la BLA sont des neurones *Rxfp-3* positifs. D'autre part, l'amygdale joue un rôle clé dans la composante émotionnelle de la douleur, du fait qu'elle ajoute l'information émotionnelle au message nociceptif d'une part, et qu'elle module les voies descendantes de l'autre (Veinante et al., 2013).

Nos résultats montrent que l'injection d'A2 et celle du peptide 5 au niveau de la BLA induisent une analgésie thermique et une analgésie mécanique dans un modèle de douleur inflammatoire persistante. Ces analgésies sont annulées dans les 2 cas par l'ajout de l'antagoniste R3 (B1-22) R. Aucune modification n'a été observée chez les souris contrôle. Nos résultats suggèrent alors que la relaxine-3 module la douleur en activant RXFP-3 au niveau de la BLA, dans le cas de douleur inflammatoire persistante.

En effet la BLA reçoit des inputs nociceptifs depuis l'ACC, le mPFC et le thalamus et projette vers la CeA (région principale des outputs de l'amygdale) soit directement soit indirectement en projetant sur ITC (intercalated mass) qui elle projette sur la CeA. La BLA projette aussi vers l'ACC, le mPFC et l'IC. La CeA reçoit aussi les informations nociceptives du noyau para brachial (PB), et projette vers le PAG pour moduler les voies descendantes de la douleur. Dans le cas de douleur chronique, on observe une augmentation des projections excitatrices du PB et de la BLA vers la CeA, alors qu'on observe une diminution des projections inhibitrices de l'ITC sur la CeA (Neugebauer, 2015; Neugebauer et al., 2004; Thompson and Neugebauer, 2017). De plus l'équipe de Huang 2019 a mis en évidence une nouvelle voie impliquée dans la douleur chronique, en effet l'étude suggère qu'en cas de douleur chronique, la BLA projette sur les interneurons GABAergiques du mPFC, qui à leur tour vont inhiber les projections du mPFC vers le PAG et par conséquent moduler les voies descendantes (Huang et al., 2019). Par conséquent, en cas de douleur chronique, on observe une hyperactivité de la BLA.

D'autre part les études de Santos et al mettent en évidence les projections du NI (principale source de fibres relaxine-3) vers l'amygdale (Santos et al., 2016) et vu que cette dernière présente une haute affinité de densité de liaison, une hypothèse serait de suggérer que la relaxine-3 en se fixant sur RXFP-3 pourrait diminuer l'hyperexcitabilité de la BLA en cas de douleur chronique, ou même inhiber les projections de la BLA, modulant ainsi les voies descendantes. Notre réflexion s'est portée sur la modulation des voies descendantes, puisque RXFP-3 n'est pas exprimé au

niveau de la moelle épinière (Liu et al., 2003) ainsi l'effet du système relaxine-3/RXFP-3 serait plus probablement dû à une modulation des voies descendantes qu'une modulation des circuits spinaux.

Par la suite notre attention s'est tournée vers la caractérisation des neurones *Rxfp-3* positifs, et nos résultats de RNAscope ont montré que la majorité des neurones exprimant l'ARNm de *Rxfp-3*, co-expriment l'ARNm de la *somatostatine* aux niveaux de BLA, CeA et BMA. Nos résultats montrent une répartition plutôt homogène des neurones *Rxfp-3* entre les différentes régions de l'amygdale avec cependant un nombre de neurones inférieur (non significativement) au niveau de la BLA. De plus, nos résultats ne montrent pas de différence d'expression entre les souris CFA et les souris NaCl. La différence observée au niveau de la BLA peut être expliquée par le fait que 85% des neurones de la BLA sont des neurones glutamatergiques versus 15 % d'interneurones GABAergiques (Polepalli et al., 2020), et vu que les interneurones SOM et PV représentent la majorité des sous-populations d'interneurones GABAergiques inhibiteurs (Kubota et al., 1994), donc la diminution observée au niveau de la BLA serait due au nombre restreint de neurones SOM présents déjà au niveau de la BLA. Peu d'études se sont attardées sur la qualification des neurones *Rxfp-3* positifs au niveau de l'amygdale et encore moins au niveau de la BLA. L'étude d'Albert-Gasco montrent que la majorité des neurones de *Rxfp-3* au sein de l'amygdale sont GABAergiques (Albert-Gasco et al., 2019). La nature GABAergique des neurones *Rxfp-3* positifs a été mise en évidence au niveau du complexe médial septal, par contre dans cette région, les neurones *Rxfp-3* positifs sont des neurones PV (Albert-Gasco et al., 2018).

Dans un deuxième temps on s'est intéressé à étudier l'effet du système relaxine-3/RXFP-3 au niveau de l'ACC. Notre choix s'est porté sur l'ACC, puisque c'est une région riche en fibre relaxine-3 (Smith et al., 2010), c'est une région cérébrale impliquée dans la composante affective de la douleur et c'est une région qui émet et reçoit des projections de la BLA (Gao et al., 2004; Neugebauer et al., 2004, 2009).

Nos résultats montrent que l'injection du peptide 5 induit une analgésie mécanique et non thermique chez les souris CFA, qui est annulée par l'ajout de R3 (B1-22) R. Cependant l'injection de R3 (B1-22) R a induit une proalgie chez les souris NaCl. Aucune étude à ce jour ne s'est penchée sur l'implication la relaxine -3 et ses analogues au niveau de l'ACC. Nos résultats mettent en

évidence la possibilité que la relaxine-3 endogène ait un tonus inhibiteur endogène, vu que l'ajout de l'antagoniste mène à une proalgie chez les souris NaCl. De plus nos résultats montrent une différence entre l'hypersensibilité mécanique et l'hypersensibilité thermique. Les différentes études menées soulignent que la différence réside aux niveaux des canaux activés présents aux niveaux des nocicepteurs, ainsi les canaux TRPV1 et TRPM8 sont activés par des stimuli thermiques, alors que TRPV2 et TRPV 4 sont activés par des stimuli mécaniques. Dans la même optique les études soulignent que la différence réside aussi au niveau de la corne dorsale de la moelle épinière et plus spécifiquement aux niveaux des neurones activés aux niveaux des différentes laminae (Cheng et al., 2017; Duan et al., 2014; Dubin and Patapoutian, 2010; Petitjean et al., 2015). Cependant aucune étude jusqu'à présent n'a soulevé la probabilité que la différence pourrait aussi résider aux niveaux des structures supra spinales. Nos résultats suggèrent alors qu'il existe au niveau du cerveau des circuits et des voies de signalisation différents pour le traitement des sensibilités mécaniques et thermiques, et que certaines voies peuvent être activées différemment en fonction des régions.

De plus nos résultats montrent une différence d'effets observés suite à l'injection des modulateurs de RXFP-3 au niveau de la BLA (analgésie mécanique et thermique) et au niveau de l'ACC (analgésie mécanique) qui peut-être due à la signalisation qui peut différer entre les régions cérébrales. Cela dit il existe très peu d'études s'attardant sur l'impact direct de l'activation de RXFP-3 (Ma et al., 2017). D'autre part la différence peut-être aussi due à la modulation d'un autre système peptidergique par le système relaxine-3/RXFP-3. En effet un des mécanismes impliqués dans l'augmentation de l'apport alimentaire suite à l'injection d'agoniste RXFP-3 serait la modulation du système oxytocine. Ainsi les études de Ganella ont montré que la sécrétion chronique d'agoniste RXFP-3 au niveau du noyau paraventriculaire mène à une diminution du taux d'ARN_m de l'oxytocine (anorexigène), menant ainsi à une levée de coupe appétit et stimulant donc la prise alimentaire (Ganella et al., 2013). Une hypothèse serait donc la signalisation relaxine-3/RXFP-3 pourrait moduler la signalisation d'un autre système peptidergique impliqué dans la modulation de la douleur.

Par la suite, tout comme pour l'amygdale, notre attention s'est tournée vers la caractérisation des neurones *Rxfp-3* positifs, et nos résultats de RNAscope ont montré que la majorité des neurones exprimant l'ARN_m de *Rxfp-3*, co-expriment l'ARN_m de la *somatostatine* au niveau de l'ACC,

avec une augmentation significative de la surface marquée de Rxfp-3 chez les souris CFA par rapport aux souris NaCl. La caractérisation spécifique des neurones Rxfp-3 au niveau de l'ACC n'a fait l'objet d'aucune étude pour le moment. Cependant la nature GABAergique des neurones Rxfp-3 au niveau de l'ACC est en accord avec littérature qui souligne la nature GABAergique des neurones Rxfp-3 au niveau de l'amygdale et du médial septal (Albert-Gasco et al., 2019; Albert-Gascó et al., 2018). La différence observée entre souris CFA et souris NaCl peut être expliquée par la possibilité d'une stimulation des neurones Rxfp-3 par la douleur.

2.2. Mécanismes impliqués dans la modulation de la douleur inflammatoire persistante par le système relaxine/RXFP-1

Le système relaxine/RXFP-1 fut l'objet de plusieurs études s'attardant sur le potentiel antifibrotique et vasodilatateur de ce système. Cependant très peu d'études s'attardent sur les fonctions de ce système au niveau du système nerveux central, ainsi ce système serait impliqué dans l'osmolarité plasmatique (Sunn et al., 2002), la consommation d'eau (Summerlee et al., 1998) et la mémoire (Ma et al., 2005). Les datas du Allen Brain Atlas montrent une répartition assez importante des fibres de relaxine dans les régions cérébrales impliquées dans la douleur, par conséquent on a décidé d'étudier l'effet de ce système sur la modulation de la douleur. Pour ce fait on a injecté les analogues de la relaxine dans le ventricule latéral (LV) des souris, notre choix s'est porté sur le LV pour avoir un effet global. L'injection ICV (intracérébroventriculaire) de H2 et de B7.33 mènent à une analgésie mécanique et thermique chez les souris CFA, l'analgésie mécanique est annulée par l'ajout de l'antagoniste alors que l'analgésie thermique ne l'est pas. Aucune étude pour le moment ne s'est attardée sur l'implication du système relaxin/RXFP-1 dans la douleur, néanmoins nos résultats soulèvent encore une fois l'hypothèse de la présence de circuits cérébraux différents pour le traitement des sensibilités thermiques et des sensibilités mécaniques. En effet, l'analgésie thermique n'étant pas reversée par l'ajout de l'antagoniste peut être la résultante de plusieurs hypothèses : une première hypothèse pourrait être que les agonistes de RXFP-1 activent un autre système de transduction, tel que les récepteurs glucocorticoïdes (Dschietzig et al., 2009a, 2009b); cependant vu que l'analgésie est rapide, cette hypothèse fut rejetée. Une deuxième hypothèse serait que l'effet serait dû à l'interaction d'H2 avec RXFP-3 (van der Westhuizen et al., 2010), mais vu que l'analgésie thermique persiste même avec B7.33 qui est un agoniste spécifique de RXFP-1 (Praveen et al., 2019), cette hypothèse fut rejetée. Une autre hypothèse se base sur le

potentiel d'H2B d'activer une voie de signalisation spécifique en fonction du type cellulaire, par conséquent il existerait donc des voies de signalisation différentes au sein de populations cellulaires différentes pour traiter les douleurs thermiques (Bokiniec et al., 2018; Filingeri, 2016) et les douleurs mécaniques (Duan et al., 2014; Moehring et al., 2018; Peirs et al., 2015).

Par la suite, notre attention s'est tournée vers la caractérisation des neurones *Rxfp-1* dans des régions impliquées dans les voies descendantes, puisque les tests de comportement reposent sur les réflexes spinaux. L'expression de *Rxfp-1* est bien caractérisée chez les rats (Ma et al., 2005, 2006), ce qui n'est pas le cas chez la souris, où la répartition de l'expression n'est décrite qu'au niveau de l'Allen Brain Atlas et qui serait plus restreinte que chez le rat. On a décidé d'étudier la répartition des neurones *Rxfp-1* dans l'ACC qui est une région connue pour son rôle facilitateur dans la transmission de la douleur (Chen et al., 2018; Sellmeijer et al., 2018; Tan et al., 2017), dans le CLA qui de par ses riches connexions corticales, constitue un hub de réseau central, coordonnant l'activité des circuits corticaux (Goll et al., 2015; Zingg et al., 2014) et dans le SUB dont les projections modulent les réponses nociceptives aux niveaux des neurones du PFC (Nakamura et al., 2010). Nos résultats montrent que l'ARNm de *RXFP-1* est abondant dans l'ACC et le CLA, soulignant ainsi le rôle de ces régions dans la modulation des voies descendantes. De plus nos résultats montrent que dans la majorité des régions, les neurones *Rxfp-1* positifs sont des neurones excitateurs exprimant l'ARNm de *CamKII*, nos résultats mettent en évidence alors un nouveau système de signalisation pour contrôler les outputs de ces régions.

La répartition cérébrale de la relaxine reste très peu explorée, c'est pourquoi on s'est attardé dans cette thèse pour présenter, pour la première fois, un mapping de la répartition générale de la relaxine au niveau de cerveau de souris, en ayant recours à l'immunohistochimie. Nos résultats sont en accord avec la littérature (Ma and Gundlach, 2007; Ma et al., 2005) et montrent que les neurones à relaxine sont surtout localisés dans les régions impliquées dans la douleur. Ainsi nous retrouvons de la relaxine dans les régions corticales, l'hypothalamus et les structures du mésencéphale. De plus nos résultats montrent que la majorité de la relaxine se trouve au niveau du corps cellulaire, soulignant ainsi le rôle neuromodulateur paracrine/autocrine de la relaxine. Davantage, nos résultats mettent en évidence des projections de relaxine vers des régions riche en *RXFP-1*, soulignant ainsi le transport de la relaxine tout au long de l'axone. Plus particulièrement nos résultats montrent des connections réciproques entre l'ACC et le CLA (Wang et al., 2017) pouvant contenir de la relaxine, et de même nos résultats montrent que la BLA émet des outputs

relaxines vers l'ACC et le CLA. De plus nos résultats soulignent la nature inhibitrice des neurones à relaxine, puisqu'ils co-expriment l'ARNm de GAD65.

En conclusion, nos résultats montrent que le système relaxine/RXFP-1 induit une analgésie thermique et mécanique dans le cas de douleur inflammatoire persistante, cependant aucune différence neuroanatomique n'est observée entre souris CFA et souris NaCl, par conséquent l'effet de ce système serait dû soit à l'altération de la signalisation RXFP-1 dans des conditions de douleurs inflammatoires persistantes, soit à la nécessité du système à être stimulé et devenir sensible à l'activation de RXFP-1, comme c'est le cas d'autres neuropeptides jouant un rôle particulier dans des conditions physiopathologiques (Hökfelt et al., 2018).

3. Modulation de la douleur au niveau cérébral par des systèmes peptidergiques

Récemment les études se penchent d'avantages sur le potentiel thérapeutique des neuropeptides, puisqu'ils présenteraient moins d'effets secondaires vu que leurs voies de signalisation sont plus distinctes (Hoyer and Bartfai, 2012). Du fait que la douleur chronique représente toujours un fléau mondial sous-traité, les recherches de traitements efficaces ciblant simultanément la douleur et ses comorbidités demeurent toujours actives. Par conséquent l'objectif de cette thèse était de tester la capacité des neuropeptides de la famille relaxine à moduler la douleur au niveau cérébral. Il est cependant important de souligner que les effets des neuropeptides sur la douleur ont été principalement étudiés au niveau de la transmission sensorielle au niveau de la corne dorsale et assez peu d'études s'attardent sur les effets au niveau cérébral.

Les résultats de cette thèse soulignent pour la première fois la modulation de la douleur au niveau cérébral par les systèmes peptidergiques relaxine/RXFP-1 et relaxine-3/RXFP-3. Les neuropeptides de la famille relaxine s'ajoutent alors à la liste des nouveaux systèmes peptidergiques modulant la douleur.

Les neuropeptides modulent le plus souvent la douleur en modulant les voies descendantes. Ainsi les études de Roca-Lapirot montrent que l'injection intra-CeA de CCK (cholécystokinine) chez des rats CFA induit une analgésie, et que cet effet serait médié par les projections CCK2 (récepteur de CCK) de la CeA au PAG pour éventuellement inhiber la décharge des neurones spinaux (Roca-Lapirot et al., 2019). Vu l'absence de RXFP-3 au niveau de la moelle, l'analgésie

observée avec le système relaxine-3/RXFP-3 serait le plus probablement due à une modulation des voies descendantes, à l'instar de CCK. Un autre neuropeptide modulant les voies descendantes est la neurotensine (NT) qui peut soit faciliter soit inhiber la douleur en fonction qu'elle soit injectée dans le PAG ou la RVM (Kleczkowska and Lipkowski, 2013). Cette variation d'effet observée avec la NT serait probablement due à une activation de voies descendantes distinctes et indépendantes pour la facilitation de la douleur et pour l'inhibition de la douleur (Kleczkowska and Lipkowski, 2013), ainsi les études d'Urban et al et celles de Smith et al montrent qu'il s'agirait plutôt de voies descendantes projetant vers le funicule ventrolatéral pour la facilitation, et celles projetant vers le funicule dorsolatéral pour l'inhibition de la douleur (Smith et al., 1997; Urban et al., 1999). Nous avons observé une variation de modulation avec nos résultats, plus précisément mes résultats montrent que la modulation de la douleur par la relaxine-3 est région dépendante, ainsi on observe une analgésie mécanique et thermique chez les souris CFA après injection dans la BLA, alors qu'on observe uniquement une analgésie mécanique chez les souris CFA après injection de la relaxine-3 dans l'ACC. Cette variation souligne encore une fois l'hypothèse de la présence de voies de signalisation distinctes pour la sensibilité mécanique et la sensibilité thermique, impliquant des régions cérébrales différentes.

En effet, il n'existe pas au niveau du cerveau une région spécifique qui traite la douleur mais plutôt une matrice renfermant plusieurs régions. De ce fait les neuropeptides peuvent moduler la douleur en agissant aux niveaux de plusieurs régions. Ainsi l'injection intra-CeA de la galanine induit une analgésie mécanique et thermique, via son récepteur GalR1 qui active la voie PKC (Li et al., 2017). Cette même analgésie mécanique et thermique est observée suite à l'injection de galanine au niveau du nucleus accumbens (NAc), toujours via activation de GalR1 (Zhang et al., 2019). La galanine induit aussi une analgésie thermique et mécanique après injection dans l'ACC mais via l'activation du récepteur GalR2 (Zhang et al., 2017). Nos résultats montrent une analgésie mécanique et thermique suite à l'injection de relaxine-3 au niveau de la BLA et seule une analgésie mécanique au niveau de l'ACC, une hypothèse serait que cette différence serait due à l'activation d'un autre récepteur. Cependant on a eu recours à des modulateurs spécifiques de RXFP-3 donc cette hypothèse ne tient pas, et on revient à l'hypothèse de l'implication des voies différentes aux niveaux de populations cellulaires différentes pour le traitement des modalités mécaniques et thermiques.

Parmi les mécanismes impliqués dans la modulation de la douleur par les neuropeptides, on peut aussi citer le « feed forward inhibition » au cours duquel les neurones excitateurs excitent les cellules inhibitrices, qui inhibent ensuite un groupe de neurones excitateurs postsynaptiques en dehors de la zone des neurones excitateurs initialisants (Kee et al., 2015). L'effet antinociceptif du neuropeptide S (NPS) repose sur le concept du feed forward inhibition, puisque le NPS inhibe l'activité de la CeA en se fixant sur son récepteur au niveau de l'ITC (Medina et al., 2014). Comme déjà expliqué dans l'introduction, la CeA reçoit des inputs depuis la BLA (inputs excitateurs) et depuis l'ITC (inputs inhibiteurs), donc le NPS en activant les projections de l'ITC sur la CeA, induit une diminution de l'activité de la CeA modulant ainsi les voies descendantes. Ce mode d'action nous pousse à suggérer un éventuel mécanisme d'action du système relaxine-3/RXFP-3 qui induit une analgésie mécanique et thermique après injection dans la BLA. Ainsi, et vu que la BLA projette vers l'ITC, une hypothèse serait que la relaxine-3 stimule les projections de la BLA vers l'ITC, ce qui par conséquent augmentera le nombre de projections inhibitrices depuis l'ITC vers la CeA, menant donc à une diminution de l'activité de la CeA.

Un autre mécanisme d'action des neuropeptides serait de stimuler des régions cérébrales à libérer des NP dans la circulation. Ainsi les travaux d'Eliava montrent que dans un modèle de douleur inflammatoire persistant, la modulation de la douleur par l'oxytocine (OT) peut être soit due à la libération d'OT des projections au niveau de la moelle épinière et inhibant par la suite les fibres afférentes, soit par stimulation des neurones du noyau supraoptique à libérer d'OT au niveau de la circulation pour moduler les fibres C aux niveaux des ganglions de la racine dorsale (Eliava et al., 2016). Pour le système relaxine-3/RXFP-3, on sait qu'il n'existe pas de relaxine-3 au niveau de la moelle épinière (Liu et al., 2003) et on sait qu'elle est strictement exprimée dans le système nerveux, par conséquent il n'existe pas de parallélisme avec les mécanismes d'OT. Cependant, pour le système relaxine/RXFP-1, on sait que la relaxine circule dans le sang et peut se fixer sur ces récepteurs présents aux niveaux du cœur, des reins et du système reproductif, et vu qu'il existe très peu d'études sur la relaxine et RXFP-1 au niveau du système nerveux central et périphérique, une hypothèse sur le mécanisme d'action de cette dernière serait semblable à celui d'OT, avec une stimulation de la libération de relaxine au niveau périphérique.

Il est vrai que les neuropeptides représentent des nouvelles pistes thérapeutiques intrigantes en raison du nombre d'effets secondaires restreint auquel on s'attend, cependant certains obstacles tels que une durée de demi-vie courte ou l'impossibilité de traverser la barrière hémato-encéphalique ralentissent ce processus. C'est en effet le cas de l'oxytocine (OT), dont les études ont souligné son effet antinociceptif aux niveaux de différentes régions cérébrales telles que l'amygdale, PAG, raphe magnus (Poisbeau et al., 2018). Ainsi les travaux de Han et al montrent que l'injection intra-CeA d'OT induit une analgésie mécanique et thermique chez les rats, qui est annulée par l'ajout de l'antagoniste (Han and Yu, 2009), puis en 2016 les études de Cragg suggèrent que le récepteur de l'OT au niveau de la CeA médie un certain tonus analgésique dans un mode de douleur arthritique (Cragg et al., 2016). Les études de Yang montrent que les stimuli nocifs stimulent le taux d'OT au niveau du PAG et que l'injection locale d'OT au niveau du PAG augmente le seuil mécanique chez des rats naïfs (Yang et al., 2011a, 2011b). Et pour ce qui est du raphe magnus, l'injection intra-raphe d'OT induit une forte analgésie et diminue aussi le taux d'anxiété (Wang et al., 2003; Yoshida et al., 2009). Malgré les différentes études qui soulignent à plusieurs reprises l'effet antinociceptif d'OT, ainsi que le potentiel d'OT d'agir sur les comorbidités émotionnelles de la douleur chronique, l'oxytocine n'a pas été retenue en tant que piste thérapeutique éventuelle à cause de son temps de demi-vie assez court, et le manque de perméabilité à travers la barrière hémato-encéphalique (BHE). Les études de Hilfiger et al ont mis en évidence l'effet antinociceptif d'un nouvel agoniste du récepteur de l'oxytocine qui présente un temps de demi-vie significativement plus long et une meilleure perméabilité au niveau de la BHE (Hilfiger et al., 2020). Ce nouvel agoniste représente donc une nouvelle piste thérapeutique intéressante. Le même problème est observé avec les peptides de la famille relaxine qui ont un temps de demi-vie assez restreint et une faible perméabilité à travers la BHE vu leur taille. Cependant pour le système relaxine-3/RXFP-3, nos collaborateurs au Florey Institute ont développé le nouveau peptide 5 qui est plus petit que l'agoniste A2 et dont les effets sur la douleur miment ceux observés avec l'A2 (comme montré dans mes résultats). De même pour le système relaxine/RXFP-1, l'agoniste B7.33 formé uniquement d'une seule chaîne, est plus petit que H2 et mime les effets observés avec H2 (comme montré dans mes résultats). La petite taille de ces peptides permet de les présenter en tant que produits sur lesquels on peut travailler pour allonger le temps de demi-vie, dans le but d'ouvrir la voie à une stratégie de développement de médicaments pour le traitement de la douleur.

Conclusion et perspectives

Vingt pourcent de la population souffrent de douleur chronique ; douleur souvent accompagnée de comorbidités émotionnelles telles que l'anxiété et la dépression (Treede et al., 2015). Les études de Ryan et Zhang montrent que la relaxine-3 a un effet anxiolytique (Ryan et al., 2013; Zhang et al., 2015), d'autre part les neuropeptides sont de plus en plus envisagés en étant que pistes thérapeutiques prometteuses (Hoyer and Bartfai, 2012), par conséquent l'objectif de cette thèse est d'étudier l'effet des systèmes relaxine-3/RXFP-3 et relaxine/RXFP-1 sur la modulation douleur. Nos résultats mettent en évidence la modulation de la douleur par les 2 systèmes. Ainsi les injections locales des agonistes de RXFP-3 dans la BLA et dans l'ACC induisent des analgésies mécaniques et thermiques dans un modèle de douleur inflammatoire persistante, il serait donc pertinent d'étudier l'effet des injections locales des analogues de relaxine-3 aux niveaux d'autres régions spécifiques telles que la CeA, vu que nos résultats ont montré une forte concentration d'ARNm de *Rxfp-3* au niveau de la CeA. De plus nos résultats ont souligné un éventuel rôle analgésique de la relaxine-3 endogène au niveau de l'ACC, puisque l'injection de l'antagoniste de RXFP-3 au niveau de l'ACC a induit une proalgie chez les souris NaCl, par conséquent il serait intéressant d'envisager d'étudier l'effet endogène de la relaxine-3 en ayant recours à des souris KO relaxine-3, sur lesquelles seront performées les tests de comportement douloureux. De plus nos résultats suggèrent que l'effet de la relaxine-3 sur la douleur passe par la BLA et/ou ACC et qui serait dû à la modulation des voies descendantes, il serait donc intéressant de confirmer l'implication des voies descendantes en ayant recours à l'électrophysiologie « single unit » au niveau de la moelle épinière. Le travail de cette thèse a permis de mettre la lumière sur un éventuel circuit impliqué dans le contrôle de la douleur, il serait donc pertinent d'explorer en profondeur ce circuit en ayant recours à l'optogénétique qui permettra d'avoir un regard plus fonctionnel sur ce circuit.

Pour ce qui est de la modulation de la douleur par le système relaxine/RXFP-1, nos résultats montrent une analgésie mécanique et thermique suite à l'injection ICV des agonistes de RXFP-1. Vu que l'injection ICV induit un effet global, l'étape suivante serait d'étudier l'effet des injections locales de relaxine sur la douleur, comme ce que nous avons fait dans cette étude pour la relaxine-3. Nos résultats montrent qu'il existe des régions cérébrales riches en RXFP-1 et fibres relaxine,

telles que l'ACC et le CLA, par conséquent il serait pertinent de commencer les injections locales dans ces régions.

Finalement, nos résultats suggèrent qu'il existe des voies de signalisation et des régions cérébrales distinctes pour le traitement de la douleur mécanique et la douleur thermique, ce qui peut constituer une piste à investiguer, d'autant plus que les données déjà présentes dans la littérature s'attardent plutôt sur les différences aux niveaux spinal et périphérique.

En conclusion, le but de la médecine et des sciences paramédicales étant toujours celui de promouvoir le meilleur intérêt de l'être humain et de limiter sa souffrance, les données présentées dans le travail ci-dessus peuvent constituer une matrice de travaux de recherches sur des pistes thérapeutiques éventuelles.

Références:

- Abboud, C., Duveau, A., Bouali-Benazzouz, R., Massé, K., Mattar, J., Brochoire, L., Fossat, P., Boué-Grabot, E., Hleihel, W., and Landry, M. (2020). Animal models of pain: Diversity and benefits. *J Neurosci Methods* 108997.
- Albert-Gascó, H., García-Avilés, Á., Moustafa, S., Sánchez-Sarasua, S., Gundlach, A.L., Olucha-Bordonau, F.E., and Sánchez-Pérez, A.M. (2017). Central relaxin-3 receptor (RXFP3) activation increases ERK phosphorylation in septal cholinergic neurons and impairs spatial working memory. *Brain Struct Funct* 222, 449–463.
- Albert-Gascó, H., Ma, S., Ros-Bernal, F., Sánchez-Pérez, A.M., Gundlach, A.L., and Olucha-Bordonau, F.E. (2018). GABAergic Neurons in the Rat Medial Septal Complex Express Relaxin-3 Receptor (RXFP3) mRNA. *Front Neuroanat* 11.
- Albert-Gasco, H., Sanchez-Sarasua, S., Ma, S., García-Díaz, C., Gundlach, A.L., Sanchez-Perez, A.M., and Olucha-Bordonau, F.E. (2019). Central relaxin-3 receptor (RXFP3) activation impairs social recognition and modulates ERK-phosphorylation in specific GABAergic amygdala neurons. *Brain Struct Funct* 224, 453–469.
- Apps, R., and Strata, P. (2015). Neuronal circuits for fear and anxiety - the missing link. *Nat. Rev. Neurosci.* 16, 642.
- Bair, M.J., Robinson, R.L., Katon, W., and Kroenke, K. (2003). Depression and pain comorbidity: a literature review. *Arch. Intern. Med.* 163, 2433–2445.
- Banerjee, A., Shen, P.-J., Ma, S., Bathgate, R.A.D., and Gundlach, A.L. (2010). Swim stress excitation of nucleus incertus and rapid induction of relaxin-3 expression via CRF1 activation. *Neuropharmacology* 58, 145–155.
- Basbaum, A.I., Bautista, D.M., Scherrer, G., and Julius, D. (2009). Cellular and molecular mechanisms of pain. *Cell* 139, 267–284.
- Bathgate, R. a. D., Halls, M.L., van der Westhuizen, E.T., Callander, G.E., Kocan, M., and Summers, R.J. (2013). Relaxin family peptides and their receptors. *Physiol. Rev.* 93, 405–480.
- Bathgate, R.A., Ivell, R., Sanborn, B.M., Sherwood, O.D., and Summers, R.J. (2006a). International Union of Pharmacology LVII: recommendations for the nomenclature of receptors for relaxin family peptides. *Pharmacological Reviews* 58, 7–31.
- Bathgate, R.A.D., Samuel, C.S., Burazin, T.C.D., Layfield, S., Claasz, A.A., Reytomas, I.G.T., Dawson, N.F., Zhao, C., Bond, C., Summers, R.J., et al. (2002). Human relaxin gene 3 (H3) and the equivalent mouse relaxin (M3) gene. Novel members of the relaxin peptide family. *The Journal of Biological Chemistry* 277, 1148–1157.

Bathgate, R.A.D., Hsueh, A.J.W., and Sherwood, O.D. (2006b). CHAPTER 16 - Physiology and Molecular Biology of the Relaxin Peptide Family. In Knobil and Neill's Physiology of Reproduction (Third Edition), J.D. Neill, ed. (St Louis: Academic Press), pp. 679–768.

Bathgate, R.A.D., Kocan, M., Scott, D.J., Hossain, M.A., Good, S.V., Yegorov, S., Bogerd, J., and Gooley, P.R. (2018). The relaxin receptor as a therapeutic target - perspectives from evolution and drug targeting. *Pharmacol. Ther.* *187*, 114–132.

Becerra, L., Breiter, H.C., Wise, R., Gonzalez, R.G., and Borsook, D. (2001). Reward circuitry activation by noxious thermal stimuli. *Neuron* *32*, 927–946.

Beitz, A.J. (1982). The sites of origin brain stem neurotensin and serotonin projections to the rodent nucleus raphe magnus. *J. Neurosci.* *2*, 829–842.

Bernard, J.F., and Besson, J.M. (1990). The spino(trigemino)pontoamygdaloid pathway: electrophysiological evidence for an involvement in pain processes. *Journal of Neurophysiology* *63*, 473–490.

Binder, C., Hagemann, T., Husen, B., Schulz, M., and Einspanier, A. (2002). Relaxin enhances in-vitro invasiveness of breast cancer cell lines by up-regulation of matrix metalloproteases. *Mol Hum Reprod* *8*, 789–796.

Bliss, T.V.P., Collingridge, G.L., Kaang, B.-K., and Zhuo, M. (2016). Synaptic plasticity in the anterior cingulate cortex in acute and chronic pain. *Nat. Rev. Neurosci.* *17*, 485–496.

Bokiniec, P., Zampieri, N., Lewin, G.R., and Poulet, J.F. (2018). The neural circuits of thermal perception. *Curr Opin Neurobiol* *52*, 98–106.

Bornhövd, K., Quante, M., Glauche, V., Bromm, B., Weiller, C., and Büchel, C. (2002). Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. *Brain* *125*, 1326–1336.

Bouhassira, D., Lantéri-Minet, M., Attal, N., Laurent, B., and Touboul, C. (2008). Prevalence of chronic pain with neuropathic characteristics in the general population. *Pain* *136*, 380–387.

Bourgeois, L., Gauriau, C., and Bernard, J.-F. (2001). Projections from the nociceptive area of the central nucleus of the amygdala to the forebrain: a PHA-L study in the rat. *European Journal of Neuroscience* *14*, 229–255.

Bourne, S., Machado, A.G., and Nagel, S.J. (2014). Basic Anatomy and Physiology of Pain Pathways. *Neurosurgery Clinics of North America* *25*, 629–638.

Bragin, E.O., Yeliseeva, Z.V., Vasilenko, G.F., Meizerov, E.E., Chuvin, B.T., and Durinyan, R.A. (1984). Cortical projections to the periaqueductal grey in the cat: a retrograde horseradish peroxidase study. *Neurosci. Lett.* *51*, 271–275.

- Breivik, H., Collett, B., Ventafridda, V., Cohen, R., and Gallacher, D. (2006). Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain* *10*, 287–333.
- Brennand, J.E., Calder, A.A., Leitch, C.R., Greer, I.A., Chou, M.M., and MacKenzie, I.Z. (1997). Recombinant human relaxin as a cervical ripening agent. *Br J Obstet Gynaecol* *104*, 775–780.
- Brodin, E., Ernberg, M., and Olgart, L. (2016). Neurobiology: General considerations—from acute to chronic pain. *Nor. Tannlegeforen. Tid* *126*, 28–33.
- Burma, N.E., Leduc-Pessah, H., Fan, C.Y., and Trang, T. (2017). Animal models of chronic pain: Advances and challenges for clinical translation. *J. Neurosci. Res.* *95*, 1242–1256.
- Callander, G.E., and Bathgate, R. a. D. (2010). Relaxin family peptide systems and the central nervous system. *Cell. Mol. Life Sci.* *67*, 2327–2341.
- Chan, L.J., Hossain, M.A., Samuel, C.S., Separovic, F., and Wade, J.D. (2011). The relaxin peptide family—structure, function and clinical applications. *Protein Pept. Lett.* *18*, 220–229.
- Chen, J., Kuei, C., Sutton, S.W., Bonaventure, P., Nepomuceno, D., Eriste, E., Sillard, R., Lovenberg, T.W., and Liu, C. (2005). Pharmacological characterization of relaxin-3/INSL7 receptors GPCR135 and GPCR142 from different mammalian species. *The Journal of Pharmacology and Experimental Therapeutics* *312*, 83–95.
- Chen, T., Koga, K., Descalzi, G., Qiu, S., Wang, J., Zhang, L.-S., Zhang, Z.-J., He, X.-B., Qin, X., Xu, F.-Q., et al. (2014). Postsynaptic potentiation of corticospinal projecting neurons in the anterior cingulate cortex after nerve injury. *Mol Pain* *10*, 33.
- Chen, T., Taniguchi, W., Chen, Q.-Y., Tozaki-Saitoh, H., Song, Q., Liu, R.-H., Koga, K., Matsuda, T., Kaito-Sugimura, Y., Wang, J., et al. (2018). Top-down descending facilitation of spinal sensory excitatory transmission from the anterior cingulate cortex. *Nat Commun* *9*, 1886.
- Chen, T.-Y., Li, X., Hung, C.-H., Bahudhanapati, H., Tan, J., Kass, D.J., and Zhang, Y. (2020). The relaxin family peptide receptor 1 (RXFP1): An emerging player in human health and disease. *Mol Genet Genomic Med* *8*, e1194.
- Cheng, L., Duan, B., Huang, T., Zhang, Y., Chen, Y., Britz, O., Garcia-Campmany, L., Ren, X., Vong, L., Lowell, B.B., et al. (2017). Identification of spinal circuits involved in touch-evoked dynamic mechanical pain. *Nat Neurosci* *20*, 804–814.
- Ciaramella, A. (2019). Psychopharmacology of chronic pain. *Handb Clin Neurol* *165*, 317–337.
- Costigan, M., Scholz, J., and Woolf, C.J. (2009). Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu. Rev. Neurosci.* *32*, 1–32.
- Cragg, B., Ji, G., and Neugebauer, V. (2016). Differential contributions of vasopressin V1A and oxytocin receptors in the amygdala to pain-related behaviors in rats. *Mol Pain* *12*.

- Crofford, L.J. (2015). Chronic Pain: Where the Body Meets the Brain. *Trans. Am. Clin. Climatol. Assoc.* *126*, 167–183.
- Davis, K.D., Taylor, S.J., Crawley, A.P., Wood, M.L., and Mikulis, D.J. (1997). Functional MRI of pain- and attention-related activations in the human cingulate cortex. *J. Neurophysiol.* *77*, 3370–3380.
- Deuis, J.R., Dvorakova, L.S., and Vetter, I. (2017). Methods Used to Evaluate Pain Behaviors in Rodents. *Front Mol Neurosci* *10*.
- Diepenhorst, N.A., Petrie, E.J., Chen, C.Z., Wang, A., Hossain, M.A., Bathgate, R.A.D., and Gooley, P.R. (2014). Investigation of Interactions at the Extracellular Loops of the Relaxin Family Peptide Receptor 1 (RXFP1). *J Biol Chem* *289*, 34938–34952.
- Dschietzig, T., Bartsch, C., Wessler, S., Baumann, G., and Stangl, K. (2009a). Autoregulation of human relaxin-2 gene expression critically involves relaxin and glucocorticoid receptor binding to glucocorticoid response half-sites in the relaxin-2 promoter. *Regul Pept* *155*, 163–173.
- Dschietzig, T., Bartsch, C., Baumann, G., and Stangl, K. (2009b). RXFP1-inactive relaxin activates human glucocorticoid receptor: further investigations into the relaxin-GR pathway. *Regul Pept* *154*, 77–84.
- Duan, B., Cheng, L., Bourane, S., Britz, O., Padilla, C., Garcia-Campmany, L., Krashes, M., Knowlton, W., Velasquez, T., Ren, X., et al. (2014). Identification of spinal circuits transmitting and gating mechanical pain. *Cell* *159*, 1417–1432.
- Dubin, A.E., and Patapoutian, A. (2010). Nociceptors: the sensors of the pain pathway. *J. Clin. Invest.* *120*, 3760–3772.
- Dum, R.P., Levinthal, D.J., and Strick, P.L. (2009). The Spinothalamic System Targets Motor and Sensory Areas in the Cerebral Cortex of Monkeys. *J. Neurosci.* *29*, 14223–14235.
- Eliava, M., Melchior, M., Knobloch-Bollmann, H.S., Wahis, J., da Silva Gouveia, M., Tang, Y., Ciobanu, A.C., Triana Del Rio, R., Roth, L.C., Althammer, F., et al. (2016). A New Population of Parvocellular Oxytocin Neurons Controlling Magnocellular Neuron Activity and Inflammatory Pain Processing. *Neuron* *89*, 1291–1304.
- Ellison, D.L. (2017). Physiology of Pain. *Crit Care Nurs Clin North Am* *29*, 397–406.
- Eto, K., Wake, H., Watanabe, M., Ishibashi, H., Noda, M., Yanagawa, Y., and Nabekura, J. (2011). Inter-regional Contribution of Enhanced Activity of the Primary Somatosensory Cortex to the Anterior Cingulate Cortex Accelerates Chronic Pain Behavior. *J. Neurosci.* *31*, 7631–7636.
- Evans, B.A., John, M., Fowler, K.J., Summers, R.J., Cronk, M., Shine, J., and Tregear, G.W. (1993). The mouse relaxin gene: nucleotide sequence and expression. *Journal of Molecular Endocrinology* *10*, 15–23.

- Feng, S., Agoulnik, I.U., Truong, A., Li, Z., Creighton, C.J., Kaftanovskaya, E.M., Pereira, R., Han, H.D., Lopez-Berestein, G., Klonisch, T., et al. (2010). Suppression of relaxin receptor RXFP1 decreases prostate cancer growth and metastasis. *Endocr Relat Cancer* *17*, 1021–1033.
- Filingeri, D. (2016). Neurophysiology of Skin Thermal Sensations. *Compr Physiol* *6*, 1429.
- Ford, B., Holmes, C.J., Mainville, L., and Jones, B.E. (1995). GABAergic neurons in the rat pontomesencephalic tegmentum: codistribution with cholinergic and other tegmental neurons projecting to the posterior lateral hypothalamus. *The Journal of Comparative Neurology* *363*, 177–196.
- Ganella, D.E., Callander, G.E., Ma, S., Bye, C.R., Gundlach, A.L., and Bathgate, R. a. D. (2013). Modulation of feeding by chronic rAAV expression of a relaxin-3 peptide agonist in rat hypothalamus. *Gene Ther.* *20*, 703–716.
- Gao, Y.-J., Ren, W.-H., Zhang, Y.-Q., and Zhao, Z.-Q. (2004). Contributions of the anterior cingulate cortex and amygdala to pain- and fear-conditioned place avoidance in rats. *PAIN* *110*, 343–353.
- Goll, Y., Atlan, G., and Citri, A. (2015). Attention: the claustrum. *Trends Neurosci* *38*, 486–495.
- Goto, M., Swanson, L.W., and Canteras, N.S. (2001). Connections of the nucleus incertus. *The Journal of Comparative Neurology* *438*, 86–122.
- Gunnarsen, J.M., Crawford, R.J., and Tregear, G.W. (1995). Expression of the relaxin gene in rat tissues. *Mol Cell Endocrinol* *110*, 55–64.
- Halls, M.L., and Cooper, D.M.F. (2010). Sub-picomolar relaxin signalling by a pre-assembled RXFP1, AKAP79, AC2, β -arrestin 2, PDE4D3 complex. *The EMBO Journal* *29*, 2772.
- Halls, M.L., Bathgate, R.A.D., and Summers, R.J. (2006). Relaxin family peptide receptors RXFP1 and RXFP2 modulate cAMP signaling by distinct mechanisms. *Mol. Pharmacol.* *70*, 214–226.
- Halls, M.L., van der Westhuizen, E.T., Bathgate, R. a. D., and Summers, R.J. (2007). Relaxin family peptide receptors--former orphans reunite with their parent ligands to activate multiple signalling pathways. *Br. J. Pharmacol.* *150*, 677–691.
- Halls, M.L., Hewitson, T.D., Moore, X.-L., Du, X.-J., Bathgate, R.A.D., and Summers, R.J. (2009). Relaxin activates multiple cAMP signaling pathway profiles in different target cells. *Ann N Y Acad Sci* *1160*, 108–111.
- Han, Y., and Yu, L.-C. (2009). Involvement of oxytocin and its receptor in nociceptive modulation in the central nucleus of amygdala of rats. *Neurosci Lett* *454*, 101–104.

- Han, L., Luo, J., Bai, S., Jia, Y., Chen, X., Zhao, Y., Chen, L., Zhu, X., Li, Y., Jiang, Y., et al. (2017). Combined Assessment of Relaxin and B-Type Natriuretic Peptide Improves Diagnostic Value in Patients With Congestive Heart Failure. *Am. J. Med. Sci.* *354*, 480–485.
- Han, S., Soleiman, M.T., Soden, M.E., Zweifel, L.S., and Palmiter, R.D. (2015). Elucidating an Affective Pain Circuit that Creates a Threat Memory. *Cell* *162*, 363–374.
- Haugaard-Kedström, L.M., Shabanpoor, F., Hossain, M.A., Clark, R.J., Ryan, P.J., Craik, D.J., Gundlach, A.L., Wade, J.D., Bathgate, R.A.D., and Rosengren, K.J. (2011). Design, synthesis, and characterization of a single-chain peptide antagonist for the relaxin-3 receptor RXFP3. *J. Am. Chem. Soc.* *133*, 4965–4974.
- Haugaard-Kedström, L.M., Lee, H.S., Jones, M.V., Song, A., Rathod, V., Hossain, M.A., Bathgate, R.A.D., and Rosengren, K.J. (2018). Binding conformation and determinants of a single-chain peptide antagonist at the relaxin-3 receptor RXFP3. *J Biol Chem* *293*, 15765–15776.
- van Hecke, O., Torrance, N., and Smith, B.H. (2013). Chronic pain epidemiology and its clinical relevance. *Br J Anaesth* *111*, 13–18.
- Heinricher, M.M., Tavares, I., Leith, J.L., and Lumb, B.M. (2009). Descending control of nociception: Specificity, recruitment and plasticity. *Brain Res Rev* *60*, 214–225.
- Hilfiger, L., Zhao, Q., Kerspern, D., Inquimbert, P., Andry, V., Goumon, Y., Darbon, P., Hibert, M., and Charlet, A. (2020). A Nonpeptide Oxytocin Receptor Agonist for a Durable Relief of Inflammatory Pain. *Sci Rep* *10*, 3017.
- Hisaw, F.L. (1926). Experimental relaxation of the pubic ligament of the guinea pig. *Proceedings of the Society for Experimental Biology and Medicine* *23*, 661–663.
- Hoare, B.L., Bruell, S., Sethi, A., Gooley, P.R., Lew, M.J., Hossain, M.A., Inoue, A., Scott, D.J., and Bathgate, R.A.D. (2018). Multi-Component Mechanism of H2 Relaxin Binding to RXFP1 through NanoBRET Kinetic Analysis. *IScience* *11*, 93–113.
- Hocher, B., Ziebig, R., Krause, R., Asmus, G., Neumayer, H.-H., Liefeldt, L., and Stasch, J.-P. (2004). Relaxin is an independent risk factor predicting death in male patients with end-stage kidney disease. *Circulation* *109*, 2266–2268.
- Hojo, K., Hossain, M.A., Tailhades, J., Shabanpoor, F., Wong, L.L.L., Ong-Pålsson, E.E.K., Kastman, H.E., Ma, S., Gundlach, A.L., Rosengren, K.J., et al. (2016). Development of a Single-Chain Peptide Agonist of the Relaxin-3 Receptor Using Hydrocarbon Stapling. *J Med Chem* *59*, 7445–7456.
- Hökfelt, T., Barde, S., Xu, Z.-Q.D., Kuteeva, E., Rüegg, J., Le Maitre, E., Risling, M., Kehr, J., Innatko, R., Theodorsson, E., et al. (2018). Neuropeptide and Small Transmitter Coexistence: Fundamental Studies and Relevance to Mental Illness. *Front Neural Circuits* *12*, 106.

- Hopkins, E.J., Bathgate, R.A., and Gooley, P.R. (2005). The Human LGR7 Low-Density Lipoprotein Class A Module Requires Calcium for Structure. *Annals of the New York Academy of Sciences* 1041, 27–34.
- Hopkins, E.J., Layfield, S., Ferraro, T., Bathgate, R.A.D., and Gooley, P.R. (2007). The NMR solution structure of the relaxin (RXFP1) receptor lipoprotein receptor class A module and identification of key residues in the N-terminal region of the module that mediate receptor activation. *J Biol Chem* 282, 4172–4184.
- Hossain, M.A., Samuel, C.S., Binder, C., Hewitson, T.D., Tregear, G.W., Wade, J.D., and Bathgate, R.A.D. (2010). The chemically synthesized human relaxin-2 analog, B-R13/17K H2, is an RXFP1 antagonist. *Amino Acids* 39, 409–416.
- Hossain, M.A., Kocan, M., Yao, S.T., Royce, S.G., Nair, V.B., Siwek, C., Patil, N.A., Harrison, I.P., Rosengren, K.J., Selemidis, S., et al. (2016). A single-chain derivative of the relaxin hormone is a functionally selective agonist of the G protein-coupled receptor, RXFP1. *Chem Sci* 7, 3805–3819.
- Hoyer, D., and Bartfai, T. (2012). Neuropeptides and neuropeptide receptors: drug targets, and peptide and non-peptide ligands: a tribute to Prof. Dieter Seebach. *Chem. Biodivers.* 9, 2367–2387.
- Hsu, S.Y.T. (2003). New insights into the evolution of the relaxin-LGR signaling system. *Trends in Endocrinology and Metabolism: TEM* 14, 303–309.
- Hsu, S.Y., Nakabayashi, K., Nishi, S., Kumagai, J., Kudo, M., Sherwood, O.D., and Hsueh, A.J.W. (2002). Activation of orphan receptors by the hormone relaxin. *Science* 295, 671–674.
- Huang, J., Gadotti, V.M., Chen, L., Souza, I.A., Huang, S., Wang, D., Ramakrishnan, C., Deisseroth, K., Zhang, Z., and Zamponi, G.W. (2019). A neuronal circuit for activating descending modulation of neuropathic pain. *Nature Neuroscience* 22, 1659–1668.
- Hudson, P., John, M., Crawford, R., Haralambidis, J., Scanlon, D., Gorman, J., Tregear, G., Shine, J., and Niall, H. (1984). Relaxin gene expression in human ovaries and the predicted structure of a human preprorelaxin by analysis of cDNA clones. *The EMBO Journal* 3, 2333–2339.
- Janak, P.H., and Tye, K.M. (2015). From circuits to behaviour in the amygdala. *Nature* 517, 284–292.
- John, M.J., Borjesson, B.W., Walsh, J.R., and Niall, H.D. (1981). Limited sequence homology between porcine and rat relaxins: implications for physiological studies. *Endocrinology* 108, 726–729.
- Johnson, M.R., Okokon, E., Collins, W.P., Sharma, V., and Lightman, S.L. (1991). The Effect of Human Chorionic Gonadotropin and Pregnancy on the Circulating Level of Relaxin. *J Clin Endocrinol Metab* 72, 1042–1047.

- Jongen-Rêlo, A.L., and Amaral, D.G. (1998). Evidence for a GABAergic projection from the central nucleus of the amygdala to the brainstem of the macaque monkey: a combined retrograde tracing and in situ hybridization study. *Eur. J. Neurosci.* *10*, 2924–2933.
- Julius, D., and Basbaum, A.I. (2001). Molecular mechanisms of nociception. *Nature* *413*, 203–210.
- Kee, T., Sanda, P., Gupta, N., Stopfer, M., and Bazhenov, M. (2015). Feed-Forward versus Feedback Inhibition in a Basic Olfactory Circuit. *PLoS Comput Biol* *11*.
- Kleczkowska, P., and Lipkowski, A.W. (2013). Neurotensin and neurotensin receptors: characteristic, structure-activity relationship and pain modulation--a review. *Eur J Pharmacol* *716*, 54–60.
- Koga, K., Li, X., Chen, T., Steenland, H.W., Descalzi, G., and Zhuo, M. (2010). In vivo whole-cell patch-clamp recording of sensory synaptic responses of cingulate pyramidal neurons to noxious mechanical stimuli in adult mice. *Mol Pain* *6*, 62.
- Kong, R.C.K., Shilling, P.J., Lobb, D.K., Gooley, P.R., and Bathgate, R.A.D. (2010). Membrane receptors: structure and function of the relaxin family peptide receptors. *Mol. Cell. Endocrinol.* *320*, 1–15.
- Kubota, Y., Hattori, R., and Yui, Y. (1994). Three distinct subpopulations of GABAergic neurons in rat frontal agranular cortex. *Brain Research* *649*, 159–173.
- Kumar, J.R., Rajkumar, R., Jayakody, T., Marwari, S., Hong, J.M., Ma, S., Gundlach, A.L., Lai, M.K.P., and Dawe, G.S. (2017). Relaxin' the brain: a case for targeting the nucleus incertus network and relaxin-3/RXFP3 system in neuropsychiatric disorders. *Br. J. Pharmacol.* *174*, 1061–1076.
- Kwon, M., Altin, M., Duenas, H., and Alev, L. (2014). The role of descending inhibitory pathways on chronic pain modulation and clinical implications. *Pain Pract* *14*, 656–667.
- Lam, M., Royce, S.G., Samuel, C.S., and Bourke, J.E. (2018). Serelaxin as a novel therapeutic opposing fibrosis and contraction in lung diseases. *Pharmacol Ther* *187*, 61–70.
- Latremoliere, A., and Woolf, C.J. (2009). Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. *The Journal of Pain: Official Journal of the American Pain Society* *10*, 895.
- LeDoux, J.E. (2000). Emotion circuits in the brain. *Annu. Rev. Neurosci.* *23*, 155–184.
- Lee, J.H., Koh, S.Q., Guadagna, S., Francis, P.T., Esiri, M.M., Chen, C.P., Wong, P.T.-H., Dawe, G.S., and Lai, M.K.P. (2016). Altered relaxin family receptors RXFP1 and RXFP3 in the neocortex of depressed Alzheimer's disease patients. *Psychopharmacology (Berl.)* *233*, 591–598.

- Leon-Cabrera, S., Solís-Lozano, L., Suárez-Álvarez, K., González-Chávez, A., Béjar, Y.L., Robles-Díaz, G., and Escobedo, G. (2013). Hyperleptinemia is associated with parameters of low-grade systemic inflammation and metabolic dysfunction in obese human beings. *Front Integr Neurosci* 7.
- Li, S.-Y., Huo, M.-L., Wu, X.-Y., Huang, Y.-Q., Wang, L., Zhang, X., Jiang, Y.-M., Zhang, M.-L., Wang, L.-L., and Yu, L.-C. (2017). Involvement of galanin and galanin receptor 1 in nociceptive modulation in the central nucleus of amygdala in normal and neuropathic rats. *Scientific Reports* 7, 15317.
- Liu, C., Chen, J., Sutton, S., Roland, B., Kuei, C., Farmer, N., Sillard, R., and Lovenberg, T.W. (2003). Identification of relaxin-3/INSL7 as a ligand for GPCR142. *The Journal of Biological Chemistry* 278, 50765–50770.
- Lu, W.-Y., Xiong, Z.-G., Lei, S., Orser, B.A., Dudek, E., Browning, M.D., and MacDonald, J.F. (1999). G-protein-coupled receptors act via protein kinase C and Src to regulate NMDA receptors. *Nature Neuroscience* 2, 331–338.
- Ma, S., and Gundlach, A.L. (2007). Relaxin-family peptide and receptor systems in brain: insights from recent anatomical and functional studies. *Adv. Exp. Med. Biol.* 612, 119–137.
- Ma, S., and Gundlach, A.L. (2015). Ascending control of arousal and motivation: role of nucleus incertus and its peptide neuromodulators in behavioural responses to stress. *J. Neuroendocrinol.* 27, 457–467.
- Ma, W., and Peschanski, M. (1988). Spinal and trigeminal projections to the parabrachial nucleus in the rat: electron-microscopic evidence of a spino-ponto-amygdalian somatosensory pathway. *Somatosens Res* 5, 247–257.
- Ma, S., Roozendaal, B., Burazin, T.C.D., Tregear, G.W., McGaugh, J.L., and Gundlach, A.L. (2005). Relaxin receptor activation in the basolateral amygdala impairs memory consolidation. *Eur. J. Neurosci.* 22, 2117–2122.
- Ma, S., Shen, P.-J., Burazin, T.C.D., Tregear, G.W., and Gundlach, A.L. (2006). Comparative localization of leucine-rich repeat-containing G-protein-coupled receptor-7 (RXFP1) mRNA and [33P]-relaxin binding sites in rat brain: restricted somatic co-expression a clue to relaxin action? *Neuroscience* 141, 329–344.
- Ma, S., Bonaventure, P., Ferraro, T., Shen, P.-J., Burazin, T.C.D., Bathgate, R. a. D., Liu, C., Tregear, G.W., Sutton, S.W., and Gundlach, A.L. (2007). Relaxin-3 in GABA projection neurons of nucleus incertus suggests widespread influence on forebrain circuits via G-protein-coupled receptor-135 in the rat. *Neuroscience* 144, 165–190.
- Ma, S., Olucha-Bordonau, F.E., Hossain, M.A., Lin, F., Kuei, C., Liu, C., Wade, J.D., Sutton, S.W., Nuñez, A., and Gundlach, A.L. (2009). Modulation of hippocampal theta oscillations and spatial memory by relaxin-3 neurons of the nucleus incertus. *Learn. Mem.* 16, 730–742.

- Ma, S., Smith, C.M., Blasiak, A., and Gundlach, A.L. (2017). Distribution, physiology and pharmacology of relaxin-3/RXFP3 systems in brain. *British Journal of Pharmacology* *174*, 1034–1048.
- MacLennan, A.H., Green, R.C., Grant, P., and Nicolson, R. (1986). Ripening of the human cervix and induction of labor with intracervical purified porcine relaxin. *Obstet Gynecol* *68*, 598–601.
- Mantyh, P.W. (1983). Connections of midbrain periaqueductal gray in the monkey. II. Descending efferent projections. *J. Neurophysiol.* *49*, 582–594.
- Matsumoto, M., Kamohara, M., Sugimoto, T., Hidaka, K., Takasaki, J., Saito, T., Okada, M., Yamaguchi, T., and Furuichi, K. (2000). The novel G-protein coupled receptor SALPR shares sequence similarity with somatostatin and angiotensin receptors. *Gene* *248*, 183–189.
- McDonald, G.A., Sarkar, P., Rennke, H., Unemori, E., Kalluri, R., and Sukhatme, V.P. (2003). Relaxin increases ubiquitin-dependent degradation of fibronectin in vitro and ameliorates renal fibrosis in vivo. *American Journal of Physiology - Renal Physiology* *285*, F59–F67.
- McGowan, B.M., Stanley, S.A., Smith, K.L., Minnion, J.S., Donovan, J., Thompson, E.L., Patterson, M., Connolly, M.M., Abbott, C.R., Small, C.J., et al. (2006). Effects of acute and chronic relaxin-3 on food intake and energy expenditure in rats.
- McGowan, B.M., Minnion, J.S., Murphy, K.G., Roy, D., Stanley, S.A., Dhillo, W.S., Gardiner, J.V., Ghatei, M.A., and Bloom, S.R. (2014). Relaxin-3 stimulates the neuro-endocrine stress axis via corticotrophin-releasing hormone. *The Journal of Endocrinology* *221*, 337–346.
- McGowan, B.M.C., Stanley, S.A., Smith, K.L., White, N.E., Connolly, M.M., Thompson, E.L., Gardiner, J.V., Murphy, K.G., Ghatei, M.A., and Bloom, S.R. (2005). Central relaxin-3 administration causes hyperphagia in male Wistar rats. *Endocrinology* *146*, 3295–3300.
- McMahon, S.B., and Bevan, S. (2005). Inflammatory mediators and modulators of pain. *Wall and Melzack's Textbook of Pain E-Dition* 49–72.
- Medalla, M., and Barbas, H. (2012). The Anterior Cingulate Cortex May Enhance Inhibition of Lateral Prefrontal Cortex Via m2 Cholinergic Receptors at Dual Synaptic Sites. *J Neurosci* *32*, 15611–15625.
- Medina, G., Ji, G., Grégoire, S., and Neugebauer, V. (2014). Nasal application of neuropeptide S inhibits arthritis pain-related behaviors through an action in the amygdala. *Mol Pain* *10*, 32.
- Melzack, R., and Wall, P.D. (1965). Pain mechanisms: a new theory. *Science* *150*, 971–979.
- Millan, M.J. (2002). Descending control of pain. *Prog. Neurobiol.* *66*, 355–474.
- Moehring, F., Halder, P., Seal, R.P., and Stucky, C.L. (2018). Uncovering the Cells and Circuits of Touch in Normal and Pathological Settings. *Neuron* *100*, 349–360.

- Nakamura, H., Katayama, Y., and Kawakami, Y. (2010). Hippocampal CA1/subiculum-prefrontal cortical pathways induce plastic changes of nociceptive responses in cingulate and prelimbic areas. *BMC Neurosci* *11*, 100.
- Neugebauer, V. (2015). 15. Amygdala pain mechanisms. *Handb Exp Pharmacol* *227*, 261–284.
- Neugebauer, V., and Li, W. (2002). Processing of nociceptive mechanical and thermal information in central amygdala neurons with knee-joint input. *J. Neurophysiol.* *87*, 103–112.
- Neugebauer, V., Li, W., Bird, G.C., and Han, J.S. (2004). The Amygdala and Persistent Pain. *Neuroscientist* *10*, 221–234.
- Neugebauer, V., Galhardo, V., Maione, S., and Mackey, S.C. (2009). Forebrain Pain Mechanisms. *Brain Res Rev* *60*, 226–242.
- Nistri, S., and Bani, D. (2003). Relaxin receptors and nitric oxide synthases: search for the missing link. *Reproductive Biology and Endocrinology* *1*, 5.
- O'Connor, W.B., Cain, G.D., and Zarrow, M.X. (1966). Elongation of the interpubic ligament in the little brown bat (*Myotis lucifugus*). *Proc Soc Exp Biol Med* *123*, 935–937.
- Olucha-Bordonau, F.E., Teruel, V., Barcia-González, J., Ruiz-Torner, A., Valverde-Navarro, A.A., and Martínez-Soriano, F. (2003). Cytoarchitecture and efferent projections of the nucleus incertus of the rat. *The Journal of Comparative Neurology* *464*, 62–97.
- Olucha-Bordonau, F.E., Albert-Gascó, H., Ros-Bernal, F., Rytova, V., Ong-Pålsson, E.K.E., Ma, S., Sánchez-Pérez, A.M., and Gundlach, A.L. (2018). Modulation of forebrain function by nucleus incertus and relaxin-3/RXFP3 signaling. *CNS Neurosci Ther* *24*, 694–702.
- Omi, E.C., Zhao, S., Shanks, R.D., and Sherwood, O.D. (1997). Evidence that systemic relaxin promotes moderate water consumption during late pregnancy in rats. *J Endocrinol* *153*, 33–40.
- Orr, P.M., Shank, B.C., and Black, A.C. (2017). The Role of Pain Classification Systems in Pain Management. *Critical Care Nursing Clinics of North America* *29*, 407–418.
- Osheroff, P.L., and Ho, W.H. (1993). Expression of relaxin mRNA and relaxin receptors in postnatal and adult rat brains and hearts. Localization and developmental patterns. *J. Biol. Chem.* *268*, 15193–15199.
- Osheroff, P.L., and Phillips, H.S. (1991). Autoradiographic localization of relaxin binding sites in rat brain. *Proc. Natl. Acad. Sci. U.S.A.* *88*, 6413–6417.
- Ossipov, M.H., Dussor, G.O., and Porreca, F. (2010). Central modulation of pain. *J. Clin. Invest.* *120*, 3779–3787.
- Ossipov, M.H., Morimura, K., and Porreca, F. (2014). Descending pain modulation and chronification of pain. *Curr Opin Support Palliat Care* *8*, 143–151.

Pape, H.-C., and Pare, D. (2010). Plastic Synaptic Networks of the Amygdala for the Acquisition, Expression, and Extinction of Conditioned Fear. *Physiol Rev* *90*, 419–463.

Parikh Ashish, Patel Divyang, McTiernan Charles F., Xiang Wenyu, Haney Jamie, Yang Lei, Lin Bo, Kaplan Aaron D., Bett Glenna C.L., Rasmusson Randall L., et al. (2013). Relaxin Suppresses Atrial Fibrillation by Reversing Fibrosis and Myocyte Hypertrophy and Increasing Conduction Velocity and Sodium Current in Spontaneously Hypertensive Rat Hearts. *Circulation Research* *113*, 313–321.

Patil, N.A., Rosengren, K.J., Separovic, F., Wade, J.D., Bathgate, R.A.D., and Hossain, M.A. (2017). Relaxin family peptides: structure-activity relationship studies. *Br. J. Pharmacol.* *174*, 950–961.

Peirs, C., Williams, S.-P.G., Zhao, X., Walsh, C.E., Gedeon, J.Y., Cagle, N.E., Goldring, A.C., Hioki, H., Liu, Z., Marell, P.S., et al. (2015). Dorsal Horn Circuits for Persistent Mechanical Pain. *Neuron* *87*, 797–812.

Pertovaara, A. (2006). Noradrenergic pain modulation. *Prog. Neurobiol.* *80*, 53–83.

Petitjean, H., Pawlowski, S.A., Fraine, S.L., Sharif, B., Hamad, D., Fatima, T., Berg, J., Brown, C.M., Jan, L.-Y., Ribeiro-da-Silva, A., et al. (2015). Dorsal Horn Parvalbumin Neurons Are Gate-Keepers of Touch-Evoked Pain after Nerve Injury. *Cell Reports* *13*, 1246–1257.

Pintalhão, M., Castro-Chaves, P., Vasques-Novoa, F., Gonçalves, F., Mendonça, L., Fontes-Carvalho, R., Lourenço, P., Almeida, P., Leite-Moreira, A., and Bettencourt, P. (2017). Relaxin serum levels in acute heart failure are associated with pulmonary hypertension and right heart overload. *Eur J Heart Fail* *19*, 218–225.

Poisbeau, P., Grinevich, V., and Charlet, A. (2018). Oxytocin Signaling in Pain: Cellular, Circuit, System, and Behavioral Levels. *Curr Top Behav Neurosci* *35*, 193–211.

Polepalli, J.S., Gooch, H., and Sah, P. (2020). Diversity of interneurons in the lateral and basal amygdala. *Npj Science of Learning* *5*, 1–9.

Pomrenze, M.B., Millan, E.Z., Hopf, F.W., Keiflin, R., Maiya, R., Blasio, A., Dadgar, J., Kharazia, V., De Guglielmo, G., Crawford, E., et al. (2015). A Transgenic Rat for Investigating the Anatomy and Function of Corticotrophin Releasing Factor Circuits. *Front Neurosci* *9*, 487.

Porro, C.A., Cettolo, V., Francescato, M.P., and Baraldi, P. (1998). Temporal and Intensity Coding of Pain in Human Cortex. *Journal of Neurophysiology* *80*, 3312–3320.

Praveen, P., Kocan, M., Valkovic, A., Bathgate, R., and Hossain, M.A. (2019). Single chain peptide agonists of relaxin receptors. *Molecular and Cellular Endocrinology* *487*, 34–39.

Qu, X., Chen, L., Sun, L., Chen, C., Gao, Z., Huang, W., and Zhou, H. (2019). Serum relaxin level predicts recurrence of atrial fibrillation after radiofrequency catheter ablation. *Heart Vessels* *34*, 1543–1551.

Queneau, P., Serrie, A., Trèves, R., and Bontoux, D. (2018). Les douleurs chroniques en France. Recommandations de l'Académie nationale de médecine pour une meilleure prise en charge des malades. *Douleurs : Évaluation - Diagnostic - Traitement* 19, 265–272.

Rainville, P., Duncan, G.H., Price, D.D., Carrier, B., and Bushnell, M.C. (1997). Pain affect encoded in human anterior cingulate but not somatosensory cortex. *Science* 277, 968–971.

Ralston, H.J. (2005). Pain and the primate thalamus. *Prog. Brain Res.* 149, 1–10.

Reddi, D., Curran, N., and Stephens, R. (2013). An introduction to pain pathways and mechanisms. *Br J Hosp Med* 74, C188–C191.

Renn, C.L., and Dorsey, S.G. (2005). The physiology and processing of pain: a review. *AACN Clin Issues* 16, 277–290; quiz 413–415.

Roca-Lapirot, O., Fossat, P., Ma, S., Egron, K., Trigilio, G., López-González, M.-J., Covita, J., Bouali-Benazzouz, R., Favereaux, A., Gundlach, A.L., et al. (2019). Acquisition of analgesic properties by the cholecystokinin (CCK)/CCK2 receptor system within the amygdala in a persistent inflammatory pain condition. *Pain* 160, 345–357.

Ryan, P.J., Büchler, E., Shabanpoor, F., Hossain, M.A., Wade, J.D., Lawrence, A.J., and Gundlach, A.L. (2013). Central relaxin-3 receptor (RXFP3) activation decreases anxiety- and depressive-like behaviours in the rat. *Behav. Brain Res.* 244, 142–151.

Sah, P., Faber, E.S.L., Lopez De Armentia, M., and Power, J. (2003). The Amygdaloid Complex: Anatomy and Physiology. *Physiological Reviews* 83, 803–834.

Samuel, C.S., Tian, H., Zhao, L., and Amento, E.P. (2003). Relaxin Is a Key Mediator of Prostate Growth and Male Reproductive Tract Development. *Laboratory Investigation* 83, 1055–1067.

Sandkühler, J. (2009). Models and Mechanisms of Hyperalgesia and Allodynia. *Physiological Reviews* 89, 707–758.

Santos, F.N., Pereira, C.W., Sánchez-Pérez, A.M., Otero-García, M., Ma, S., Gundlach, A.L., and Olucha-Bordonau, F.E. (2016). Comparative Distribution of Relaxin-3 Inputs and Calcium-Binding Protein-Positive Neurons in Rat Amygdala. *Front Neuroanat* 10, 36.

Schwabe, C., McDonald, J.K., and Steinetz, B.G. (1976). Primary structure of the A chain of porcine relaxin. *Biochemical and Biophysical Research Communications* 70, 397–405.

Scott, D.J., Layfield, S., Riesewijk, A., Morita, H., Tregear, G.W., and Bathgate, R. a. D. (2004). Identification and characterization of the mouse and rat relaxin receptors as the novel orthologues of human leucine-rich repeat-containing G-protein-coupled receptor 7. *Clin Exp Pharmacol Physiol* 31, 828–832.

Scott, D.J., Layfield, S., Yan, Y., Sudo, S., Hsueh, A.J.W., Tregear, G.W., and Bathgate, R.A.D. (2006). Characterization of novel splice variants of LGR7 and LGR8 reveals that receptor

signaling is mediated by their unique low density lipoprotein class A modules. *J Biol Chem* 281, 34942–34954.

Sellmeijer, J., Mathis, V., Hugel, S., Li, X.-H., Song, Q., Chen, Q.-Y., Barthas, F., Lutz, P.-E., Karatas, M., Luthi, A., et al. (2018). Hyperactivity of Anterior Cingulate Cortex Areas 24a/24b Drives Chronic Pain-Induced Anxiodepressive-like Consequences. *J. Neurosci.* 38, 3102–3115.

Sethi, A., Bruell, S., Patil, N., Hossain, M.A., Scott, D.J., Petrie, E.J., Bathgate, R.A.D., and Gooley, P.R. (2016). The complex binding mode of the peptide hormone H2 relaxin to its receptor RXFP1. *Nat Commun* 7, 11344.

Shabanpoor, F., Akhter Hossain, M., Ryan, P.J., Belgi, A., Layfield, S., Kocan, M., Zhang, S., Samuel, C.S., Gundlach, A.L., Bathgate, R.A.D., et al. (2012). Minimization of human relaxin-3 leading to high-affinity analogues with increased selectivity for relaxin-family peptide 3 receptor (RXFP3) over RXFP1. *J Med Chem* 55, 1671–1681.

Sherwood, O.D. (2004). Relaxin's physiological roles and other diverse actions. *Endocr Rev* 25, 205–234.

Shyu, B.-C., and Vogt, B.A. (2009). Short-term synaptic plasticity in the nociceptive thalamic-anterior cingulate pathway. *Mol Pain* 5, 51.

Sikes, R.W., and Vogt, B.A. (1992). Nociceptive neurons in area 24 of rabbit cingulate cortex. *Journal of Neurophysiology* 68, 1720–1732.

Smith, C.M., Shen, P.-J., Banerjee, A., Bonaventure, P., Ma, S., Bathgate, R.A.D., Sutton, S.W., and Gundlach, A.L. (2010). Distribution of relaxin-3 and RXFP3 within arousal, stress, affective, and cognitive circuits of mouse brain. *The Journal of Comparative Neurology* 518, 4016–4045.

Smith, C.M., Ryan, P.J., Hosken, I.T., Ma, S., and Gundlach, A.L. (2011). Relaxin-3 systems in the brain—The first 10 years. *Journal of Chemical Neuroanatomy* 42, 262–275.

Smith, C.M., Walker, A.W., Hosken, I.T., Chua, B.E., Zhang, C., Haidar, M., and Gundlach, A.L. (2014). Relaxin-3/RXFP3 networks: an emerging target for the treatment of depression and other neuropsychiatric diseases? *Front Pharmacol* 5, 46.

Smith, D.J., Hawranko, A.A., Monroe, P.J., Gully, D., Urban, M.O., Craig, C.R., Smith, J.P., and Smith, D.L. (1997). Dose-dependent pain-facilitatory and -inhibitory actions of neurotensin are revealed by SR 48692, a nonpeptide neurotensin antagonist: influence on the antinociceptive effect of morphine. *J Pharmacol Exp Ther* 282, 899–908.

Sudo, S., Kumagai, J., Nishi, S., Layfield, S., Ferraro, T., Bathgate, R.A.D., and Hsueh, A.J.W. (2003). H3 relaxin is a specific ligand for LGR7 and activates the receptor by interacting with both the ectodomain and the exoloop 2. *The Journal of Biological Chemistry* 278, 7855–7862.

- Summerlee, A.J., Hornsby, D.J., and Ramsey, D.G. (1998). The dipsogenic effects of rat relaxin: The effect of photoperiod and the potential role of relaxin on drinking in pregnancy. *Endocrinology* *139*, 2322–2328.
- Sun Junhui, Hao Weidong, Fillmore Natasha, Ma Hanley, Springer Danielle, Yu Zu-Xi, Sadowska Agnieszka, Garcia Andrew, Chen Ruoyan, Muniz-Medina Vanessa, et al. (2019). Human Relaxin-2 Fusion Protein Treatment Prevents and Reverses Isoproterenol-Induced Hypertrophy and Fibrosis in Mouse Heart. *Journal of the American Heart Association* *8*, e013465.
- Sunn, N., Egli, M., Burazin, T.C.D., Burns, P., Colvill, L., Davern, P., Denton, D.A., Oldfield, B.J., Weisinger, R.S., Rauch, M., et al. (2002). Circulating relaxin acts on subfornical organ neurons to stimulate water drinking in the rat. *Proc Natl Acad Sci U S A* *99*, 1701–1706.
- Sutton, S.W., Bonaventure, P., Kuei, C., Nepomuceno, D., Wu, J., Zhu, J., Lovenberg, T.W., and Liu, C. (2005). G-protein-coupled receptor (GPCR)-142 does not contribute to relaxin-3 binding in the mouse brain: further support that relaxin-3 is the physiological ligand for GPCR135. *Neuroendocrinology* *82*, 139–150.
- Sutton, S.W., Shelton, J., Smith, C., Williams, J., Yun, S., Motley, T., Kuei, C., Bonaventure, P., Gundlach, A., Liu, C., et al. (2009). Metabolic and neuroendocrine responses to RXFP3 modulation in the central nervous system. *Ann N Y Acad Sci* *1160*, 242–249.
- Tan, L.L., Pelzer, P., Heintl, C., Tang, W., Gangadharan, V., Flor, H., Sprengel, R., Kuner, T., and Kuner, R. (2017). A pathway from midcingulate cortex to posterior insula gates nociceptive hypersensitivity. *Nat Neurosci* *20*, 1591–1601.
- Tan, Y.Y., Wade, J.D., Tregear, G.W., and Summers, R.J. (1999). Quantitative autoradiographic studies of relaxin binding in rat atria, uterus and cerebral cortex: characterization and effects of oestrogen treatment. *Br J Pharmacol* *127*, 91–98.
- Tanaka, M., Iijima, N., Miyamoto, Y., Fukusumi, S., Itoh, Y., Ozawa, H., and Ibata, Y. (2005). Neurons expressing relaxin 3/INSL 7 in the nucleus incertus respond to stress. *The European Journal of Neuroscience* *21*, 1659–1670.
- Teerlink, J.R., Cotter, G., Davison, B.A., Felker, G.M., Filippatos, G., Greenberg, B.H., Ponikowski, P., Unemori, E., Voors, A.A., Adams, K.F., et al. (2013). Serelaxin, recombinant human relaxin-2, for treatment of acute heart failure (RELAX-AHF): a randomised, placebo-controlled trial. *The Lancet* *381*, 29–39.
- Thompson, J.M., and Neugebauer, V. (2017). Amygdala Plasticity and Pain. *Pain Res Manag* *2017*, 8296501.
- Treede, R.-D., Rief, W., Barke, A., Aziz, Q., Bennett, M.I., Benoliel, R., Cohen, M., Evers, S., Finnerup, N.B., First, M.B., et al. (2015). A classification of chronic pain for ICD-11. *Pain* *156*, 1003.

- Treede, R.-D., Rief, W., Barke, A., Aziz, Q., Bennett, M.I., Benoliel, R., Cohen, M., Evers, S., Finnerup, N.B., First, M.B., et al. (2019). Chronic pain as a symptom or a disease: the IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11). *PAIN* 160, 19–27.
- Urban, M.O., Coutinho, S.V., and Gebhart, G.F. (1999). Biphasic Modulation of Visceral Nociception by Neurotensin in Rat Rostral Ventromedial Medulla. *J Pharmacol Exp Ther* 290, 207–213.
- Vanegas, H., and Schaible, H.-G. (2004). Descending control of persistent pain: inhibitory or facilitatory? *Brain Res. Brain Res. Rev.* 46, 295–309.
- Veinante, P., Yalcin, I., and Barrot, M. (2013). The amygdala between sensation and affect: a role in pain. *J Mol Psychiatry* 1, 9.
- Vertes, R.P. (2005). Hippocampal theta rhythm: a tag for short-term memory. *Hippocampus* 15, 923–935.
- Walensky, L.D., and Bird, G.H. (2014). Hydrocarbon-Stapled Peptides: Principles, Practice, and Progress. *J. Med. Chem.* 57, 6275–6288.
- Wang, J.-W., Lundeberg, T., and Yu, L.-C. (2003). Antinociceptive role of oxytocin in the nucleus raphe magnus of rats, an involvement of mu-opioid receptor. *Regul Pept* 115, 153–159.
- Wang, Q., Ng, L., Harris, J.A., Feng, D., Li, Y., Royall, J.J., Oh, S.W., Bernard, A., Sunkin, S.M., Koch, C., et al. (2017). Organization of the connections between claustrum and cortex in the mouse. *J Comp Neurol* 525, 1317–1346.
- Weiss, G. (1989). Relaxin in the male. *Biol Reprod* 40, 197–200.
- van der Westhuizen, E.T., Christopoulos, A., Sexton, P.M., Wade, J.D., and Summers, R.J. (2010). H2 relaxin is a biased ligand relative to H3 relaxin at the relaxin family peptide receptor 3 (RXFP3). *Mol Pharmacol* 77, 759–772.
- Wilkinson, T.N., Speed, T.P., Tregear, G.W., and Bathgate, R.A.D. (2005a). Coevolution of the relaxin-like peptides and their receptors. *Annals of the New York Academy of Sciences* 1041, 534–539.
- Wilkinson, T.N., Speed, T.P., Tregear, G.W., and Bathgate, R.A.D. (2005b). Evolution of the relaxin-like peptide family. *BMC Evol. Biol.* 5, 14.
- Williams, L.S., Jones, W.J., Shen, J., Robinson, R.L., Weinberger, M., and Kroenke, K. (2003). Prevalence and impact of depression and pain in neurology outpatients. *J. Neurol. Neurosurg. Psychiatry* 74, 1587–1589.

- Wong, L.L.L., Scott, D.J., Hossain, M.A., Kaas, Q., Rosengren, K.J., and Bathgate, R.A.D. (2018). Distinct but overlapping binding sites of agonist and antagonist at the relaxin family peptide 3 (RXFP3) receptor. *J. Biol. Chem.* *293*, 15777–15789.
- Woolf, C.J. (1983). Evidence for a central component of post-injury pain hypersensitivity. *Nature* *306*, 686–688.
- Woolf, C.J., and Salter, M.W. (2000). Neuronal Plasticity: Increasing the Gain in Pain. *Science* *288*, 1765–1768.
- Yam, M.F., Loh, Y.C., Tan, C.S., Khadijah Adam, S., Abdul Manan, N., and Basir, R. (2018). General Pathways of Pain Sensation and the Major Neurotransmitters Involved in Pain Regulation. *Int J Mol Sci* *19*.
- Yamamura, H., Iwata, K., Tsuboi, Y., Toda, K., Kitajima, K., Shimizu, N., Nomura, H., Hibiya, J., Fujita, S., and Sumino, R. (1996). Morphological and electrophysiological properties of ACCx nociceptive neurons in rats. *Brain Res.* *735*, 83–92.
- Yang, J., Liang, J.-Y., Li, P., Pan, Y.-J., Qiu, P.-Y., Zhang, J., Hao, F., and Wang, D.-X. (2011a). Oxytocin in the periaqueductal gray participates in pain modulation in the rat by influencing endogenous opiate peptides. *Peptides* *32*, 1255–1261.
- Yang, J., Li, P., Liang, J.-Y., Pan, Y.-J., Yan, X.-Q., Yan, F.-L., Hao, F., Zhang, X.-Y., Zhang, J., Qiu, P.-Y., et al. (2011b). Oxytocin in the periaqueductal grey regulates nociception in the rat. *Regul Pept* *169*, 39–42.
- Yegorov, S., Good-Avila, S.V., Parry, L., and Wilson, B.C. (2009). Relaxin family genes in humans and teleosts. *Annals of the New York Academy of Sciences* *1160*, 42–44.
- Yoshida, M., Takayanagi, Y., Inoue, K., Kimura, T., Young, L.J., Onaka, T., and Nishimori, K. (2009). Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. *J Neurosci* *29*, 2259–2271.
- Zhang, C., Chua, B.E., Yang, A., Shabanpoor, F., Hossain, M.A., Wade, J.D., Rosengren, K.J., Smith, C.M., and Gundlach, A.L. (2015). Central relaxin-3 receptor (RXFP3) activation reduces elevated, but not basal, anxiety-like behaviour in C57BL/6J mice. *Behavioural Brain Research* *292*, 125–132.
- Zhang, M.-L., Wang, H.-B., Fu, F.-H., and Yu, L.-C. (2017). Involvement of galanin and galanin receptor 2 in nociceptive modulation in anterior cingulate cortex of normal rats and rats with mononeuropathy. *Scientific Reports* *7*, 45930.
- Zhang, Q., Liu, S.-H., Erikson, M., Lewis, M., and Unemori, E. (2002). Relaxin activates the MAP kinase pathway in human endometrial stromal cells. *J Cell Biochem* *85*, 536–544.

Zhang, Y., Gao, Y., Li, C.-Y., Dong, W., Li, M.-N., Liu, Y.-N., Dong, Y., and Xu, S.-L. (2019). Galanin plays a role in antinociception via binding to galanin receptors in the nucleus accumbens of rats with neuropathic pain. *Neurosci Lett* 706, 93–98.

Zhao, L., Roche, P.J., Gunnarsen, J.M., Hammond, V.E., Tregear, G.W., Wintour, E.M., and Beck, F. (1999). Mice without a functional relaxin gene are unable to deliver milk to their pups. *Endocrinology* 140, 445–453.

Zhao, Z.-Q., Chiechio, S., Sun, Y.-G., Zhang, K.-H., Zhao, C.-S., Scott, M., Johnson, R.L., Deneris, E.S., Renner, K.J., Gereau, R.W., et al. (2007). Mice lacking central serotonergic neurons show enhanced inflammatory pain and an impaired analgesic response to antidepressant drugs. *J. Neurosci.* 27, 6045–6053.

Zingg, B., Hintiryan, H., Gou, L., Song, M.Y., Bay, M., Bienkowski, M.S., Foster, N.N., Yamashita, S., Bowman, I., Toga, A.W., et al. (2014). Neural networks of the mouse neocortex. *Cell* 156, 1096–1111.

(1979). Pain terms: a list with definitions and notes on usage. Recommended by the IASP Subcommittee on Taxonomy. *Pain* 6, 249.

Annexe 1:

Review article

Animal models of pain: diversity and benefits

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Abstract

Chronic pain is a maladaptive neurological disease that remains a major health problem. A deepening of our knowledge on mechanisms that cause pain is a prerequisite to developing novel treatments. A large variety of animal models of pain has been developed that recapitulate the diverse symptoms of different pain pathologies. These models reproduce different pain phenotypes and remain necessary to examine the multidimensional aspects of pain and understand the cellular and molecular basis underlying pain conditions.

In this review, we propose an overview of animal models, from simple organisms to rodents and non-human primates and the specific traits of pain pathologies they model. We present the main behavioral tests for assessing pain and investigating the underpinning mechanisms of chronic pathological pain. The validity of animal models is analysed based on their ability to mimic human clinical diseases and to predict treatment outcomes. Refine characterization of pathological phenotypes also requires to consider pain globally using specific procedures dedicated to study emotional comorbidities of pain. We discuss the limitations of pain models when research findings fail to be translated from animal models to human clinics. But we also point to some recent successes in analgesic drug development that highlight strategies for improving the predictive validity of animal models of pain. Finally, we emphasize the importance of using assortments of preclinical pain models to identify pain subtype mechanisms, and to foster the development of better analgesics.

Highlights:

- Evaluation tools to assess chronic pain and its comorbidities
- Inventory of a large diversity of pain models including rodents, non-human primates pain models and simple organisms
- Focus on inflammatory and neuropathic pain models
- Limitations of the predictive validity of animal models of pain
- Translational successes from animal model studies to develop new analgesics

Introduction

Pain is a vital physiological function that protects organisms against potential damage. Acute nociceptive pain is a normal function of the nervous system that provides important sensory information about the environment and reacts to harmful stimuli such as noxious heat, extreme cold, chemical irritants, and mechanical tissue damage. These noxious stimuli activate peripheral nociceptors, triggering action potentials that propagate along sensory axons to the dorsal horn of the spinal cord where nociceptive inputs are processed and relayed to the brain. In turn, the activation of specific brain areas produces a broad array of sensory, emotional, autonomic, and motor responses that shape our experience and perception of pain (Basbaum et al., 2009; Burma et al., 2017).

In contrast to acute pain, chronic pain is a maladaptive disease that heightens the sensitivity to sensory stimulation (Costigan et al., 2009b; Woolf and Salter, 2000b). Chronic pain results from abnormal functioning of the nervous system, with pain persisting far beyond the resolution of the primary injury. Pain hypersensitivity manifests as spontaneous pain (pain in the absence of an external stimulus), allodynia (pain resulting from an innocuous stimulus), and/or hyperalgesia (an exaggerated pain response to a noxious stimulus). Chronic pain is a major health problem that negatively impacts the quality-of-life of sufferers and exacts enormous socio-economic costs with a prevalence of around 8% of the general population (Bouhassira et al., 2008b). In the European Union, it is estimated that 20% of the population would suffer from chronic pain during lifespan (Alshami, 2014; Breivik et al., 2006b), and it is among the most significant risk factors for suicide.

Chronic pain drastically diminishes quality of life and causes enormous socio-economic costs. Besides high costs for disease management, chronic pain is associated with major impacts on daily activities and quality of life (Groenewald and Palermo, 2015) and high productivity losses due to work absences (Mayer et al., 2019) partly due to common co-morbidities such as depression (Phillips, 2006). The estimated direct and indirect healthcare costs for chronic pain disorders in European Member States vary between two and three percent of GDP across the EU (Breivik et al., 2013). For 2016, this estimate would result in up to €441 billion. In the USA, ~100 million people suffer from pain costing ~\$600 billion/year in health care and lost productivity (Walker et al., 2014). These costs are reported to exceed those estimated for heart disease, cancer and diabetes (Breivik et al., 2013). However, chronic pain is poorly managed with treatment success rates around 30% (Ossipov et al.,

2014b), mainly because chronic pain mechanisms remain poorly understood (Basbaum et al., 2009b; Cordero-Erausquin et al., 2016; Dolique et al., 2010; Kuner and Flor, 2016), and patients often suffer from comorbid disorders such as anxiety and depression (Attal et al., 2011).

It is important to recognize that there is not one overarching, singular condition called chronic pain but rather, there are multiple aetiologies of pain, each resulting from different pathologies and differing in the clinical presentation of signs and symptoms (Burma et al., 2017). Pain is usually subdivided in different categories as a function of the mechanism of injury. Nociceptive pain represents the normal response to noxious insult or injury of tissues such as skin, muscles, visceral organs, joints, tendons, or bones. Inflammatory pain results of activation and sensitization (peripheral and/or central) of the nociceptive pain pathway by a variety of mediators released at a site of tissue inflammation. Neuropathic pain arises from damage to the nervous system itself, central or peripheral, either from disease, injury, or pinching. Other types of pain were characterized, e.g. cancer pain or dysfunctional pain when no biological cause is identified. To address differences in the presentation of pain symptoms across chronic pain conditions, an array of preclinical animal models has been developed to recapitulate the underlying pathology, duration, and comorbidities of pain phenotypes (Mogil, 2009). This variety of preclinical pain models is essential for understanding the molecular and cellular mechanisms that underlie distinct pain conditions. The development of novel and more efficacious therapies requires a thorough understanding of the mechanistic underpinnings of chronic pain and the design and testing of new drugs.

Rodents are employed in an large majority of preclinical pain studies (Mogil, 2009). However, the use of alternate vertebrates and invertebrates, such as zebrafish, fruit flies (*Drosophila* sp.) and nematodes (*Caenorhabditis elegans*) can also be advantageous for screening assays and for studying the genetic and molecular mechanisms of acute and chronic pain (Gonzalez-Nunez and Rodríguez, 2009; Milinkeviciute et al., 2012; Way and Chalfie, 1989). Each organism confers a distinct advantage for studying pain; the behavioral complexity of the rodents allows the analysis of the affective components of pain (Johansen et al., 2001; Panksepp and Lahvis, 2011), whereas simpler organisms, such as *Drosophila*, can facilitate the discovery of novel molecular players involved in the detection of noxious stimuli (Caldwell and Tracey, 2010; Mogil et al., 2010).

Another important aspect for designing animal models is to recognize that pain is a multi-dimensional experience. Indeed, pain is processed not only at the peripheral and spinal levels, but also in higher brain structures including cortical areas underlying the affective component of pain (Liu and Chen, 2014). As pain transitions from acute to chronic and becomes pathological, the associated negative emotional state not only exacerbates sensory modalities, but also worsens the comorbidities. Indeed, anxiety is a highly common comorbidity of pain where the interactions between pain and anxiety have been demonstrated in human. In addition, chronic pain and depression are complex disorders that often coexist and increase the risk of one another (Radat et al., 2013; Steel et al., 2014; Zhu et al., 2018a). Investigations of pain and psychiatric disorders are mostly conducted separately, but more knowledge of the overlap and interactions between affective and pain circuits is key to better treatments. Refined analysis of animal models of pain and depression has become mandatory to understand interactions between pain and emotional comorbidities (Kremer et al., 2020).

The present review proposes an overview of rodent and alternate models of pain. A large panel of evaluation devices used to characterize pain-like behavior in animal models has been developed over the years, and a summary description of these tools is presented here. The review focuses mainly on the broadly studied neuropathic and inflammatory types of pain. It also highlights the necessity of specific procedures dedicated to studying emotional comorbidities to pain. Finally, it summarizes the main limitations of using animal models to mimic clinical pain in humans, but also provides examples of successful translational applications of using animal models.

1. Assessment of pain

The classical pain evaluation devices aim to assess the sensory component of pain, usually by measuring a withdrawal reflex. However, more recently, specific efforts have been made to assess pain perception and to evaluate the emotional component of pain and comorbid affections. Unlike humans, animals are incapable of verbally describing pain, therefore a battery of behavioral tests has been developed in order to assess pain-like behavior in animals. These assays can be divided into stimulus-dependent and stimulus independent tests.

Stimulus-dependent tests

In the evoked tests the latency to or the frequency of paw withdrawal is usually measured after mechanical or thermal stimulation.

Mechanical stimulation.

Mechanical hypersensitivity can be of various types, namely dynamic (triggered by brushing), static (triggered by pressure), and punctate (triggered by touch) (Deuis et al., 2017b). Dynamic mechanical hypersensitivity is measured by air puffing on the face (Miraucourt et al., 2009) or light stroking (velocity is ~ 2 cm/s) of the external lateral side of the injured hind paw with a paintbrush (Cheng et al., 2017b). The typical response of naive rodents to the dynamic mechanical stimulation is a very fast lifting of the stimulated paw aside. In experimental pain models, the response changes into sustained lifting, flinching or even licking behavior. Static mechanical sensitivity can be assessed with a pressure algometer (Deuis et al., 2017b). Testing punctate mechanical hypersensitivity generally consists of applying an increasing pressure and measuring nociception in animals. Some tests are more specific to rats (Randall-Selitto Test) and some are common to rats and mice (von Frey). These tests are described more in detail below.

von Frey test. The von Frey test, originally developed by Maximilian von Frey, is used to assess mechanical allodynia in rodents (Deuis et al., 2017c; Piel et al., 2014). The manual von Frey test consists of applying pressure of different force using calibrated von Frey filaments (ranged from 0.008 to 300 g) on the plantar surface of the hind paw. The animal is freely moving in a cage, and can rest on a penetrable grid. Once the animal is standing on its 4 legs, the filament is placed perpendicularly and pressed against the paw until it bends.

(i) Three main methods of using von Frey filaments are commonly used. The “up and down” method determines the weight of stimulus that elicits a response 50% of the times it is applied. It derives of the statistical formula used to determine LD50s (Chaplan et al., 1994; Dixon, 1980). The method is based on a series of testing with filament of different forces. The calculation takes into account six responses around the threshold and assess the 50% threshold using the formula: 50% threshold (g) = $10^{(X+kd)}/10^4$, where X = the value (in log units) of the final von Frey filament, k = tabular value for the response pattern (see Appendix 1 in (Chaplan et al., 1994)) and d = the average increment (in log units) between von Frey filaments (Deuis et al., 2017b). Recent refinements of this

methods have been published and proposed easier procedures (Bonin et al., 2014; Christensen et al., 2020). A limitation of this method is the repetitive stimulations that is time-consuming and can cause sensitization.

(ii) In the “percent” method, identical series of stimulations with von Frey filaments of increasing are applied to all animals and the percentage of withdrawal responses is assessed (Chaplan et al., 1994). Here also the limitation relates to the high number of stimulations applied to one animal.

(iii) In the “ascending” method, filaments with increasing force are used, and nociceptive behavior is considered when the animal retracts the paw, licks it or even shakes it (D’Souza et al., 2011; Papon et al., 2020). The effects of stress are very limited in this test since the animal is unrestrained and is habituated to the environment prior to the test. However, it relies on the experimenter eye and judgment thus a subjective bias has to be taken into consideration. In order to limit this subjectivity, one can use electronic von Frey where a single filament is pressed against the paw and the force is increased automatically until paw withdrawal.

Overall, despite the popularity of the von Frey test, there is no consensus on the sensation that is triggered in rodents, mainly is because the distinction between noxious and innocuous stimuli relies on subjective human judgment and can hardly be applied to an animal. Moreover, pain is an experience that can be different for human and rodents. Therefore, it is assumed that the von Frey test triggers nociception and can be used to assess unpleasantness of the stimulation, rather than pain (Abdus-Saboor et al., 2019).

Randall-Selitto Test or paw pressure test. This test is used to assess mechanical hypersensitivity in rats (Randall and Selitto, 1957). The animal is restrained and an increasing pressure is exerted on the hind paw or tail using a dome-shaped plastic tip. When the rat withdraws its paw or vocalizes, threshold is attained and the pressure is stopped (Barrot, 2012). Waiting for the animal to vocalize is not recommended since it is a synonym to great pain which may lead to injuring the animal (Muley et al., 2016). Another limitation is that rats should be habituated to being restrained in order to minimize the stress bias (Santos-Nogueira et al., 2012).

Behavioral tests and readouts must be carefully chosen according to the mechanisms under investigation. Withdrawal reflex is usually seen as a measure of spinal reflex. In contrast,

vocalizations are considered as a measure of integrated pain-like behavior (Kayser and Christensen, 2000). Since 22 kHz vocalizations are emitted in response to aversive stimuli, the use of ultrasound has been considered. However, ultrasound vocalization do not correlate to pain-like behavior and cannot be considered as a reliable readout of pain sensation in rodents (Wallace et al., 2005).

Cold stimulation.

Acetone evaporation test. Acetone evaporation test is used to assess cold allodynia in rodents (Choi et al., 1994; Colburn et al., 2007; Vissers and Meert, 2005). The evaporation of acetone on the hind paw leads to a cold stimulation that is not considered nociceptive in naive animals. Therefore, it is used to evaluate allodynia in neuropathic and inflammatory pain models (Deuis et al., 2017). The test consists of a repetitive application of acetone on the hind paw and a measurement of the latency to withdrawal (Xing et al., 2007). Alternatively, cold allodynia assessment may be achieved by monitoring the duration or the number of nocifensive responses, or scoring the severity of the response. The test is done on one paw, leaving the other as control. However, the smell of acetone can trigger an olfactory stimulus that can overlap the cold stimulation (D'Souza et al., 2011a).

Cold plate. The cold plate test is used to assess cold allodynia and hyperalgesia. It consists of measuring the latency to paw withdrawal after applying a stick of ice (wet or dry ice) on the floor of a cage just underneath the paw of a freely moving animal (Allchorne et al., 2005). Alternatively, the rodent can be standing on a plate that is cooled down to 5°C by Pelletier effect. One main disadvantage for this test is that the animal should stay in place until the temperature transfer (Barrot, 2012).

The cold plantar assay has been developed to apply a focal ramping cold stimulus to unrestrained, acclimated mice (Brenner et al., 2012). This assay has the ability to establish a detail evaluation of the nocifensive response as a function of the temperature and to measure both cold allodynia and cold anesthesia with low variability in mice. It is also highly sensitive to experimentally altered pain conditions including CFA-induced inflammation, and SNL-induced nerve injury, or morphine treatment.

Hot stimulation.

Hot plate. Hot plate test is used to assess thermal nociception in mice and rats (Woolfe and Macdonald, 1944). The test consists of placing a freely moving animal on a hot metallic surface (with a constant temperature at 50-55°C) and measuring the latency for the animal to exhibit nocifensive behavior (paw withdrawal or jumping). For ethical reasons, it is recommended to stop the test as soon as the animal withdraws the paw. The latency should not be measured on the forepaws since they are more often used to explore and groom. Consequently, it is more reliable to observe hind paw withdrawal. In order to avoid tissue damage, explorers should determine a cut-off time if the animal doesn't respond. Another variant of the hot plate is the dynamic hot plate test where the temperature of the metallic surface increases gradually until pain-like behavior (D'Souza et al., 2011; Barrot, 2012; Burma et al., 2017; Deuis et al., 2017).

The dynamic hot plate (Ogren and Berge, 1984) differs of the classical hot plate test in the sense that the animal is submitted to a ramp of increasing temperatures, usually starting at 42°C until a nocifensive response is observed. By assessing the response temperature, the dynamic hot plate test gives an estimate of thermal allodynia.

Hargreaves or plantar test: Hargreaves test is used to assess heat pain threshold in mice and rats (Hargreaves et al., 1988). The test consists of applying an infrared heat source on the hind paw of an unrestrained animal in a clear box which rests on a glass floor. The latency to withdrawal is automatically measured and displayed. The animal should be acclimatized to the box prior to the experiment to minimize movement not related to nociception. Compared to the hot plate test, the Hargreaves test offers the possibility to address each hind paw and thus using one as control (Hargreaves et al., 1988; Harvey and Dickenson, 2009). In 2013, Banik and Kabadi have developed a modified Hargreaves for rats where they fix the time and measure the temperature at which the paw is withdrawn (Banik and Kabadi, 2013). The authors apply a constant temperature for 10s, then they increase the temperature by 2.5 °C and apply it for another 10 seconds until paw withdrawal (with temperature starting at 35°C up to a maximum of 70°C).

Tail Flick test : The tail flick test is used to assess heat pain threshold in mice and rats (D'Amour and Smith, 1941). The test consists of measuring the latency of the tail to flick after a heat stimulation. The tail is either dipped in a hot water bath with a constant water temperature between 46° and 52°C or the tail is exposed to a light beam. This test is easy and quick to perform if the animal is habituated

to being loosely restrained, but there is a risk of jeopardizing animal's thermoregulation since the tail plays an important role in rodents' thermoregulation (Barrot, 2012; Deuis et al., 2017c).

Stimulus-independent tests

When in pain, animals are capable of developing a spontaneous behavior that is not a consequence of thermal nor mechanical stimulation. This type of behavior is believed to be more relevant clinically although it must be kept in mind that rodents being prey animals, they tend to hide states of discomfort. Throughout the years a series of methods have been developed to assess these non-evoked behaviors. In this review the grimace scale, weight bearing, conditioned place preference and burrowing will be discussed (for a more thorough review on spontaneous behavior please refer to Tappe-Theodor and Küner (Tappe-Theodor and Küner, 2014).

Grimace scale and unbiased behavioral analysis.

The grimace scale test in rodents consists of observing 5 facial features: orbital tightening, nose bulge, cheek bulge, ear position, and whisker position (Langford et al., 2010; Sotocinal et al., 2011). These features are scored from 0 (normal) to 2 (severe). It is classically used to assess acute pain since animals are capable of adapting to long-lasting pain, and therefore no longer express facial changes (Langford et al., 2010). Moreover, facial expression is being used to assess emotions associated with pain (Dolensek et al., 2020).

Beyond the grimace scale test, high-throughput, unbiased approaches that accurately measure non stimulus-evoked pain are currently under development. Increasing the dimensionality of pain assays has the potential to increase the predictive validity of translational pain therapeutics and the transfer from bench to bedside. The speed of rodent responses to sensory stimulations is in the range of the millisecond and cannot be captured only by the eye experimenter. It requires the development of automated systems to monitor pain-like behavior, possibly coupled with statistical modelling and machine-learning (Abdus-Saboor et al., 2019). Automated behavioral analysis can be performed in a dedicated apparatus (Behavioral Spectrometer, Behavioral Instruments) or in a home cage (HomeCageScan, CleverSys; PhenoTyper, Noldus) and captured a broad array of various motor behaviors. It also computes distance traveled and velocity (Brodkin et al., 2014; Roughan et al., 2016).

The challenge is to ensure that the combination of measured behaviors is representative of pain states since each individual behavior is not specific to pain. Comparisons with well-established assays, or series of individual assays, are necessary prior to generalize the use of automated, unbiased paradigms to assess pain conditions in rodents.

Weight bearing.

The test consists of measuring weight repartition on the rodent's hind limbs. In normal conditions the weight is equally distributed on both hind limbs, whereas in inflammatory or neuropathic pain models, the weight is shifted towards the non-painful paw. Weight bearing is assessed either statically (the animal is standing in inclined cage with the 2 paws relying on 2 separate pressure detectors) or dynamically (the animal is freely moving on pressure sensitive floor) (Muley et al., 2016; Burma et al., 2017; Deuis et al., 2017).

Conditioned place-preference (CPP).

CPP test is used to assess ongoing pain in rodents (Leite-Almeida et al., 2015). The original protocol was developed in rats by King et al. (King et al., 2009). The test consists of 2 compartments with identical dimensions but with different floors and walls. One compartment is associated with pain relief and both compartments communicate through a middle chamber (Blanco-Gandía et al., 2018; King et al., 2009). Prior to the test, animals are preconditioned for 3 days where they are left moving freely between both compartments, the ones developing a preference to one compartment are excluded. Afterwards animals are conditioned for 4 days by pairing each of the two compartments with either drug or vehicle administration. The purpose is to associate one compartment with pain and the other one with analgesia. On the test day, animals are left uninjected and moving freely. The time spent in each compartment is recorded (King et al., 2009; Sellmeijer et al., 2018). The animals tend to spend more time in the pain-relief compartment (Vuralli et al., 2019). The test assesses spontaneous pain in an indirect way. To avoid biases in the compartment choice, it is important to maintain the same environment during the whole procedure (Narita et al., 2006).

Burrowing

Burrowing is a spontaneous behavior that has been used in rodents to assess non-evoked pain conditions. A burrow, filled with appropriate substrate, is placed in the home cage and the amount of

material displaced by the animal within a predefined time interval is measured by the experimenter (Deacon, 2006). It is a very simple, objective, test, showing a high level of reproducibility. It has been validated for various models of inflammatory and neuropathic pain (Deuis et al., 2017b).

Assessment of pain comorbidities

When chronic pain lasts for more than 3 months and is considered as an illness, it is often associated with emotional or psychiatric comorbidities. In spite of its prevalence and impact on patients quality of life, chronic pain and emotional disorders are still poorly managed and current therapies are often inadequate (Finnerup et al., 2010). Although many reports suggest that typical anti-depressants are successful in treating pain, their success rate remains lower than 50% (Kroenke et al., 2009). Moreover, anti-depressants have anti-nociceptive effects but are less convincing in preventing the affective component of pain, i.e., pain perception (Boyce-Rustay et al., 2010). Therefore, simply targeting either pain or emotional disorders is not effective and there is a need to identify new common mechanisms. An increased understanding of mechanisms that underlie the overlap of pain with comorbid emotional states are key factors in the development of new therapies (Navratilova et al., 2016). For this purpose, the identification of agents that are active against both pain and emotional pathologies is a priority since these agents are likely to target common mechanisms, engaging overlapping neuronal circuits and underlying both disorders. Such research at the frontier between pain studies and psychiatry requires elaborate animal models and complex analysis to discriminate between the various components of the global pathology. Some highlights on comorbidities between chronic pain and psychiatric disorders are proposed below. A thorough evaluation of pain and anxiety/depression models was recently proposed (Kremer et al., 2020).

Anxiety-like behavior.

In the following tests the time and frequency of staying in one compartment or another is measured.

Elevated plus maze test (EPM). The EPM test is considered as the standard test for anxiety-like behavior in rodents (Leite-Almeida et al., 2015). It was first developed in rats by Pellow et al. in 1985 (Pellow et al., 1985) and by Lister et al. in 1987 for mice use (Lister, 1987). The test consists of a cross-shaped maze that is elevated from the floor with 2 open arms that are perpendicular to 2 closed

arms (Vuralli et al., 2019). The animal is placed in the centre and left to explore the premises for 5 minutes (Narita et al., 2006). The test relies on the quandary between the animal innate tendency to explore and its fear of open, lit and elevated places (Campos et al., 2013). Anxious animals spend less time in open arm. Indeed, inflamed animals exhibit anxious-like behavior just hours after pain induction and lasts for 3-4 weeks, whereas animal models of neuropathic pain develop this kind of behavior 3-4 weeks after the surgery and it lasts for 4 more weeks (Leite-Almeida et al., 2015). It is important to notice that the animal gets familiar with the cross shaped maze, thus introducing some bias (Tucker and McCabe, 2017). Therefore, the elevated zero maze was developed as it consists of a circular-shaped maze (Shepherd et al., 1994). One more pitfall is that the presence of the experimenter interferes with the results. Consequently, animals are videotaped for unbiased data (Vuralli et al., 2019).

Open field test (OF). The OF test represents the oldest test to assess anxiety-like behavior in rodents (Leite-Almeida et al., 2015). The test consists of placing the animal in the center of a square with high walls and let the animal explore the area freely for 5 minutes. The concept of this test is similar to the EPM, as it relies on the dilemma between exploratory behavior and fear of open spaces. Anxious animals tend to stay next to the wall and avoid the center, in fact mouse models of inflammatory pain exhibit anxiety-like behavior from 2 to 28 days after induction of the inflammation whereas neuropathic pain models may develop this kind of behavior 2 to 8 weeks after surgery or may not. This great variability could be linked to the type of neuropathy or to the test protocol; this is why it is not recommended to use this test as the sole determinant of anxiety-like behavior in neuropathic pain models (Kremer et al., 2020).

Light and dark box test (LDB): The LDB test was first developed by Crawley and Goodwin in the 1980's (Crawley and Goodwin, 1980). The box is divided into two compartments, one small and dark and the other big and brightly lit (twice as big as the small dark compartment) (Bourin and Hascoët, 2003). The animal is placed in the light compartment and left to explore the box freely for 5 minutes (Vuralli et al., 2019). The LDB concept is the same as the EPM and OF, as it is based on the conflict between the animal exploratory behavior and its aversion to light places. Anxious animal tend to avoid the light compartment. In fact mouse models of inflammatory pain exhibit anxiety-like behavior

from 1 to 28 days after induction of the inflammation whereas this kind of behavior may develop 4 to 8 weeks after surgery in neuropathic pain models (Kremer et al., 2020).

Depression-like behavior.

Forced swim test (FST). The FST is the most commonly used test to assess depression-like behavior in rodents. It was first developed by Porsolt in 1977 (Porsolt et al., 1977a, 1977b). The test consists of dropping the animal in a water filled cylinder for 5 minutes and measure the total duration of immobility (Leite-Almeida et al., 2015). When faced with the inability to escape (closed water cylinder), the animal stays immobile as a possible manifestation of depression-like behavior. Increased immobility is observed in mouse models of inflammatory pain between 4 to 35 days post induction and between 4 to 8 weeks post-surgery in neuropathic pain models (Kremer et al., 2020).

Tail suspension test (TST): TST is a depression-like behavior test applicable only in mice and is considered as a more sensitive variant of FST (Steru et al., 1985). It consists of suspending the mice by their tail for 6 minutes and measuring their duration of immobility. Increased immobility is observed in inflamed mice between 7 days (complete Freund's adjuvant model in mice, see §2) to 41 weeks (osteoarthritis model in mice, see §2) post induction and between 2 weeks to 2 months post-surgery in animal models of neuropathic pain (Kremer et al., 2020).

Sucrose preference test (SPT): The SPT developed by Katz (Katz, 1982), is a depression-like behavior test that assesses anhedonia which refers to the inability to feel pleasure. The animal has the freedom to drink either water or a sweet solution. Animal models of depression have a decrease ratio of usage of sweet solution over water. In fact inflammatory pain mouse models have their ratio decreased between 2 days to 4 weeks post induction and between 1 week to 10 weeks post-surgery in neuropathic pain models (Kremer et al., 2020).

2. Models of inflammatory pain

Two important parameters to be considered in animal models of pain are the method of injury and the endpoint measurement. The most appropriate models, whether an injury, application of chemical agents, or other manipulations, should be based on 1) understanding the clinical disease presentation and pathology (i.e. face validity); 2) producing nociception by recapitulating the mechanisms of

specific clinical conditions (i.e. construct validity). Measures of nociceptive behavior must not only detect pain-like responses but also do so in a manner consistent with the clinical experience of pain (Gregory et al., 2013). Measures of reflexive behaviors such as withdrawal thresholds to noxious stimuli have been used for decades to examine mechanisms of pain. These have clearly proven useful in advancing our understanding of the physiological basis of nociception, and the identification of neurotransmitters, receptors, intracellular messengers, genes, and circuits implicated in pain-specific mechanisms. They led to better understanding of existing pharmacologic and non-pharmacologic pain treatments (Basbaum et al., 2009b; Woolf, 1983b, 2011). In addition, past studies in rodent models of acute nociception and chronic pain indicated that the pharmacologic action (i.e. efficacy, potency, duration of action) of a broad spectrum of analgesics to reduce reflexive sensory responses have demonstrated relevance to human analgesia (Yaksh, 2002; Gregory et al., 2013).

Chronic pain can be the consequence of persistent inflammation (Burma et al., 2017). Injured peripheral tissue releases an inflammatory soup made of pro-inflammatory molecules comprising bradykinin, prostaglandin, cytokines and chemokines. This peripheral inflammation stimulates primary afferent neurons and causes peripheral sensitization. This can be accompanied by central sensitization due to the release in the spinal cord of neurotransmitters and neuromodulators such as glutamate, substance P or BD. This sensitization is transient in case of acute inflammation, whereas it becomes long lasting in chronic pain diseases (Ji et al., 2014). In the following, classical animal models of inflammatory pain will be listed first (**Table 1**) and followed by specific models for specific pathologies. As a matter of fact some animal models are specifically developed for well-defined pathologies. In this review, animal models of pain commonly used for osteoarthritis (which is the most common form of arthritis) and rheumatoid arthritis will be detailed.

Pain model induced by capsaicin

Capsaicin is a proto-alkaloid present in chili peppers and is considered to be the main irritant responsible for hot sensation (Ilie et al., 2019). Capsaicin induces a neurogenic inflammation. In fact capsaicin binds to transient receptor potential vanilloid 1 (TRPV-1) present on free neuronal endings of primary sensory neurons which will leads to the peripheral release of several inflammatory mediators: calcitonin gene-related peptide (CGRP), neurokinin A/B, somatostatin, vasoactive

intestinal peptide (VIP), and substance P (Ilie et al., 2019; Muley et al., 2016). These neuropeptides will thus induce the release of pro-inflammatory cytokines by nearby mast cells, endothelial cells, epithelial cells and immune cells. The local inflammation occurs with hyperalgesia. Administration of capsaicin is accompanied by 2 types of hyperalgesia, a primary one that is believed to be a consequence of peripheral sensitization and responds to thermal and mechanical stimuli, and a secondary one which is a result of central sensitization and responds to mechanical stimulation (Frias and Merighi, 2016; Ilie et al., 2019; Muley et al., 2016). Capsaicin effects are dose dependent. In fact, a high concentration or a continuous administration can lead to desensitization of TRPV1 resulting in an analgesic effect.

This inflammatory induced pain model, obtained after intra-plantar injection of capsaicin, is used in mice and rats (Carter and Francis, 1991; Caterina et al., 2000; Drewes et al., 2003; Laird et al., 2001a; Lynn et al., 1992) to mimic skin inflammation and inflammatory bowel disease. It has also been used to assess the efficiency of non-steroid anti-inflammatory drugs (diclofenac) (NSAID), or anti-epileptic drugs (gabapentin) (Muley et al., 2016).

Pain model induced by formalin

The formalin test is commonly used since it was created in 1977 (Dubuisson and Dennis, 1977). It consists of an injection (sub-cutaneous or intra-plantar) of a diluted solution (0,05-5%) of formaldehyde (Tjølsen et al., 1992). This pain model is characterized by a biphasic response. A short (0-5 minutes) first phase, where pain is believed to be a consequence of direct activation of primary sensory neurons, is followed by a second prolonged phase (10-40 minutes), where pain is the result of spinal cord inflammation within the dorsal horn, leading to central sensitization (Hunskar and Hole, 1987; Tjølsen et al., 1992; Raboisson and Dallel, 2004). Among the mechanisms involved in this model, it has been shown that formalin activates transient receptor potential ankyrin 1 (TRPA1) (McNamara et al., 2007) more particularly in phase 2. Interleukin-33 and its receptor ST2 are also believed to be involved in inducing pain in both phases. In fact, Il-33/ST2 pathway activation leads to activating mitogen-activated protein kinase (MAPK) signalling pathway cascades that are implicated in central sensitization (Han et al., 2015a; McNamara et al., 2007). In phase 1, pain-like behavior (paw licking and lifting, vocalization) can be observed. It is inhibited by lidocaine. In

contrast, in phase 2, pain is alleviated by NSAID, morphine and gabapentin (McNamara et al., 2007). The formalin induced model is used in rats (Dubuisson and Dennis, 1977; McNamara et al., 2007) and mice (Hunskar and Hole, 1987; Han et al., 2013) has largely contributed to better understanding of pain mechanisms (central and peripheral) (Muley et al., 2016).

Pain model induced by complete Freund's adjuvant (CFA)

CFA is a suspension of heat-killed *Mycobacterium tuberculosis* in paraffin oil (Barrot, 2012) that is frequently used for inducing inflammatory pain. CFA is injected subcutaneously in the hind paw or intra-articular. When injected in the hind paw, the inflammation occurs 24 hours after the injection and lasts for one to two weeks (Bas et al., 2016; Ren and Dubner, 1999). However, after intra-articular injection, the inflammation takes 7 days to occur (Muley et al., 2016). The mechanisms leading to CFA-induced inflammatory pain are still to be deepened but it is known that the injection of CFA leads to the release of pro-inflammatory mediators (PGE2, TNF alpha, IL-1) that induces synovitis, bone resorption and eventual degeneration. Inflammatory mediators are responsible of neuronal sensitization that is responsible for joint pain (Muley et al., 2016). CFA-induced pain models are frequently used to assess inflammatory pain in mice and rats (Fernandes et al., 2011; Keeble et al., 2005; Knight et al., 1992; McDougall et al., 1995; Nisar et al., 2015; Simjee et al., 2007; Uematsu et al., 2011). For example, the CFA model is considered a robust model to study arthritis, and particularly rheumatic arthritis (RA) since it mimics the synovitis and bone resorption observed in human RA but does not mimic cartilage alteration (Bas et al., 2016).

Pain model induced by carrageenan

This model is mainly used to study inflammatory pain, more specifically joint inflammation (Mert et al., 2018). Carrageenan is used to induce transient joint inflammation, where hyperalgesia and allodynia are observed (Fehrenbacher et al., 2012; Winter et al., 1962). A carrageenan solution with a concentration centered between 0.5-2 percent is injected subcutaneously, the swelling is observed 3-5 hours after the injection and it lasts for 24 hours (Otterness and Moore, 1988; Winter et al., 1962).

Pain model induced by zymosan

This self-resolving model is used to study acute inflammation (Cash et al., 2009). Indeed, 30 minutes after injecting zymosan, which is an insoluble polysaccharide component of the cell walls of *Saccharomyces cerevisiae*, animals develop edema and inflammation as well as thermal and mechanical hyperalgesia (Doherty et al., 1985; Meller and Gebhart, 1997). Thermal and mechanical hyperalgesia are dose dependent, and spontaneous pain is observed with greater doses (Doherty et al., 1985; Meller and Gebhart, 1997).

Osteoarthritis pain models

Arthritis is a medical condition in which the inflammation of the joint leads to chronic pain and movement limitation. In 2012, 52.5 million USA adults suffered from arthritis. This number is thought to increase and reach 78.4 million by year 2040 (Hootman et al., 2016). Osteoarthritis (OA) is a progressive musculoskeletal disease that leads to the failure of the entire joint (Teeples et al., 2013; Alshami, 2014). Novel treatment that tackles pain and helps increase activity is of necessity and therefore animal models of pain are needed to deepen the understanding of the underlying physiopathology of OA.

Chemically induced models

A wide range of chemicals are used to induce OA-like models, including papain, trypsin, carrageenan, kaolin, hyaluronidase, collagenase, sodium urate and mono-iodoacetate (MIA) (D'Souza et al., 2011a; Fang and Beier, 2014). Notably, MIA is the most commonly used model to assess OA pain and one of the best models to study symptom-modifying OA drugs (D'Souza et al., 2011b). MIA injection was first used by Dieter Kalbhen (Kalbhen, 1987). The mechanism of action of MIA consists of inhibiting the glyceraldehyde-3-phosphate dehydrogenase in chondrocytes; thus inhibiting the glycolytic pathway and eventually leading to chondrocyte apoptosis, subchondral bone necrosis and cartilage degeneration, consequently mimicking the morphology of end stage OA (D'Souza et al., 2011a; Fang and Beier, 2014). Subsequent to MIA injection, there are 2 phases. The first one consists of an early (few days after) inflammation, where pain is reversed by non-steroidal anti-inflammatory drugs (NSAIDs). In the second phase, the joint is destructed and the expression of the nerve injury

marker activating transcription factor-3 (ATF-3) is increased in the L5 dorsal root ganglion. The pain during this phase may be lowered by the administration of morphine/gabapentin. MIA injection is used mainly in rats and also in mice and guinea pigs. In rats and mice the clinical outcomes are characterized by mechanical hypersensitivity in hind paw, alteration of sleep, and locomotive deficit, whereas in guinea pig mechanical allodynia is observed with altered weight bearing (D'Souza et al., 2011a; Fang and Beier, 2014; Malfait et al., 2013). The intra-articular (knee) injection of the above-listed chemicals is a low-cost procedure that leads to rapid severe joint degeneration. Unfortunately the clinical relevance of this model to model OA is not as accurate as with other procedures.

Surgically induced models.

Surgically induced models are obtained after joint surgery that is consequently unstable and altered. The cartilage degeneration, which is proportional to the degree of joint instability, depends on the joint structure. The most common procedures are performed on mice knees and leads to a fast and reproducible disease progression.

Anterior cruciate ligament transection (ACLT). It has been used for decades on large animals and nowadays this procedure is performed on rodents (Gomis et al., 2007; Stoop et al., 2001). The post-surgery physiopathology evolves over time with changes in chondrocyte physiology at 4 weeks post-surgery, mild cartilage destruction at 8 weeks, and osteophytes formed at 12 weeks post-surgery. When ACLT is combined to the removal of medial meniscus, the joint destruction and osteophytes formation are observed only at 4 weeks post-surgery. It is important to mention that in human OA, support structures are not all ruptured (D'Souza et al., 2011; Teeple et al., 2013).

Meniscectomy. The meniscectomy in rodents could be partial or total, lateral or medial, unilateral or bilateral (Bendele, 1987; Bove et al., 2006; Knights et al., 2012). At 4 weeks post-surgery, the cartilage starts to get damage and OA lesions progressively develop from 8 to 12 weeks post-surgery (Knights et al., 2012). This procedure is questionable since there is no consistency in the amount of meniscus that is being removed (Teeple et al., 2013).

Destabilisation of medial meniscus (DMM). The DMM is very common in mice (Glasson et al., 2007). Transection of the medial meniscotibial ligament leads to mild instability with cartilage destruction, subchondrial bone sclerosis and osteophyte formation. Cartilage lesions appear at 2 weeks post-surgery and progress for 16 weeks after this period. This procedure is the most common

since it is reliable, reproducible and structurally similar to human OA. Furthermore, pain is reversed by standard analgesics. Finally, the disease progression is slower when compared to others (D'Souza et al., 2011a).

Streptococcal cell wall (SCW)-induced arthritis

This model is used to mimic RA in rats. It consists of an intra-articular injection of *Streptococcus pyogenes* cell walls. A single injection leads to an acute inflammation within one day, whereas multiple injections lead to chronic RA (Bessis et al., 2017; van den Broek et al., 1988).

Osteoarthritis in companion animals

OA is a natural occurring disease in cats, dogs and horses and appears to be very similar to human disease. Studies in these large animals, although less used than small animal models, are often required in translational research (Lascelles et al., 2018; McCoy, 2015). Dogs are considered as an excellent model to study human OA and remain the most used, especially in preclinical trials (Aragon et al., 2007; McCoy, 2015; Pelletier et al., 2010). However, natural racehorse OA is the best-suited model to investigate the pathophysiology of post-traumatic osteoarthritis in humans, especially in athletes (McCoy, 2015; McIlwraith et al., 2012). In particular, the stifle joints articular cartilage, the most frequent altered tissue in OA suffering horses, is highly similar to the human knee cartilage. Although some pain research studies have been carried out in induced-OA companion animals, OA-natural companion models are, along with cancer-natural models (see section 4), valid models for modelling human pain (Klinck et al., 2017).

Thermal and mechanical nociception and pain tests commonly used in rodents have been adapted for these large animal models, such as facial expression analysis with the Grimace Scales (de Grauw and van Loon, 2016; Holden et al., 2014). For OA large animal models, subjective lameness scoring and kinetic gait analysis are also a widely method to assess OA pain (Moreau et al., 2014). Clinical metrology instruments (CMIs), questionnaires designed for pet owners, have improved the assessment of sensory and affective effects of pain in companion animals. Several OA CMIs exist for dogs, e.g. The “Canine Brief Pain Inventory” (CBPI) and “Liverpool Osteoarthritis” (LOAD), and cats, e.g. ‘The Owner Behavior Watch’ (OBW) and ‘The Feline Musculoskeletal Pain Index’ (FMPI). Although these CMIs are subjective methods to assess pain, they are validated by comparative

analysis with objective tests for diagnosis and for outcome measurements in clinical research (Stadig et al., 2019; Walton et al., 2013). The positive therapeutic outcome of the anti-NGF antibodies in OA suffering dogs and cats is a good example of the translational positive values of companion animal models (see § 10; (Gruen et al., 2016; Kelly et al., 2019; Lascelles et al., 2018; Sanga et al., 2017)).

Rheumatoid arthritis models

An average of 0.5-1% of the general population suffer from rheumatoid arthritis (RA) (Bas et al., 2016). RA is a chronic autoimmune disorder characterized by T cells' activation of macrophages and release of pro-inflammatory cytokines, e.g. tumour necrosis factor α (TNF α), interleukin (IL) 1, 6 and 17. This results in inflammation of the synovial membrane as well as cartilage and bone erosion (Bas et al., 2016; Caplazi et al., 2015). Immunotherapy is the most commonly used treatment for RA but the underlying cause of T cells reactivation is still unknown. Therefore, animal models are still needed and should new lights on pathophysiological mechanisms.

Collagen-induced arthritis (CIA)

CIA is the most frequently used models for RA since there is a good resemblance with human RA with the presence of rheumatoid factor and anti-citrullinated peptide antibody (Bas et al., 2016; Caplazi et al., 2015). The experimental procedure consists of an intra-dermal (ID) injection of a combination of CFA and collagen II, which constitutes the major form of articular cartilage, leading to chronic polyarthritis in periarticular joints (Bas et al., 2016; Caplazi et al., 2015; Courtenay et al., 1980; Fischer et al., 2017).

Collagen antibody-induced arthritis (CAIA)

CAIA is obtained after intravenous or intraperitoneal injection of a mixture of anti-CII antibodies, followed by lipopolysaccharide (LPS) injection 3 days after (Bas et al., 2016; Caplazi et al., 2015; Nandakumar et al., 2003). Inflammation is at its maximum 8 days after the injection and lasts for a month (Lynn et al., 1992). This model is frequently used because of its histological similarities with human RA. In addition, bone and cartilage degradation as well as synovitis and pannus are observed in CAIA (Fischer et al., 2017). In this model, mechanical and thermal (hot and cold) hypersensitivity are observed. CAIA can be implanted in rodents that are unsuitable for CIA. However the

inflammation observed is innate and not related to B cell nor T cell activation (Caplazi et al., 2015; Fischer et al., 2017).

3. Models of migraine pain

Migraine has not classically been considered an inflammatory disease probably because it is not obviously associated with heat, redness, and swelling. Instead, a vascular etiology was proposed and the prevailing theory of migraine for most of the twentieth century, held that pain results from an abnormal dilatation of intracranial blood vessels, leading to mechanical excitation of sensory fibers. However, in recent years, advancements in the neurobiology of migraine headache have shifted the emphasis away from vascular smooth muscle toward mechanisms related to inflammation (Waeber and Moskowitz, 2005). Accumulating data have come, in large part, from basic science research utilizing small animal models of migraine-related pain (Akerman et al., 2017; Harriott et al., 2019; Vuralli et al., 2019c). Although the vascular system plays a crucial role in the headache associated with migraine that distinguishes it from somatic or visceral pain, a neuronal involvement in the aetiology of migraine has increasingly been considered (Olesen et al., 2009). Several lines of evidence suggest that activation of trigeminal nociceptors innervating meningeal tissues is central to the initiation of migraine pain. Once activated, trigeminovascular afferents release neuropeptides that, in turn, may mediate additional release of mast cell contents and other immune mediators (Harriott et al., 2019). Exposure of perivascular fibers to inflammatory agents released in the vicinity of sensory fibers alters their sensitivity and leads to the sensation of head pain. Animal models ultimately seek to reproduce the etiology of migraine with various experimental paradigms described below.

Dural stimulation

Peripheral stimulation of meningeal nerve terminals can be achieved electrically or chemically. The direct electrical stimulation of meningeal nerve terminals (Zagami et al., 1990) derived from the demonstration in human that it results in pain often referred to the face. Such stimulation elicits the polysynaptic activation of the central projection sites of these afferents in the trigeminal nucleus caudalis and ascending projections throughout the brain (Benjamin et al., 2004). These studies have

been key in identifying major migraine-related pain processing in preclinical research (Akerman et al., 2013). Direct electrical stimulation is however a nonrecoverable procedure and other protocols are necessary to mimic chronic activation of the dura.

Indeed, direct application of inflammatory soup on the exposed dura mater induces cephalic hypersensitivity (Melo-Carrillo and Lopez-Avila, 2013). This chemical stimulation elicits the expression of pronociceptive markers in the trigeminal ganglia (Lukács et al., 2015). This paradigm authorizes repeated applications producing a long-lasting central sensitization that mimics the pathophysiology of migraine (Munro et al., 2017). Less invasive refinements of this paradigm make it compatible with behavioral testing in conscious freely-moving animals.

Electrophysiology is a common readout for the various procedures of dura mater direct stimulation. It is a powerful assay method that allowed identifying effective mechanisms such as the triptans and CGRP receptor antagonists, while predicting failure of neurokinin (NK)1 receptor antagonists (Akerman et al., 2013).

Trigeminal neuron stimulation

The stereotaxic insertion of electrodes allows to directly stimulate trigeminal ganglion neurons which causes release of CGRP from perivascular afferent terminals to the dura mater. Short (3-5 min) and long (30 min) stimulation paradigms have been used with the latter inducing morphological changes (Knyihár-Csillik et al., 1997). Notably, neurochemical and morphological changes are sensitive to triptan and dihydroergotamine, thus making the inhibition of peripheral neuropeptide release a plausible mechanism of anti-nociceptive action (Buzzi et al., 1991).

In vivo application of algogenic substances

The most widely used models in preclinical migraine research rely on systemic infusion of nitric oxide donors, e.g. glycerol trinitrate (GTN) (Munro et al., 2017). Intraperitoneal administration is replaced in some studies by intravenous infusion that can directly activate the trigeminovascular system (Ramachandran et al., 2014). Beside acute administration, other authors have developed repeated intermittent intraperitoneal administration (Pradhan et al., 2014), or intradermal injection of

GTN in the hindpaw (Ferrari et al., 2016). Both variants have clinical relevance, resulting in progressive and sustained basal hyperalgesia and showing sumatriptan sensitivity, respectively. Interestingly in these models, the effects of algogenic substances are not restricted to specific peripheral or central nervous system sites but are likely to act more broadly in migraine-related structures (Harriott et al., 2019).

Genetic models

Among the rare genetic models of migraine, mouse models expressing gain of function missense mutations of the CACNA1A faithfully recapitulate the symptoms of familial hemiplegic migraine (FHM), a rare monogenic migraine subtype associated with aura. Notably, CACNA1A, as well as other genes implicated in FHM, encode ion channel and transporter subunits that play important roles in neurotransmission.

Cortical spread depression model

The aura is associated with migraine in up to 30% of patients. The aura results from a wave of intense excitation across the visual cortex that has been attributed to cortical spreading depression (CSD) process. CSD is a slowly propagating depolarizing wave that can be induced experimentally in rodents where it is initiated by injection of KCl into the cortex (Munro et al., 2017). CSD triggers neuronal activation within the trigeminal ganglion and in higher brain regions (Cui et al., 2015). Moreover, CSD also increased activation of microglia that is a key mechanism relevant to central sensitization and contributing to pain sensitivity.

4. Models of neuropathic pain.

Neuropathic pain (NP) is a painful syndrome caused by central or peripheral lesion of the nervous system. It is highly disabling, affecting 7-8% of general population (Bouhassira et al., 2008b). NP elicits sensory alteration including dysesthesia and paresthesia, spontaneous pain, increase pain sensation for innocuous stimuli (allodynia) and increase pain sensation for noxious stimuli (hyperalgesia). Animal models of NP are developed, mainly in rodents, to recapitulate one or several

symptoms of NP aiming at deciphering the underlying mechanisms. Larger animals, e.g. cat, have also been used to study NP mechanisms (Koyama et al., 1993). NP models are distinguished according to the localization, central or peripheral, of the nerve injury.

Models of central NP

Central pain syndrome (CPS) classified as neuropathic pain is defined as “pain initiated or caused by a primary lesion or dysfunction of the central nervous system” (International Association for the Study of the Pain, Merskey and Bogduk, 1994) or more recently as “pain arising as a direct consequence of a lesion or disease affecting the central somatosensory system” (Treede et al., 2008). CPS patients display somatosensory abnormalities (hyposensitivity to thermal and noxious stimuli), which is the universal feature of this syndrome, due to the spinothalamoparietal thermoalgesic pathway dysfunctions (Canavero and Bonicalzi, 2007). CPS patients suffer from dysesthesia and painful burning sensation whose localization in body regions, and intensity and duration, widely differ, depending of the syndrome etiology. CPS onset can be delayed several months after CNS damage but is a lifetime condition in the majority of cases (Berić, 1998; Canavero and Bonicalzi, 2007). CPS is mostly induced by stroke (referred as central post-stroke pain), multiple sclerosis or brain/spinal cord injury (SCI). Diseases displaying a CPS-like component such as Parkinson’s disease are referred as central pain-allied conditions (CPAC). Depending of the origins of the lesions, patients suffer from brain central pain (brain-brainstem lesion) or cord central pain (spinal cord lesion).

CPS has been mostly studied in rodents by performing SCI, either targeting individual fiber tracts, or extensively affecting the cord. Electrolytic lesions (one or two) of the rat spinothalamic tract afferents are performed using tungsten or quartz-insulated platinum or electrodes (Wang and Thompson, 2008; Masri et al., 2009). The electrode is targeted unilaterally to the ventrolateral spinal quadrant in the lower cervical or thoracic vertebral level (Masri et al., 2009; Wang and Thompson, 2008). Demyelinating lesions have also been carried out by the injection of lysophosphatidylcholine solution through a glass pipette inserted into the region of the rat spinothalamic tract (Wang and Thompson, 2008). CCP rodent models allowed to identify the biological bases of this syndrome (Masri et al., 2009; Naseri et al., 2013; Quiton et al., 2010; Wang and Thompson, 2008) and to test therapeutic approaches (Lucas et al., 2011; Wang and Thompson, 2008).

Other models are generated by a more extensive SCI, not only restricted to fiber tracts, e.g. spinal cord compression, partial or complete sections, photo-chemically induced ischemia, spinal cord crushing, contusion, or application of excitatory neurotoxins (**Table 1**). Even if paralysis is the core symptom of this model due to the lesion of descending motor tracts, other features of SCI impair quality of life such as pain (Caplazi et al., 2015; Fischer et al., 2017; Simpson et al., 2012). Extensive lesions of the spinal cord are associated with spontaneous and evoked pain resulting in both hyperalgesia and allodynia (Campbell and Meyer, 2006; Schneider et al., 2017; Starkey et al., 2009).

Contusive SCI is usually obtained by using a weight-drop technique (Siddall et al., 1995). In this model, rats developed allodynia few days after SCI, that lasts up to 30 days after motor recovery (Siddall et al., 1995). Spinal hemi-section mimics chronic central pain after spinal cord lesion (Christensen et al., 1996). This model offers advantages of maintaining the injured site separate from the intact side. It is obtained by performing a hemi section of a thoracic segment, usually cranial to L1 dorsal root entry. Spinal hemi-section induces changes in withdrawal thresholds in response to both mechanical and thermal stimuli that persist for 5-6 months. In this model, NP develops both below and above the SCI on both sides. This model recapitulates the NP in Brown–Sequard Syndrome, a spinal cord hemi-section injury, where chronic pain also develops on both sides (Koehler and Endtz, 1986).

Photochemical injury, developed by Watson and Colleagues (Watson et al., 1985), presents the advantage to reproduce a mechanical trauma of the spinal cord without surgery. It consists of intravenous injection of the photosensitizing dye, Rose Bengal or erythrosin B, followed by an irradiation. This reaction induces thrombosis leading to ischemia and tissue necrosis of the spinal cord (Gaviria et al., 2002). Injury induces a strong allodynia in different areas innervated by the ischemic spinal segments (caudal trunk, hind limbs, and hind paws) and a hypersensitivity to cold but not to heat stimuli.

Models of peripherally-induced NP

Various models have been proposed to copy consequences of radiculopathies or post-operative nerve lesions (**Table 1**). Classical models are obtained by lesioning peripheral nerves, e.g. the infra-orbital nerve (face neuropathy) or the sciatic nerve (hindlimb neuropathy). Peripheral NP models

elicit peripheral but also central effects, including spontaneous discharges from afferent neurons, nociceptor sensitization and spinal and cortical reorganization.

Nerve transection.

The sciatic nerve total transection is achieved by a complete axotomy of the sciatic nerve removing approximately 5mm of the sciatic nerve in rats or mice. This model mimics the clinical symptoms of “phantom limb” that occur in humans after transversal spinal lesion. The limitation of this axotomy model is the motor impairment, which hampers behavioral evaluation by testing withdrawal latencies, and causes frequent autotomy. For ethical considerations, these axotomy models are now replaced by partial nerve injury in which autotomy is strongly reduced or totally suppressed.

Partial nerve lesion.

Several partial nerve lesion models have been developed over the last 20 years. These models differ according to their procedure, their reproducibility, the inflammatory component and the duration of NP symptoms. Chronic constriction injury (CCI) (Bennett and Xie, 1988) consists of four loose knots around the sciatic nerve or the infra-orbital nerve. This model involves both neuropathic and inflammatory components triggered by suture thread (Costa et al., 2005). CCI elicits spontaneous pain and pain hypersensitivity that occurs 24 hours after surgery and persists for a duration of at least 7 weeks. Variability in responses occurs upon variation in the tightness of ligature and thread used. Cuffing of sciatic nerve (“cuff” model) allows a standardized and reproducible chronic constriction injury; it is obtained by the implantation of a section of polyethylene tubing placed around the common branch of the sciatic nerve of rats or mice (Pitcher et al., 1999; Benbouzid et al., 2008; Yalcin et al., 2014b). Animals develop heat-hyperalgesia lasting for 3 weeks and mechanical allodynia for at least 2 months (Benbouzid et al., 2008). This model is of interest to study anxio-depressive comorbidities associated with NP that lasts for weeks after removal of hypersensitivity to pain (Yalcin et al., 2014b). Partial sciatic nerve injury (PNI), developed in rats by Seltzer and colleagues (Seltzer et al., 1990), consists of tight partial ligation of the dorsal third or half of sciatic nerve, peroneal or tibial branch or both. Pain symptoms occur within 1 week after surgery and continue up to 6 weeks post-injury. PNI has been adapted in mice by Malmberg and Basbaum (Malmberg and Basbaum, 1998) and showed pain symptoms one day after injury. The thermal allodynia resolves by 49 days, but the mechanical allodynia persists for the duration of the study (70 days). Spinal Nerve Ligation

(SNL) was developed in rats by Kim and Chung (Kim and Chung, 1992). It consists of a unilateral and tight ligation of lumbar spinal nerves L5 and L6 distal to dorsal root ganglia. Within 24 to 48 hours after surgery, this model develops a mechanical allodynia, thermal hyperalgesia and spontaneous pain that persist for 4 months without any autotomy. Although the surgery is complex, it is a highly reproducible method with little damage to surrounding tissue (Dobremez et al., 2005; Fossat et al., 2010; Kim and Chung, 1992; LaBuda and Little, 2005; Laffray et al., 2012). SNL model is also available in mice (Kiso et al., 2008) where mechanical allodynia started at day 1 and lasted for at least 2 months after surgery. Spared nerve injury (SNI) corresponds to the lesion of two of the three terminal branches of the sciatic nerve that is obtained by the ligation of common peroneal and tibial nerves leaving the remaining sural nerve intact (Bourquin et al., 2006; Decosterd and Woolf, 2000). Mechanical and thermal responsiveness is increased in the territory of spared sural nerve. SNI model displays early and persistent pain-like behaviors; it is easy to perform and highly reproducible. Caudal trunk resection is performed by lesioning the caudal trunk in rat or mice (Na et al., 1994; Sung et al., 2000). Allodynia and hyperalgesia appear within a day of nerve injury and last for weeks (Back et al., 2003). In rats and mice, this model offers several advantages by allowing behavioral tests, thermal and mechanical stimuli to be easily performed at the same spot on the tail.

These models of peripheral neuropathy induce pain-like behavior as a direct consequence of nerve lesion (i.e. sciatic nerve for the hindlimb or infra-orbital nerve for the face). These models have the ability to recapitulate some particular neuropathic syndromes in human. For instance, CCI, SNI and PNI targeting the sciatic nerve are models of complex regional pain syndromes type II (CRPS) that same sensory disorders similar to those described in patients, e.g. touch hypersensitivity, mechanical hyperalgesia, spontaneous pain, and pain irradiatio. Moreover, in the case of CCI, the strong inflammatory component mimics some features of the chronic low back pain syndromes in human. Finally, SNL that targets dorsal roots of the sciatic nerve models sciatica or radiculopathy (defined as pain resulting from injury or disease of the cervical, thoracic, lumbar or sacral nerve roots). Although these models present interesting features, there is still today a large gap between the number of molecules effective in preclinical studies and their poor outcome in clinical trials. Several explanations can be evoked such as the genetic homogeneity of the models (inbred mouse/rat), the low diversity of the behavioral tests generally used (i.e. withdrawal reflexes), the similarities between the different preclinical models (nerve injury modelling mononeuropathy) and the almost exclusive

use of male rodents. Future challenges will be to determine precisely which mechanisms is targeted by a given drug, and which patients will be relevant for treatment (Rice et al., 2018). For this purpose, the relevance of pain assessment in animal models, and the similarities between animal behavior and human symptoms are critical.

Models of poly-neuropathy (PN)

Notably, studies on animal models of mononeuropathic pain most largely report sensory gain (Rice et al., 2018). However, traumatic nerve injury is most often characterized by a loss of sensitivity to non-painful stimuli, and a moderate sensory gain to painful stimuli (Gierthmühlen et al., 2012). In addition, most of clinical trials involve patients with polyneuropathy (Rice et al., 2019). In human clinics, polyneuropathies (peripheral neuropathies) are the most common type of disorder of the peripheral nervous system in adults, and specifically in the elderly, with an estimated prevalence of 5–8%, depending on age. A sensory loss profile is reported in the majority of patients with common polyneuropathies for example: chemotherapy-induced (Ventzel et al., 2018), diabetic (Raputova et al., 2017) and HIV-associated associated (Phillips et al., 2014) polyneuropathies, and spinal cord injury (Finnerup et al., 2003) or postherpetic neuralgia (Fields et al., 1998). Recently, relevant disease models have been developed and validated, especially those related to drug-induced neuropathy, diabetic and HIV-associated neuropathy and autoimmune neuropathy. Some examples of these models are briefly discussed in the following.

Chemotherapy-induced neuropathy

Peripheral neuropathy is a dose-limiting side effect for a number of chemotherapeutic agents including platinum analogues, microtubule-directed agents (taxol, vincaalkaloids), suramin or thalidomide. Cisplatin neurotoxicity is very common and can be mimicked in mice and rats by repeated injections of platinum for several weeks. Mechanical and thermal hyperalgesia and allodynia appear in the 2–4 weeks following the beginning of treatment (Authier et al., 2003). Oxaliplatin is another chemotherapeutic drug used for the treatment of gastrointestinal tract tumours, especially in colorectal cancer (Ibrahim et al., 2004). Oxaliplatin induces acute side effects in 80% of the patients, and can evolve to a long-term neuropathy in a fraction of the affected patients (around 15-20%). Mouse models of chemotherapy-induced neuropathy are generated by repeated (e.g. twice a week for

'weeks) intravenous injection of oxaliplatin in the tail vein (Marmioli et al., 2017). Chronic treatment with oxaliplatin has been reported to induce shrinkage of Dorsal Root Ganglia (DRG) neurons and axonopathy of myelinated fibres in animal models (Renn et al., 2011), and impairs sensory neuron arborisation, indicating that oxaliplatin neurotoxicity occurs mainly in sensory neurons (López-González et al., 2018).

Diabetic neuropathy

Diabetic neuropathy is a progressive disease that leads to structural changes in the nerve causing hyperalgesia. Various animal models, with possible different pathogenesis underlying mechanisms, exist and highlight the importance of establishing clear criteria for models of diabetic neuropathy in rodents.

The most common metabolic models are the streptozotocin (STZ) diabetic rats and mice (mainly Swiss, C57/Bl6 or CD1) (Biessels et al., 2014). Depending on the severity of anticipated disease, diabetes is induced by a single intraperitoneal or intravenous dose of 40–80 mg STZ/kg (rat), or 150–200 mg STZ/kg (mouse) body weight, or of lower doses given to mouse over consecutive days. STZ neurotoxicity was reported to induce early behavioral and electro-physiological changes followed by distal nerve fiber loss, axonal atrophy and myelin thinning after many months of diabetes. However, STZ toxicity may be indirectly responsible for neuropathy (Davidson et al., 2009).

A large variety of genetic model have been also proposed to recapitulate the pathophysiology of diabetes. The BB/Wor and BBZDR/Wor rats model type 1 and type 2 diabetes respectively. The BB/Wor rats lack T lymphocytes expressing the RT6 alloantigen, and develop auto-immune attack of the pancreas and spontaneous onset of type 1 diabetes in males between the ages of 70–80 days (Yang and Santamaria, 2006). The BBZDR/Wor-rat spontaneously develops insulin resistance that is preceded by obesity and these animals model type 2 diabetes (Tirabassi et al., 2004). In mouse, there are two common genetic models of type 1 diabetes: the non-obese diabetic (NOD) mouse and the Akita mouse, representing spontaneous auto-immune type 1 diabetes (NOD) and a mutation of the insulin-2 gene, respectively. There is currently relatively little consistent information on the neuropathy in these animal models (Biessels et al., 2014). Models of type 2 diabetes, such as db/db (leptin receptor mutation) and ob/ob (leptin mutation) mice, have been available for many years

(Wang et al., 2014). Both models develop diabetes at 4–6 weeks of age with hyperinsulinemia and hyperlipidemia. Early electrophysiological changes, and later structural abnormalities have been reported during the progression of the disease. The main limitation of both the db/db and ob/ob models is that death occurs at 24–30 weeks of diabetes in the absence of insulin supplementation.

Autoimmune neuropathy

Autoimmune neuropathies comprise a diverse group of conditions resulting from an immune attack on the peripheral nervous system. The prototypic immune neuropathy is the heterogeneous Guillain-Barré syndrome (GBS), the most common acute paralytic disorder in industrialized countries. It is classically considered as an acute inflammatory demyelinating polyneuropathy (Fricker et al., 2008). The pathological substrate for GBS has been well established with immune cell infiltration, demyelination with or without axonal damage in the peripheral nervous system (PNS), nevertheless molecular and cellular mechanisms require adapted animal models to be fully understood. In the classical models of experimental allergic neuritis (EAN), the auto-immune aetiology of GBS was reinforced using homogenate of peripheral nerve tissue in adjuvant (Waksman and Adams, 1956). Classical EAN is induced in susceptible rats (the Lewis rat strain is the most sensitive to auto-immune conditions) or mice by active immunization (complete Freund's adjuvant) with peripheral myelin extract, or the whole or partial purified P2 myelin protein (Hahn, 1996). Adoptive transfer EAN is induced by injection into naive animals of antigen-specific T cells obtained from lymph nodes of EAN syngenic animals (Toyka, 1999). Although EAN has provided valuable information, it has been criticized for its artificial manipulation (Yang et al., 2014). Therefore, genetic models have been raised that develop spontaneous autoimmune peripheral polyneuropathy caused by B7-2 (Salomon et al., 2001) or CD4 (Yang et al., 2014) depletion in mouse.

Infectious neuropathy

Infectious neuropathies include several diseases associated with infectious micro-organisms targeting the central (e.g. neurosyphilis, viral meningitis) or the peripheral (e.g. leprosy, HIV, Lyme disease, hepatitis C) nervous system. Here we provide examples of animal models of the two most common neuropathies induced by an infectious agent, leprosy and HIV-associated neuropathy. Leprosy is the most common sensory multiple mononeuropathy, still largely present in developing countries. Leprosy is caused by the non-toxic non-cytolytic *Mycobacterium leprae*, an obligate

intracellular parasite. The proliferation of *M. leprae* in the mouse footpad (MFP) has been widely used to test bactericidal activity (Ji et al., 2006) and to define the role of immunity in leprosy after MFP infection in immune-deficient mice (Fricker et al., 2008; Rambukkana et al., 2002). However, the development of more representative models of human leprosy is still necessary to better account for a real leprosy neuropathy.

In HIV-associated neuropathy, peripheral sensory structures (DRG, peripheral nerves and cutaneous terminals) are affected. Distal sensory polyneuropathy (DSP) is the most common HIV-associated neuropathy and is present in one third of HIV-infected patients. Classical animal models rely on the postulated direct toxicity of secreted viral proteins (gp120 and Tat) on DRG and axons leading to degeneration and the development of DSP. Gp120 can be delivered acutely through an intradermal injection, or chronically, directly to the sciatic nerve, with a carrier matrix, thus inducing allodynia and hyperalgesia (Herzberg and Sagen, 2001). HIV infection may also heightens the vulnerability to antiretrovirus-induced neurotoxicity. Indeed, in transgenic mice continuously releasing gp120 from astrocytes and Schwann cells, food supplementation with the antiretroviral agent didanosine results in small unmyelinated fiber degeneration and hyperalgesia (Keswani et al., 2006). Similarly, the intravenous injection of a common protease inhibitor, indinavir, triggers hindpaw mechanical hypersensitivity in rats (Huang et al., 2017). Notably, DSP in these models is responsive to analgesic compounds, e.g. gabapentin.

5. Models of cancer pain

Several mammalian and non-mammalian animal models have been developed to improve our understanding of cancer biology (Schachtschneider et al., 2017). However, cancer pain, which is experienced by human patients and animals in advanced stages of cancer, has only been addressed in some of these models.

Immunocompromised and immunocompetent rodents (mouse and rat) are commonly used to study cancer pain. Because of its large size, rat model is better suited for manipulations and injections into bones compared to mouse. However, mice are more widely used compared to rats mostly due to the possibility to address cancer pain in transgenic mice either in knock-out or -in overexpressing gene mice (Ghilardi et al., 2005a; Lindsay et al., 2005). Larger animals also used are dogs and less

commonly cats as they develop spontaneous tumors such as mammary, prostate, head and neck squamous cell carcinoma (HNSCC), squamous cell carcinoma (SCC). In dogs, cancer is the number one cause of mortality with osteosarcoma (OSA) being the most common primary bone tumor, and a painful one. Dogs and cats display similar cancer biology when compared with humans and are therefore appropriate models for human cancer (MacEwen, 1990; Simmons et al., 2015). Methods used to quantify cancer pain are various and dependent of the animal species used. In rodents, the von Frey assay remains most widely used although non-evoked pain tests are also applied (**Table 2**). Cancer pain in dogs is addressed mostly using owner-completed questionnaires and activity sensory function measure assays (Brown et al., 2015; Monteiro et al., 2018) (**Table 2**).

Bone cancer leads to the most painful conditions with patients suffering from chronic pain and breakthrough pain. Moreover, as bone is the major site of metastasis in advanced stage cancer, e.g. mammary and prostate cancers, it is expected that bone cancer induced pain (CIBP) models are the first cancer pain models to be developed. Metastatic bone cancer pain models were developed via intravenous, intra-cardiac (left ventricle of the heart), or orthotopic injections of cancer cells (Arguello et al., 1988; Liepe et al., 2005; Yoneda et al., 1994). However, assessment of bone induced pain cancer has been proven difficult in these models due to the differences in size, tissue/organ localization of the metastases. The first primary bone cancer pain model was developed in 1998 by injection of NCTC 2472 fibrosarcoma cells into mouse intramedullary space of long bone, i.e. femur (Schwei et al., 1999). The first rat model was developed a few years later by injection of MRMT1 mammary carcinoma into the intramedullary space of tibia (Medhurst et al., 2002). A model of breakthrough cancer pain defined as an intermittent episode of extreme pain has been recently developed (Tang et al., 2016). Non-bone cancer models were also developed using orthotopic injections (see **Table 2**). The differences between these different rodent models depend on the site of injection and the type of cancer cell injected (**Table 2**). Development of these diverse models allowed pharmacological interventions with preclinical positive outcome (reviewed in (Slosky et al., 2015)). Indeed, analgesic effects of resiniferatoxin, a TRPV1 activator, were demonstrated on intractable osteosarcoma suffering dogs and in phase I clinical trials on patients with intractable cancer (Brown et al., 2015). Moreover, the use of various animal models has validated the anti-NGF therapy for cancer pain management (Jimenez-Andrade et al., 2011; Sevcik et al., 2005). Tanezumab and Fulranumab, two NGF antibodies, safety and efficiency have been tested in phase I and phase II

clinical trials (Slatkin et al., 2016; Sopata et al., 2015). A phase III trial using Tanezumab is currently in progress to determine whether Tanezumab is effective in the treatment of cancer pain due to bone metastasis in patients already taking background opioid therapy (see ClinicalTrials.gov).

The use of these different models has provided insight into the mechanisms involved in cancer pain (reviewed in (Falk and Dickenson, 2014)) and brought *in vivo* evidence of the implication of tumor secreting proteins, e.g. endothelin 1, (Tang et al., 2016; Wacnik et al., 2001)), microRNAs (Bali et al., 2013; Elramah et al., 2017), neurotrophins, receptors (e.g. osteoprotegerin, (Honore et al., 2002)), and TRPV1 (Ghilardi et al., 2005a; Sapio et al., 2018). In particular, the implication of the purinergic P2X receptor family and purinergic pathway in cancer pain has been shown using these rodent models (Falk et al., 2019; Gilchrist et al., 2005; Guedon et al., 2016; Wu et al., 2012).

6. Models of visceral pain

Visceral pain includes pain emanating from organs localized into the thoracic, pelvic and abdominal regions. This type of pain is poorly localized, often affecting two or more visceral organs. In particular, gastrointestinal (GI) pain is common in various disorders including irritable bowel syndrome, inflammatory bowel diseases (IBD) (Crohn's disease and ulcerative colitis), pancreatitis, kidney stones, biliary disorders or associated with cancer. Several factors have been identified as contributing to visceral pain, such as stress, early life influence, microbiota, genetic predispositions, epigenetic changes and miRNA regulation ((Fuentes and Christianson, 2018; Larauche et al., 2012; Lomax et al., 2019; Louwies et al., 2019; van Thiel et al., 2020; Zhang and Banerjee, 2015). However, the mechanisms of visceral pain are complex and identifying its cause and the correct treatments for patient remains a challenge (Drewes et al., 2020). Numerous animal models, mostly rodent models, have been developed to mimic GI diseases (reviewed in (Goyal et al., 2014; Greenwood-Van Meerveld and Johnson, 2017; Larauche et al., 2012)). However, visceral pain has only been investigated in a small number of these available models (Schwartz and Gebhart, 2014). Some examples of naturally occurring or induced visceral pain are given in the **Table 3**.

Visceral pain associated diseases naturally occur in dogs, and pancreatitis is the most frequent visceral pain condition (Catanzaro et al., 2016). Pain is addressed by posture, movement, behavioral,

physiological changes and reaction to abdominal palpation (Catanzaro et al., 2016; Mansfield and Beths, 2015).

Classically, visceral pain can be induced by hollow organ isobaric distension using latex balloon inserted into the esophagus or colon that is distended over a certain period of (Ness and Gebhart, 1988; O'Mahony et al., 2012; Ozaki et al., 2002). Colonic sensitivity is then measured by behavior analysis, e.g. abdominal withdrawal reflex (AWR) and/or monitoring abdominal muscles contraction (visceromotor responses, VMR). This method is mostly used in rodents but has been adapted to bigger animals, such as dogs or horses (Ness, 1999).

One widely used technique consists of inducing GI irritation, in order to copy IBD, by intracolorectal injection or oral ingestion of irritant chemicals such as trinitrobenzene sulphonic acid, dextran sulfate sodium, dibutyltin dichloride (Hou et al., 2019; Lapointe et al., 2015; Vera-Portocarrero and Westlund, 2004; Zhu et al., 2018b). This technique derives from the writhing test developed in 1957. In this method, intraperitoneal injection of chemical irritant, such as acetic acid or phenylquinone, induced writhing responses and has been used as pharmaceutical screening tool (reviewed in (Schwartz and Gebhart, 2014)). However, its use needs to be reconsidered due to limitations and ethical issues. GI inflammation can also be induced by parasite or E.coli infection or lipopolysaccharide (LPS) injection (Nozu et al., 2018, 2019; Quick et al., 2013; Zhang et al., 2019a).

More disease-specific models, such as experimental autoimmune pancreatitis induced by mice immunization with rat prostate extracts antigen, or kidney stones obtained by the injection of dental cement into rat ureter, have been generated (Giamberardino et al., 1995; Liu et al., 2019).

Animal models have also been developed to evaluate stress-induced visceral pain. They rely on inducing stress in adult rodents by repeated water avoidance stress, partial restraint stress or in early life of mice by neonatal maternal separation (Miquel et al., 2016; Nozu et al., 2018; Zhu et al., 2019).

In all of these disease models, visceral pain is then measured using abdominal withdrawal reflex, visceromotor responses, mustard oil test, von Frey assay or “up and down” method, or animal behavior analysis such as grimace face (see **Table 3**).

The use of different rodent strains is a plus to address the importance of individual's sensitivity to stress into the induction and maintenance of GI pain (O'Malley et al., 2014). Furthermore, these assays

to induce visceral nociception/pain can be carried out in transgenic mice, enabling to highlight the implications of factors, or signaling pathways, e.g. purinergic signaling pathway in the mechanisms of visceral pain and/or to address the effects of analgesics and probiotics/prebiotics on visceral pain (Ochoa-Cortes et al., 2014; Pusceddu and Gareau, 2018; Trimble et al., 2007).

7. The particular case of non-human primates

Although rodents are mammals, their behavior and the organization of their nervous system remains different from humans. In contrast, studying pain in non-human primates offers the opportunity to investigate mechanisms, and response to analgesic treatment, that are close to what is expected in humans. However, using non-human primates undoubtedly poses acute ethical problems and little exploration of pain-related mechanisms has been done in non-human primates as compared to rodent.

Few attempts have been made to develop NP models in non-human primates. The most classical approach was the ligation of the L7 spinal nerve in rhesus monkeys. At 14 days post lesion, this resulted in the alteration in the activity of spinothalamic tract neurons that displayed increased activity in response to mechanical and thermal stimuli (Palecek et al., 1992). Accordingly, the animals exhibited behavioral responses consistent with mechanical and thermal allodynia (Carlton et al., 1994). Recently, *Macaca fascicularis* has been used to establish a model of mild injury of the sciatic nerve. This does not involve nerve transection, so that nerve transduction can still take place. Under these conditions, behavioral and physiological outcomes model the chronic and long-term nature of sciatic nerve neuropathy that is observed in clinics more closely than in classical rodent models (Guo and Gu, 2014). Sciatic nerve retained the ability of nerve signal transduction, and showed a firing rate profile consistent with injury induced-sensitization of sensory fibres. This model may serve as a non-human primate model to study functional changes of traumatic injury to the sciatic nerve. In a macaque model of chemotherapy-induced neuropathic pain, functional magnetic resonance imaging (fMRI) reported abnormal activation of pain-related brain regions including insular and secondary somatosensory cortices, suggesting that fMRI measurement combined with behavioral outcomes in macaque models could be used to test therapeutic treatments for neuropathic pain and document their modes of action (Nagasaka et al., 2020).

Interestingly, a procedure similar to the rodent CCI model failed to induce symptoms of neuropathic pain in non-human primates (Palecek et al., 1992), likely because of the thicker protective sheath of the primate spinal nerve that prevented rodent-like pathology to develop. This example highlights that specific pain-inducing procedures must be adapted for every species studied.

The use of large animal models is more common to study acute or chronic inflammatory pain (Henze and Urban, 2010). In non-human primates, the formalin test has been used as a tonic pain model with good objectivity, validity, reproducibility and quantification. Moreover, this model exhibits good predictive validity by recapitulating the analgesic effects of morphine and pethidine (Alreja et al., 1984). Similarly, topical application of capsaicin proved to be efficient in inducing transient hyperalgesia and can serve as a model to test analgesic treatments in primates (Kupers et al., 1997). Spontaneous osteoarthritis exists in rhesus monkeys but despite a good face validity, it has never been used to test effectiveness of analgesics, perhaps because it would require preliminary reliable methods to assess pain in these free-range animals (Henze and Urban, 2010). Another painful affection that develops spontaneously in non-human primates is endometriosis (Braundmeier and Fazleabas, 2009). Such spontaneous endometriosis in *cynomolgus* monkeys displays similar clinic-pathological characteristics to the human disease and was useful as an experimental model (Nishimoto-Kakiuchi et al., 2018). *Cynomolgus* monkeys have similar lesions to those in humans as revealed with MRI studies (Nishimoto-Kakiuchi et al., 2018). The implications of these findings are that well established conventional laboratory methods and parameters for assessment can be applied for evaluating disease progress and drug efficacy in this model.

8. Pain models in simple organisms

To reduce the use of rodents in pain studies and carry out high-throughput screening studies, new models have started to appear in pain research, using lower vertebrate species such as zebrafish or *Xenopus* and invertebrates such as *Drosophila* and *Caenorhabditis elegans* (*C. elegans*).

The shared advantages of these models reside in their simplicity of use including low cost and easy maintenance, large brood and egg size and rapid external development. They are genetically similar to humans and genome manipulation (random or directed mutagenesis, transgenesis, Crisp/Cas9) or

gene expression alteration (knock-down or over-expression) can be easily and quickly carried out (Chen et al., 2016; Sassen and Köster, 2015; St Johnston, 2013; Tandon et al., 2017). Genetic, chemical and behavioral screening can also be performed in multiwell-format screening (Giacomotto and Ségalat, 2010; Jorgensen and Mango, 2002; Venken and Bellen, 2014; Wheeler and Brändli, 2009). These diverse and efficient technologies together with online resources make them powerful model to human neuropathologies (de Abreu et al., 2019; Bessa et al., 2013; Fontana et al., 2018; Jeibmann and Paulus, 2009; Markaki and Tavernarakis, 2020; Pratt and Khakhalin, 2013; Vaz et al., 2019). Furthermore, the lower level of ethical constraints for *Drosophila* and *C. elegans* and zebrafish and *Xenopus* embryos and larvae under European and USA legislation places them as a replacement approach in animal experimentation and alternative promising models to study nocifensive reactions, in respect of the 3R principles (Sneddon et al., 2017).

There are numerous debates regarding the capacities of non-mammals to experience pain (Brown, 2015; Key, 2015; Sneddon, 2018, 2019; Sneddon et al., 2014; Williams et al., 2019). Indeed these species are quite distant phylogenetically from humans and their nervous system is indeed not organized as in mammals (Lee-Liu et al., 2017; Pratt and Khakhalin, 2013; Rein et al., 2002; Vaz et al., 2019; White et al., 1986; Zheng et al., 2018). The simplicity of nervous system and the lack of neocortex in zebrafish and somatocortex in *Xenopus* is one of the main arguments for no possible pain experience in these species. However, it has been established that nociceptive pathways in these four animals are similar to mammalian ones (Demin et al., 2018; Kahn-Kirby and Bargmann, 2006; Khuong and Neely, 2013; Smith and Lewin, 2009; Sneddon, 2018; Stevens, 2011, 2015; Tobin and Bargmann, 2004; Walters, 2018). This includes (1) nociceptors with morphological and functional resemblance to vertebrate ones, (2) ions channels which, based on their sequence and structure homology and functional assays, are orthologous to the mammalian TRP channels, (3) opioid system and (4) neurotransmitters of primary afferents (**Table 4**). These four species are capable of sensing mechanical, chemical and thermal noxious stimuli although only amphibians can sense cold stimuli (Sneddon, 2018).

Stereotypical behavioral responses to a noxious stimulus have been well described in these simple organisms and helped to develop numerous assays to study thermal, mechanical and chemical nociception and also neuropathic and chronic pain models (Table 5). In *Drosophila*, the classical

response of larvae to heat or mechanical noxious stimuli is the rolling (corkscrew-like) response (Milinkeviciute et al., 2012). *C.elegans* respond to a heat stimulus with the stereotypical escape behavior called Tav (thermal avoidance), which includes a stop movement followed by reversal, reposition and a forward movement to another new direction (Wittenburg and Baumeister, 1999). However, new heat avoidance assays rely on other behavior responses such as crossing a thermal barrier or distance travelled on heated assay plate (Glauser et al., 2011; Nkambeu et al., 2019). *Xenopus* nociceptive response to the application of acetic acid test (AAT) drops of dilute acetic drops (5%) to the hindlimb, a pain model developed by Pezalla in 1983 (Pezalla, 1983), is called “wipping response”. In zebrafish, nociception has been assessed by changes in behavior, such as reduction of activity, swimming distance and space use, physiological changes (opercular beat rate) or more recently by abdominal constriction like response (Costa et al., 2019; Sneddon, 2019). Some of these assays such as the hotplate or von Frey test, successfully implemented in *Drosophila* larvae and adults, are similar to the mammalian ones (Milinkeviciute et al., 2012). These nociception like assays based on behavioral responses coupled to the ease of genomic manipulation allowed the discovery of new molecular mechanisms involved in nociception and pain. As an example, *painless*, a TRP channel related to the TRPA1 channel subfamily, was identified in the first nociception study through a forward genetic screen for *Drosophila* mutants defective in heat stimulus response (Tracey et al., 2003).

It is now admitted that these four animals can be used as alternatives to mammalian models to study nocifensive behaviors. However, it remains unclear if they can be used to improve our understanding of pain integration. Pain models, such as neuropathic pain, have been developed in *Drosophila* and zebrafish (Khuong et al., 2019; Malafoglia et al., 2014) (Table 5). The AAT test, performed in *Xenopus* and zebrafish have been considered as a pain model, mimicking the rodent visceral pain protocol when injected intraperitoneally (Costa et al., 2019). These animals have also been used in analgesia and anaesthesia research and it has been proven that negative effects of noxious stimulus are blocked by the use of anaesthetics (Lopez-Luna et al., 2017; Morgan et al., 2007; Stevens, 2011). To access pain events is complicated in these four organisms, but it has shown they display cognitive processes such as memory, learning, active avoidance learning, punishment learning, pain-relief learning and if trained, opioids self-administration (Ardiel and Rankin, 2010; Bossé and Peterson, 2017; Gerber et al., 2014; Millhr et al., 1974; Rothman et al., 2016; Yang et al., 2018).

The large number of *Drosophila*, *C.elegans* and zebrafish and *Xenopus* larvae obtained from one mating combined to the absence of ethical issues for these individuals made them very attractive for large genetic and chemical screenings. Teratogenicity assays in zebrafish and *Xenopus* embryos and larvae have been used to address toxicity of pain drugs and herbal remedies and could be powerful tests for future analgesics and anaesthetics development (Chae et al., 2015; Jayasinghe and Jayawardena, 2019). High-throughput screenings have already been implemented with the aim to identify new pain drugs (Ellis et al., 2018). *Xenopus* wild type or overexpressing human proteins related to chronic pain oocytes are frequently used to screen libraries of compounds and test their specificity using electrophysiology or voltage-clamp fluorometry (Zeng et al., 2020). In particular, inhibitors of the P2X7 purinergic receptor or an ion channel involved in chemotherapy induced peripheral neuropathy have been identified using *Xenopus* oocytes (Zeng et al., 2020). Straightjacket, member of the voltage-gated calcium channel family involved in acute and chronic pain in humans, and the thermoreceptor TRPA1 were identified among 580 new pain genes in a 10,000 knockdown adult flies screen (Neely et al., 2010, 2011). Recently, a forward behavioral genetic screen allowed testing more than 600,000 transgenic tgMOR *C.elegans* mutants and identifying a novel and conserved orphan-opioid system (Wang et al., 2019). The recent development of novel behavioral assays with large numbers of animals, automated behavioral tool (such as the Fish Behavior index (FBI)), tracking software (AnyMaze®) and quantitative models should become very useful tools for assessing nociceptive responses high-throughput screening (Curtright et al., 2015; Deakin et al., 2019; Leung et al., 2016; Taylor et al., 2017). Indeed, the Multi-Worm Tracker software (Swierczek et al., 2011) was used to record the animal movement and identify *C.elegans* mutants displaying behavioral changes to opioids (Wang et al., 2019). Finally, habituation alterations have been successfully tested in these high-throughput models for neurodevelopmental disorders (Kepler et al., 2020). Such assay could be a promising approach to provide insight into nociception and pain.

The easiness of these four organisms, the ethical issues and the development of recent automated methodologies have contributed to the increase of studies on nociception and pain research. Although these animals feel nociception and can be used to test behavioral responses to thermal, chemical and mechanical noxious stimuli with a great potential for pharmacological research, for molecular mechanisms understanding and for the 3R respect, there is some limitations to their use. One of these limitations is their phylogenetic distance to humans and therefore to some molecular and cellular

mechanisms differences. For example, the P2X3 receptor involved in pain is not found in *Xenopus* genome (Bernier et al., 2018; Blanchard et al., 2019; Burnstock, 2016). The main limitation resides in the difficulty to assess pain perception, if any. However, the use of these models, especially in high-throughput screening, could be the first *in vivo* step before any experimentation on rodents or other mammals.

9. Limitations of animal models of pain

The study of pain always relied on preclinical animal models that attempted to explore the complex physiological and sensory implications of the condition. Various animal models of chronic pain aim to emulate different types of pain and they have been instrumental in the discovery and development of analgesic agents. While there is no doubt about the necessity of such models in chronic pain research, there are several limitations to their use. This is especially true when considering their failure to facilitate the translation of basic science data into effective and commercially available therapies. This is supported by the fact that there is a high rate of translational failures associated with preclinical animal models (Le Bars et al., 2001; Negus et al., 2006). One well-known failure is that of the neurokinin-1 receptor antagonists (substance P) (Hill, 2000). Several reasons have been suggested for these failures (Mogil, 2009). This debate focuses partly on the utility of animal models of pain and behavioral measures for screening new potential analgesics. One concern is the reliance of studies on reflexive measures, and it has been suggested that additional measures of supraspinal integration that use non-reflexive pain-like behaviors should be included, such as operant learning measures, spontaneous nocifensive behaviors and quality of life or physical activity measures. Another concern is the use of animal models of disease that do not reflect the clinical condition the experimenter is trying to model, such as using inflammatory pain in animals to study chronic low back pain. Finally, the insufficiency of the knowledge on fundamental neuronal mechanisms must be also emphasized. In particular, recent studies pointed to the importance of NK1 receptor signaling along the endocytotic pathway, and not only at the plasma membrane, in the transduction of nociceptive information (Jensen et al., 2017). Therefore, classical NK1 antagonists that target membrane NK1 may not be able to prevent atypical intracellular NK1 signaling.

Most molecules that successfully demonstrated analgesic effects in preclinical studies fail to produce similar effects when tested on a clinical level. One of the reasons this might occur relies on the genetic and molecular neurochemical differences between humans and animals. This makes it difficult to predict the pharmacological effect of a molecule on the clinical level. In contrast, after clinical validation, most approved drugs have proven good efficiency in reverse translational studies in animals. The difficult translatability of pain across species and transfer to clinical settings could also be due to non-optimal translational research conditions and not directly related to the failure of the model to simulate human pain mechanisms (Henze and Urban, 2010; Le Bars et al., 2001). In fact, failure in translational approaches can be due to the presence of severe side effects that appear in clinics but are not detected in preclinical studies (Mogil, 2009).

Another major cause is likely to be the heterogeneous nature of human chronic pain which can have a multitude of aetiologies. This heterogeneity clashes with the controlled environment often used in animal studies that might interfere with the translation of the research to a human population. In particular, a vast majority of animal studies utilize inbred animal. While some injury models are available, most studies only use sensory-evoked thresholds (e.g., von Frey filaments) in order to assess nociceptive sensitivity; whereas the evaluation of ongoing pain based on non-evoked stimulus (such as the grimace scale, weight bearing, CPP...) may be clinically more relevant (Deuis et al., 2017c; Negus et al., 2006). Other challenges researchers are facing include the replication of the transitional process of pain from acute to chronic in animal models. The complexity of pain mechanisms and differences between pain types (i.e. neuropathic vs. inflammatory) indicates a lack of a mechanistic delineation between conditions (Ferrari et al., 2015; Le Bars et al., 2001).

In addition, several demographic factors are implicated in pain mechanisms in humans including age. Pain mechanisms are often considered independent from age in animals, which lead to the predominant use of young mice in preclinical studies. This is questioned by the increasing evidence of the involvement of an age-dependent mechanism (Mogil, 2009). Sexual dimorphism is another demographic factor that might lead to the misrepresentation of chronic pain in animal models. This is apparent through the overuse of male subjects in studies which is a clear contrast to the high prevalence of chronic pain in females. The underutilization of female subjects in preclinical trials might provide an explanation to the lack of effective analgesic therapies for the human global population (Henze and Urban, 2010; Le Bars et al., 2001; Negus et al., 2006). Pain perception differs

between female and male; with female perceiving more pain perhaps due to high levels of estrogen and progesterone, and low testosterone levels. In fact, pain perception is modulated by the immune system that is under the control of gonadal hormones (Mogil, 2009). This difference has been highlighted in a study that indicated only male mice developed acute thermal hypersensitivity when tested in an environment linked to a painful memory (Martin et al., 2019). Furthermore it has been showed that T-cell deficient female mice needed 2-3 times the morphine dose administered to male mice (Rosen et al., 2019). Another example pointed that the hypersensitivity induced by nerve injury is different between male and female mice since the spinal mechanisms differ between the two genders (Mapplebeck et al., 2018).

Interstrain animal genotypic variabilities can also account for the failure of human chronic pain modelling in animals (Henze and Urban, 2010; Le Bars et al., 2001; Negus et al., 2006). The example of the gene encoding sodium channel Nav1.7 is illustrative. This gene is present on adrenal and pituitary gland of rodents, and its mutation is lethal. However, the situation is different in primates where this gene mutation leads to pain insensitivity (Mogil, 2009). Notably, recent studies pointed that Nav1.7 may not be critical for survival even in rodents, but appears as an key molecular component for transmitting high-threshold noxious stimuli (Gingras et al., 2014). There is also genetic variability among rodents that is also to blame for clinical failure. In fact, mice show an increase of morphine analgesia when isolated in a cage, whereas it decreases with rats. Furthermore chronic pain was shown to modulate 43 genes in rats versus only 10 in mice (Mogil, 2009).

10. Translational successes from animal model studies

It is worth noting that despite these limitations, a growing number of examples demonstrate the potential to convert new agents into widely used analgesics through pre-clinical testing in animal models. The following will consider such examples for treating neuropathic or inflammatory pain, as well as low back pain and post-operative pain.

Targeting purinergic signalling

ATP induces acute pain when applied to human skin (Bleehen and Keele, 1977; Hamilton et al., 2000) where it activates ATP-gated channels (P2X receptors), mainly P2X3 receptors that are almost exclusively expressed in nociceptive sensory neurons. P2X3 and heteromeric P2X2/3 play a key role in pain sensing and in various sensory transduction such as taste and visceral afferent sensation. Several P2X receptors expressed in neurons and/or microglia, including P2X3 and P2X4 or P2X7, are also involved in the chronic nociceptive behavior that follow nerve injury or inflammation indicating that P2X receptors represent therapeutic targets for pain syndrome (Khakh and North, 2006).

Nerve injury mouse models recently allowed the understanding of a spinal mechanism in which the purinergic receptor P2X4 expressed in microglia plays a critical role specifically in neuropathic pain but not in acute or inflammatory pain. Using a spinal nerve ligation rat model of neuropathic pain, a pioneered work showed that the increase of P2X4 expression specifically in reactive microglia within the dorsal horn of the spinal cord is determinant in tactile allodynia (Tsuda et al., 2003). The authors also showed that the pharmacological blockade of P2X4 or the expression knock-down using siRNA dramatically reduced pain-like behavior indicating that P2X4 activation is required to maintain pain hypersensitivity. The key role of P2X4 was confirmed in P2X4 knockout mice (P2X4KO) in which tactile allodynia after peripheral nerve ligation was drastically reduced (Ulmann et al., 2008). Several studies established that activation of microglial P2X4 by ambient ATP increases nociceptive signaling within the spinal cord by reducing inhibitory currents within the spinal cord. This neuronal disinhibition results from a rise in the intracellular concentration of chloride ions that suppresses GABA or Glycine mediated-inhibitory responses. This phenomenon may eventually convert inhibition into excitation in a subset of neurons, sufficient to induce hypersensitivity of spinal neurons and tactile allodynia (Coull et al., 2005; Trang et al., 2009). These changes in chloride concentration are triggered by the potassium-chloride co-transporter (KCC2) downregulation, which is mediated by the extracellular release of BDNF and the activation of Trk-B receptor located at the surface of inhibitory neurons. The neuronal hyper-excitability mediated by microglial P2X4 signaling may account for the main symptoms of neuropathic pain suggesting that the blockade of P2X4 expression or function

alleviates this debilitating condition in human. Although no commercially available P2X4-derived pain killer exists, the therapeutic potential of targeting this system is well recognized, and current research efforts are made for P2X4 drug development. TNP-ATP or 5-BDBD are P2X4 antagonists that have been commonly used to show the involvement of P2X4 in rodent nervous tissue but they are not very selective (Suurväli et al., 2017). New antagonists of rodent P2X4 (BAY-1797 or NP-1815-PX) and a highly selective human P2X4 antagonist (BX430) with no effect on rodent P2X4 were recently identified (Ase et al., 2015; Matsumura et al., 2016). Kyushu University and Nippon Chemiphar Co., Ltd. initiated a phase 1 clinical trial of the NC2600 P2X4 antagonist but no data is available since then (Bhattacharya, 2016).

An alternative to P2X4 blockade could be the enhancement of KCC2. Actually, chloride extrusion enhancers have been proposed as novel therapeutics for pain alleviation (Gagnon et al., 2013). Chloride enhancers could also be used in synergy with other pharmacological agents. Indeed, a recent study pointed to the benefit of rescuing chloride homeostasis with the KCC2-enhancer CLP25728 on the analgesic action of the benzodiazepine site ligand L838,417. This combination produced an efficient analgesia even with high doses of benzodiazepine, whereas they remained otherwise ineffective when administered alone (Lorenzo et al., 2020).

P2X7 is expressed in most of the immune cells and regulate the inflammation, thus representing a valuable therapeutic target for many diseases involving inflammation, including persistent pain (Burnstock and Knight, 2018). P2X7 antagonists such as AZ106006120 or nanobodies that are small single chain antibodies blocking or modulating P2X7 functions have been proposed as drug candidates for inflammatory disorders (Danquah et al., 2016; Mishra et al., 2016). P2X7 knockout mice showed reduced behaviorral responses to inflammation or tactile allodynia (Chessell et al., 2005). These effects seem to be mediated by the decrease of the pro-inflammatory interleukin-1 release from microglia. P2X7 is abundantly expressed in reactive microglia after CNS injury, and neuropathic pain is reduced by P2X7 antagonist such as A-740003 and A-438079. These data suggest that targeting microglial P2X7 may be useful for the treatment of chronic pain (Tsuda, 2017).

Antagonist selective of P2X3 or P2X2/3 receptors have also been shown to have anti-inflammatory and anti-nociceptive effects in different models of neuropathy and peripheral

inflammation when expressed in sensory neurons (Jarvis et al., 2002; Kuan and Shyu, 2016). Mechanical allodynia in animal models was also reduced in mouse with deletion of P2X3 gene (Cockayne et al., 2000; Souslova et al., 2000). In addition, P2X3 knockout mice exhibited a marked urinary bladder hyporeflexia and it has been showed that ATP released by the distension of the urothelium activates P2X3 expression in afferent nerves. A similar link have been established for the taste buds, the wall intestine and reflexes such as cough, indicating that P2X3 and/or P2X2/3 receptors have a more widespread role in sensory transduction. Therefore, they represent attractive targets for drugs, and not only for pain. Among the numerous selective antagonists of P2X3 discovered, some of them have been studied in clinical trials for pain-related disorders but also for other diseases such as overactive bladder, irritable bowel syndrome, or chronic cough. Gefapixant (MK-7264) significantly reduced cough frequency in patients with refractory chronic cough or unexplained chronic cough (Smith et al., 2020) and is one of the most promising P2X3 antagonists in phase 3 clinical trials for the treatment of chronic cough.

Targeting 5-HT receptors

Lumbar disc herniation is a well known aetiology of chronic lower back pain. A herniated disc induces pain by a mechanical deformation (in addition to a biochemical irritation) to the dorsal root ganglion and the nerve root. Many substances such as proinflammatory cytokines and monoamines play a role in the biochemical irritation of the nerve. Serotonin (5-HT) is one of the monoamine participating in pain modulation mainly *via* 5-HT_{2A} receptors. Nowadays, scientists are using animal models to understand the complexity of chronic pain pathways, aiming to project these hypothesis and find a curative treatment for chronic pain, and especially low back pain in humans (Kato et al., 2015).

In their paper, Hashizume H et al. evaluated the effect of a 5-HT_{2A} receptor antagonist (sarpogrelate) on lumbar disc herniation pain (Hashizume et al., 2007). They performed a study on rats that underwent surgery to place an autologous nucleus pulposus at the 4th and 5th left lumbar nerve roots in order to mimic lumbar disc herniation pain. After studying pain related behaviors, researchers found that sarpogrelate treatment considerably reduced mechanical allodynia at 5 and 8 days after drug administration (Hashizume et al., 2007). In a related experiment, the same

surgical model of rats was used to study the effect of a similar *5-HT_{2A}* receptor antagonist (saprorelate hydrochloride) on pain related behavior, and on the expression of *5-HT_{2A}* receptors in dorsal root ganglia. The results confirmed that saprorelate used at high doses (10 mg/kg) dramatically reduced mechanical withdrawal threshold evaluated by von Frey tests. In addition to decreasing nociceptive transmission, researchers confirmed that this *5-HT_{2A}* antagonist down-regulates *5-HT_{2A}* receptor expression in dorsal root ganglia (Kato et al., 2015). Furthermore, the effect of the serotonin noradrenaline reuptake inhibitor (SNRI) duloxetine was evaluated on radiculopathy in the same rat model of lumbar disc herniation pain (Handa et al., 2016). Rats received duloxetine at different doses (low dose 0.4 mg/kg, high dose 1.2 mg/kg). Results confirmed that duloxetine had a positive impact on neuropathic pain in rats probably by down regulating the expression of tumor necrosis factor alpha and nerve growth factor and microglia activation (Handa et al., 2016).

These preclinical data obtained on animal models drove clinical trials in order to demonstrate SNRI drug efficacy on chronic back pain in humans. A recent meta-analysis reported the results of 3 randomized double blind clinical studies of patients taking duloxetine or placebo for chronic low back pain (Enomoto et al., 2017). Duloxetine-treated patients (n=400) and placebos (n=451) were considered and the statistical analysis revealed that duloxetine considerably alleviated pain. Duloxetine-induced analgesic effect was mainly modulated by interaction with the pain pathway rather than with the anti-depressive effect of the drug, indicating that duloxetine primarily targeted pain-specific mechanisms and not comorbid disorders (Enomoto et al., 2017). The effectiveness and safety of duloxetine monotherapy was assessed in a Japanese population suffering from chronic low back pain. Duloxetine (60 mg daily) was effective in pain management. Moreover, duloxetine was well tolerated with mild side effects that may resolve and improve with the course of the treatment (Konno et al., 2016). These results were confirmed by independent studies with randomized, double-blind trials on chronic low back pain patients (Skljarevski et al., 2010b, 2010a).

Taken together, these studies have led to the recommendation of duloxetine in the management of chronic low back pain as a second line therapy by the American college of physicians in 2017 (Qaseem et al., 2017).

In addition, a novel dual antagonist of the GlyT2 glycine transporter and 5-HT_{2A} receptor (VVZ-149) is being tested in healthy volunteers for future applications in the management of post-operative pain (Oh et al., 2018). In case of success, the therapeutic indication for this drug may be extended beyond post-operative pain to other pain pathologies.

Targeting TRPV1 receptors

Capsaicin is a TRPV1 receptor agonist (see §2.) with anaesthetic effects that is approved as a pain killer and proved to be efficient in various types of pain. Although the analgesic properties of capsaicin were empirically known for long, animal model studies allowed to determine its mechanisms of action. Early evidence, back in the 1980s, indicated that capsaicin treatment causes an alteration of the synthesis and storage of substance P in the dorsal horn and primary afferent neurons, thus affecting nociceptive transmission (Gamse et al., 1980; Lembeck and Donnerer, 1981). Subsequently, capsaicin-mediated thermal anti-nociceptive effects were demonstrated, and the suggested mechanisms pointed to a possible suppression of axoplasmic impulses in sensory neurons (Buck et al., 1981; Gamse et al., 1982; Yaksh et al., 1979). More recent studies revealed that capsaicin alleviates pain in various animal models including post-surgical pain (Hamalainen et al., 2009) and bone cancer pain (Ghilardi et al., 2005b).

Based on animal model studies, the use of topical capsaicin for the management of human chronic pain became increasingly popular (Blair, 2018; Wagner et al., 2013). The effect of an 8% capsaicin patch were assessed on peripheral neuropathic pain in comparison with oral Pregabalin (Haanpää et al., 2016). Capsaicin treatment contributed to remarkable pain alleviation at 8 weeks in comparison with optimal Pregabalin doses. Patches are associated with less systemic side effect, rapid action and greater treatment satisfaction (Haanpää et al., 2016). Another study also indicated the superiority of 8% capsaicin patches over Pregabalin on mechanical allodynia in patients suffering from peripheral neuropathic pain (Cruccu et al., 2018). These findings were confirmed by a network meta-analysis of 25 randomized controlled trials (van Nooten et al., 2017). Consequently, Capsaicin patch is indicated in treating neuropathic and skeletomuscular pain (Chang and Quick, 2020). In addition, a recent paper supported the use of capsaicin patches to

attenuate chemotherapy-induced peripheral neuropathy that may occur in cancer patients (Anand et al., 2019).

Prolonged application of capsaicin results in the desensitization of TRPV1 channel. As a consequence, the nociceptive afferent becomes functionally silent (Lambert, 2009; Zhang and Li Wan Po, 1994). The efficacy of capsaicin-induced desensitization in treating pain pushed pharmaceutical companies to develop TRPV1 antagonists (Dray, 2008). Moreover, TRPV1 was shown to play a role in neural sensitization (Ramsey et al., 2006), and preclinical studies suggested TRPV1 antagonists may exhibit analgesic effects in inflammatory conditions (García-Martinez et al., 2002). These findings paved the way for clinical research, and at least seven orally active TRPV1 antagonist substances were under development in 2009, with many more in preclinical development (Gunthorpe and Chizh, 2009). In the same period, phase II clinical trials were performed for compounds such as GRC 6211 (Lilly/Glenmark), NDG6243 (Merck/Neurogen) and AZD1386 (AstraZeneca). However, to date, no TRPV1 antagonists are available for the clinician.

The main reasons for this failure relies in the lack of precise and rigorous preclinical evidence for using TRPV1 antagonists in pain treatment. Hence, it is necessary to optimize preclinical pharmacology to define precisely pain profiles sensitive to the compounds tested. Alternatively, refinements in preclinical studies may avoid making overenthusiastic assumptions, and help in a timely manner not to pursue clinical developments, thus saving money and resources for the pharmaceutical industry (M Keppel Hesselink, 2016).

Targeting Nerve Growth Factor

Evidence for a contribution of locally produced nerve growth factor (NGF) to joint pathology, as well as pain in arthritic joints has emerged from animal model studies. A single intraarticular (IA) injection of NGF into normal rat knees produced a direct sensitisation of nociceptors through dose-dependent, long-lasting increases in pain-like behavior, joint swelling and synovial macrophage infiltration (Ashraf et al., 2014). Other work provided evidence that NGF is released by damaged cells in arthritic joints, and elevated NGF is detected in synovium of murine OA model (Kc et al., 2016). Elevated levels of NGF has been also found in synovial fluid in dogs with naturally occurring OA (Isola et al., 2011). Administration of an anti-NGF/TrkA signalling

molecule significantly decreased pain-like behaviors in a murine model of OA (McNamee et al., 2010). In particular, anti-NGF monoclonal antibody (mAb) produced a robust analgesia that is equal to or greater than current analgesics in animal models (Enomoto et al., 2019; Xu et al., 2016).

In view of experimental preclinical findings on the role of NGF in pain, clinical trials have been initiated to demonstrate the effectiveness of the strategy targeting the NGF system (Bannwarth and Kostine, 2014, 2017; Takasusuki and Yaksh, 2011). Anti-NGF mAbs are in development as treatments for several pain conditions and three NGF-blocking drugs have been developed: tanezumab (humanised mAb; Pfizer, in collaboration with Eli Lilly), fulranumab (fully human mAb; Amgen) and fasinumab (fully human mAb; Regeneron Pharmaceuticals, in collaboration with Sanofi). The first attempts to develop clinical trials aimed at evaluating the efficacy of anti-NGF antibodies on osteoarthritis pain (Ishikawa et al., 2015; Miyagi et al., 2017). Tanezumab prevents NGF binding to its TrkA receptor resulting in pain alleviation. Although many studies emphasized Tanezumab positive effects against osteoarthritis pain, Tanezumab exhibits also serious adverse effects such as arthralgia, paraesthesias and osteonecrosis that question Tanezumab approval to treat chronic pain conditions in humans (Schnitzer et al., 2015; Walicke et al., 2018; Webb et al., 2018). After being put on a clinical hold between 2010 and 2012, trials for the development of anti-NGF mAbs in human restarted in 2015, and Tanezumab received fast track designation by the Food and Drug Administration in 2017. Tanezumab is currently in phase III studies in patients with OA of the hip and knee, chronic lower back pain, and bone cancer pain trials (Enomoto et al., 2019). Other companies are developing TrkA antagonist (Sanofi, GZ389988; Glenmark Pharmaceuticals Ltd., GBR 900). Comparisons with anti-NGF antibodies in animal models of inflammatory pain demonstrated that GBR 900's efficacy is similar as that of anti-NGF antibodies (Pharmaceuticals).

Since OA naturally occurs in cats, dogs and horses (see §2), companies are developing canine and feline anti-NGF mAbs (NexVet, Ranevetmab and Frunevetmab, respectively). Overall, significant improvement has been seen in both dogs and cats following administration of anti-NGF mAbs. Assessment was made in dogs with OA through the subjective (owned completed questionnaire) and objective (activity) measures. These results suggested a positive analgesic effect possibly greater than that expected with NSAIDs (Webster et al., 2014). Anti-NGF mAB

effectiveness was also demonstrated in dogs with pain artificially induced by kaolin injection (Gearing et al., 2013).

Targeting calcium channels

Conopeptides are interesting candidates to design novel drugs for alleviating chronic neuropathic pain. Conopeptides are extracted from the venoms of marine snails and have an effect on calcium channels in modulating pain (Patel et al., 2018; Schroeder and Craik, 2012). Efficiency of conopeptides were first assessed in animal models of pain and then transferred to human clinic (Nielsen et al., 2005). Among conopeptides, a peptide blocker of CA_V 2.2 channels (synthetic version of W-conotoxin MVIIA, named Ziconotide) has attracted much attention (Patel et al., 2018). Intrathecal administration of Ziconotide for refractory chronic pain (e.g. neuropathic pain) has proved antiallodynic and antihyperalgesic effects (McDowell and Pope, 2016; Wermeling and Berger, 2006). Ziconotide however show severe drawbacks, and various alarming side effects have been monitored, e.g. sedation, confusion, ataxia, memory impairment, vertigo, gait imbalance, hallucinations, nausea and vomiting, that limit its indications (Wermeling and Berger, 2006). Actually Ziconotide is approved as a nonopioid analgesic but it must be administered intrathecally in refractory chronic pain cases, allowing patients to benefit from a potent long lasting analgesic effect related to malignant and non-malignant aetiologies (McGivern, 2007; Wie and Derian, 2020). To facilitate the use of Ziconotide, recent studies are evaluating the possibility of administrating the drug intranasally (Manda et al., 2016).

11. Conclusion

Animal models of pain were historically viewed as reliable tools that have served to advance research in the field over the past decades. They have been instrumental in constructing a global picture of how key proteins, signalling systems, and neural circuits contribute to pain-like behaviors. However, there are limitations and caveats to these models that must be acknowledged when considering the translation of research findings from the bench to the bedside. There is not one “best model” for the study of pain. Instead, the use of different organisms has been instrumental for

integrating a comprehensive array of genetic, molecular, cellular, and behavioral data on normal and pathological pain processing in the central and peripheral nervous systems.

Classical rodent models usually display good face validity. However, it is still needed to improve construct validity by choosing refine animal models that mimic faithfully the human diseases. In particular, considering sex-differences, genetic variations, or comorbid interactions will contribute to describe a greater diversity of mechanisms that accurately account for clinical situations. In addition, defining more accurate behavioral measures will assist investigators in developing an appropriate set of experiments to better test mechanisms and potential treatments.

In contrast, using simpler organisms provides an efficient mean to achieve high throughput screening of possible key players in pain processes. These approaches are based on alterations of aversive behaviors that are considered surrogates of aberrant pain processes. Although far from the human clinics, these assays are likely to inform the construct of specific rodent models, or the design of protocols dedicated to test the actual role of these candidates.

The combination of distinct animal models may be the alternative to the lack of good predictive validity of animal models that basic research is facing so far. As long as the interpretation of the results remains constrained by their intrinsic limitations, integrating data obtained from different animal models is a powerful method to build up a comprehensive understanding of pain mechanisms and for the identification of potentially more relevant therapeutic targets.

Developing clinical assessment of pain is another important path to help defining more accurate animal models. Quantitative sensory testing, assessment of evoked *vs* resting pain, are parameters that are needed to understand how pain develops in humans. Clinical trials may also be designed to test outcomes and mechanisms derived from animal studies. Finally, an open dialogue between basic research results and clinical challenges posed by human studies is necessary to improve the translation of findings from bench to the bedside and to ensure the development of better pain therapies and pain management strategies

Bibliography

Abboud, C., Duveau, A., Bouali-Benazzouz, R., Massé, K., Mattar, J., Brochoire, L., Fossat, P., Boué-Grabot, E., Hleihel, W., and Landry, M. (2020). Animal models of pain: Diversity and benefits. *J Neurosci Methods* 108997.

Abdus-Saboor, I., Fried, N.T., Lay, M., Burdge, J., Swanson, K., Fischer, R., Jones, J., Dong, P., Cai, W., Guo, X., et al. (2019). Development of a Mouse Pain Scale Using Sub-second Behavioral Mapping and Statistical Modeling. *Cell Rep* 28, 1623-1634.e4.

de Abreu, M.S., Genario, R., Giacomini, A.C.V.V., Demin, K.A., Lakstygai, A.M., Amstislavskaya, T.G., Fontana, B.D., Parker, M.O., and Kalueff, A.V. (2019). Zebrafish as a Model of Neurodevelopmental Disorders. *Neuroscience*.

Akerman, S., Holland, P.R., and Hoffmann, J. (2013). Pearls and pitfalls in experimental in vivo models of migraine: dural trigeminovascular nociception. *Cephalalgia* 33, 577–592.

Akerman, S., Romero-Reyes, M., and Holland, P.R. (2017). Current and novel insights into the neurophysiology of migraine and its implications for therapeutics. *Pharmacol. Ther.* 172, 151–170.

Albert-Gascó, H., García-Avilés, Á., Moustafa, S., Sánchez-Sarasua, S., Gundlach, A.L., Olucha-Bordonau, F.E., and Sánchez-Pérez, A.M. (2017). Central relaxin-3 receptor (RXFP3) activation increases ERK phosphorylation in septal cholinergic neurons and impairs spatial working memory. *Brain Struct Funct* 222, 449–463.

Albert-Gascó, H., Ma, S., Ros-Bernal, F., Sánchez-Pérez, A.M., Gundlach, A.L., and Olucha-Bordonau, F.E. (2018). GABAergic Neurons in the Rat Medial Septal Complex Express Relaxin-3 Receptor (RXFP3) mRNA. *Front Neuroanat* 11.

Albert-Gasco, H., Sanchez-Sarasua, S., Ma, S., García-Díaz, C., Gundlach, A.L., Sanchez-Perez, A.M., and Olucha-Bordonau, F.E. (2019). Central relaxin-3 receptor (RXFP3) activation impairs social recognition and modulates ERK-phosphorylation in specific GABAergic amygdala neurons. *Brain Struct Funct* 224, 453–469.

Allchorne, A.J., Broom, D.C., and Woolf, C.J. (2005). Detection of cold pain, cold allodynia and cold hyperalgesia in freely behaving rats. *Mol Pain* 1, 36.

Alreja, M., Mutalik, P., Nayar, U., and Manchanda, S.K. (1984). The formalin test: a tonic pain model in the primate. *Pain* 20, 97–105.

Alshami, A.M. (2014). Knee osteoarthritis related pain: a narrative review of diagnosis and treatment. *Int J Health Sci (Qassim)* 8, 85–104.

- Anand, P., Elsaifa, E., Privitera, R., Naidoo, K., Yiangou, Y., Donatien, P., Gabra, H., Wasan, H., Kenny, L., Rahemtulla, A., et al. (2019). Rational treatment of chemotherapy-induced peripheral neuropathy with capsaicin 8% patch: from pain relief towards disease modification. *J Pain Res* *12*, 2039–2052.
- Apps, R., and Strata, P. (2015). Neuronal circuits for fear and anxiety - the missing link. *Nat. Rev. Neurosci.* *16*, 642.
- Aragon, C.L., Hofmeister, E.H., and Budberg, S.C. (2007). Systematic review of clinical trials of treatments for osteoarthritis in dogs. *J. Am. Vet. Med. Assoc.* *230*, 514–521.
- Ardiel, E.L., and Rankin, C.H. (2010). An elegant mind: Learning and memory in *Caenorhabditis elegans*. *Learn. Mem.* *17*, 191–201.
- Arguello, F., Baggs, R.B., and Frantz, C.N. (1988). A murine model of experimental metastasis to bone and bone marrow. *Cancer Res.* *48*, 6876–6881.
- Ase, A.R., Honson, N.S., Zaghdane, H., Pfeifer, T.A., and Séguéla, P. (2015). Identification and characterization of a selective allosteric antagonist of human P2X4 receptor channels. *Mol. Pharmacol.* *87*, 606–616.
- Ashraf, S., Mapp, P.I., Burston, J., Bennett, A.J., Chapman, V., and Walsh, D.A. (2014). Augmented pain behavioural responses to intra-articular injection of nerve growth factor in two animal models of osteoarthritis. *Ann. Rheum. Dis.* *73*, 1710–1718.
- Attal, N., Lanteri-Minet, M., Laurent, B., Fermanian, J., and Bouhassira, D. (2011). The specific disease burden of neuropathic pain: results of a French nationwide survey. *Pain* *152*, 2836–2843.
- Authier, N., Gillet, J.P., Fialip, J., Eschalier, A., and Coudore, F. (2003). An animal model of nociceptive peripheral neuropathy following repeated cisplatin injections. *Exp. Neurol.* *182*, 12–20.
- Back, S.K., Kim, J.S., Hong, S.K., and Na, H.S. (2003). Ascending pathways for mechanical allodynia in a rat model of neuropathic pain. *Neuroreport* *14*, 1623–1626.
- Bair, M.J., Robinson, R.L., Katon, W., and Kroenke, K. (2003). Depression and pain comorbidity: a literature review. *Arch. Intern. Med.* *163*, 2433–2445.
- Bali, K.K., Selvaraj, D., Satagopam, V.P., Lu, J., Schneider, R., and Kuner, R. (2013). Genome-wide identification and functional analyses of microRNA signatures associated with cancer pain. *EMBO Mol Med* *5*, 1740–1758.
- Banerjee, A., Shen, P.-J., Ma, S., Bathgate, R.A.D., and Gundlach, A.L. (2010). Swim stress excitation of nucleus incertus and rapid induction of relaxin-3 expression via CRF1 activation. *Neuropharmacology* *58*, 145–155.

- Banik, R.K., and Kabadi, R.A. (2013). A modified Hargreaves' method for assessing threshold temperatures for heat nociception. *J. Neurosci. Methods* 219, 41–51.
- Bannwarth, B., and Kostine, M. (2014). Targeting nerve growth factor (NGF) for pain management: what does the future hold for NGF antagonists? *Drugs* 74, 619–626.
- Bannwarth, B., and Kostine, M. (2017). Nerve Growth Factor Antagonists: Is the Future of Monoclonal Antibodies Becoming Clearer? *Drugs* 77, 1377–1387.
- Barrot, M. (2012). Tests and models of nociception and pain in rodents. *Neuroscience* 211, 39–50.
- Bas, D.B., Su, J., Wigerblad, G., and Svensson, C.I. (2016). Pain in rheumatoid arthritis: models and mechanisms. *Pain Manag* 6, 265–284.
- Basbaum, A.I., Bautista, D.M., Scherrer, G., and Julius, D. (2009a). Cellular and molecular mechanisms of pain. *Cell* 139, 267–284.
- Basbaum, A.I., Bautista, D.M., Scherrer, G., and Julius, D. (2009b). Cellular and molecular mechanisms of pain. *Cell* 139, 267–284.
- Bathgate, R. a. D., Halls, M.L., van der Westhuizen, E.T., Callander, G.E., Kocan, M., and Summers, R.J. (2013). Relaxin family peptides and their receptors. *Physiol. Rev.* 93, 405–480.
- Bathgate, R.A., Ivell, R., Sanborn, B.M., Sherwood, O.D., and Summers, R.J. (2006a). International Union of Pharmacology LVII: recommendations for the nomenclature of receptors for relaxin family peptides. *Pharmacological Reviews* 58, 7–31.
- Bathgate, R.A.D., Samuel, C.S., Burazin, T.C.D., Layfield, S., Claasz, A.A., Reytomas, I.G.T., Dawson, N.F., Zhao, C., Bond, C., Summers, R.J., et al. (2002). Human relaxin gene 3 (H3) and the equivalent mouse relaxin (M3) gene. Novel members of the relaxin peptide family. *The Journal of Biological Chemistry* 277, 1148–1157.
- Bathgate, R.A.D., Hsueh, A.J.W., and Sherwood, O.D. (2006b). CHAPTER 16 - Physiology and Molecular Biology of the Relaxin Peptide Family. In *Knobil and Neill's Physiology of Reproduction (Third Edition)*, J.D. Neill, ed. (St Louis: Academic Press), pp. 679–768.
- Bathgate, R.A.D., Kocan, M., Scott, D.J., Hossain, M.A., Good, S.V., Yegorov, S., Bogerd, J., and Gooley, P.R. (2018). The relaxin receptor as a therapeutic target - perspectives from evolution and drug targeting. *Pharmacol. Ther.* 187, 114–132.
- Becerra, L., Breiter, H.C., Wise, R., Gonzalez, R.G., and Borsook, D. (2001). Reward circuitry activation by noxious thermal stimuli. *Neuron* 32, 927–946.
- Beitz, A.J. (1982). The sites of origin brain stem neurotensin and serotonin projections to the rodent nucleus raphe magnus. *J. Neurosci.* 2, 829–842.

- Benbouzid, M., Pallage, V., Rajalu, M., Waltisperger, E., Doridot, S., Poisbeau, P., Freund-Mercier, M.J., and Barrot, M. (2008). Sciatic nerve cuffing in mice: a model of sustained neuropathic pain. *Eur J Pain* *12*, 591–599.
- Bendele, A.M. (1987). Progressive Chronic Osteoarthritis in Femorotibial Joints of Partial Medial Meniscectomized Guinea Pigs. *Vet Pathol* *24*, 444–448.
- Benjamin, L., Levy, M.J., Lasalandra, M.P., Knight, Y.E., Akerman, S., Classey, J.D., and Goadsby, P.J. (2004). Hypothalamic activation after stimulation of the superior sagittal sinus in the cat: a Fos study. *Neurobiol. Dis.* *16*, 500–505.
- Bennett, G.J., and Xie, Y.K. (1988). A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* *33*, 87–107.
- Berić, A. (1998). Central pain and dysesthesia syndrome. *Neurol Clin* *16*, 899–918.
- Bernard, J.F., and Besson, J.M. (1990). The spino(trigemino)pontoamygdaloid pathway: electrophysiological evidence for an involvement in pain processes. *Journal of Neurophysiology* *63*, 473–490.
- Bernier, L.-P., Ase, A.R., and Séguéla, P. (2018). P2X receptor channels in chronic pain pathways. *Br. J. Pharmacol.* *175*, 2219–2230.
- Bessa, C., Maciel, P., and Rodrigues, A.J. (2013). Using *C. elegans* to decipher the cellular and molecular mechanisms underlying neurodevelopmental disorders. *Mol. Neurobiol.* *48*, 465–489.
- Bessis, N., Decker, P., Assier, E., Semerano, L., and Boissier, M.-C. (2017). Arthritis models: usefulness and interpretation. *Semin Immunopathol* *39*, 469–486.
- Bhattacharya, A. (2016). Clinical optimism for antagonists targeting certain ion channels. *3*, 10–13.
- Biessels, G.J., Bril, V., Calcutt, N.A., Cameron, N.E., Cotter, M.A., Dobrowsky, R., Feldman, E.L., Fernyhough, P., Jakobsen, J., Malik, R.A., et al. (2014). Phenotyping animal models of diabetic neuropathy: a consensus statement of the diabetic neuropathy study group of the EASD (Neurodiab). *J. Peripher. Nerv. Syst.* *19*, 77–87.
- Binder, C., Hagemann, T., Husen, B., Schulz, M., and Einspanier, A. (2002). Relaxin enhances in-vitro invasiveness of breast cancer cell lines by up-regulation of matrix metalloproteases. *Mol Hum Reprod* *8*, 789–796.
- Blair, H.A. (2018). Capsaicin 8% Dermal Patch: A Review in Peripheral Neuropathic Pain. *Drugs* *78*, 1489–1500.
- Blanchard, C., Boué-Grabot, E., and Massé, K. (2019). Comparative Embryonic Spatio-Temporal Expression Profile Map of the *Xenopus* P2X Receptor Family. *Front Cell Neurosci* *13*.

- Blanco-Gandía, M.C., Aguilar, M.A., Miñarro, J., and Rodríguez-Arias, M. (2018). Reinstatement of Drug-seeking in Mice Using the Conditioned Place Preference Paradigm. *J Vis Exp*.
- Bleehen, T., and Keele, C.A. (1977). Observations on the algogenic actions of adenosine compounds on the human blister base preparation. *Pain* 3, 367–377.
- Bliss, T.V.P., Collingridge, G.L., Kaang, B.-K., and Zhuo, M. (2016). Synaptic plasticity in the anterior cingulate cortex in acute and chronic pain. *Nat. Rev. Neurosci.* 17, 485–496.
- Bokiniec, P., Zampieri, N., Lewin, G.R., and Poulet, J.F. (2018). The neural circuits of thermal perception. *Curr Opin Neurobiol* 52, 98–106.
- Bonin, R.P., Bories, C., and De Koninck, Y. (2014). A simplified up-down method (SUDO) for measuring mechanical nociception in rodents using von Frey filaments. *Mol Pain* 10, 26.
- Bornhövd, K., Quante, M., Glauche, V., Bromm, B., Weiller, C., and Büchel, C. (2002). Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. *Brain* 125, 1326–1336.
- Bossé, G.D., and Peterson, R.T. (2017). Development of an opioid self-administration assay to study drug seeking in zebrafish. *Behav. Brain Res.* 335, 158–166.
- Bouhassira, D., Lantéri-Minet, M., Attal, N., Laurent, B., and Touboul, C. (2008a). Prevalence of chronic pain with neuropathic characteristics in the general population. *Pain* 136, 380–387.
- Bouhassira, D., Lantéri-Minet, M., Attal, N., Laurent, B., and Touboul, C. (2008b). Prevalence of chronic pain with neuropathic characteristics in the general population: *Pain* 136, 380–387.
- Bourgeois, L., Gauriau, C., and Bernard, J.-F. (2001). Projections from the nociceptive area of the central nucleus of the amygdala to the forebrain: a PHA-L study in the rat. *European Journal of Neuroscience* 14, 229–255.
- Bourin, M., and Hascoët, M. (2003). The mouse light/dark box test. *Eur. J. Pharmacol.* 463, 55–65.
- Bourne, S., Machado, A.G., and Nagel, S.J. (2014). Basic Anatomy and Physiology of Pain Pathways. *Neurosurgery Clinics of North America* 25, 629–638.
- Bourquin, A.-F., Süveges, M., Pertin, M., Gilliard, N., Sardy, S., Davison, A.C., Spahn, D.R., and Decosterd, I. (2006). Assessment and analysis of mechanical allodynia-like behavior induced by spared nerve injury (SNI) in the mouse. *Pain* 122, 14.e1-14.
- Bove, S.E., Laemont, K.D., Brooker, R.M., Osborn, M.N., Sanchez, B.M., Guzman, R.E., Hook, K.E., Juneau, P.L., Connor, J.R., and Kilgore, K.S. (2006). Surgically induced osteoarthritis in the rat results in the development of both osteoarthritis-like joint pain and secondary hyperalgesia. *Osteoarthr. Cartil.* 14, 1041–1048.

- Boyce-Rustay, J.M., Zhong, C., Kohnken, R., Baker, S.J., Simler, G.H., Wensink, E.J., Decker, M.W., and Honore, P. (2010). Comparison of mechanical allodynia and the affective component of inflammatory pain in rats. *Neuropharmacology* 58, 537–543.
- Bragin, E.O., Yeliseeva, Z.V., Vasilenko, G.F., Meizerov, E.E., Chuvin, B.T., and Durinyan, R.A. (1984). Cortical projections to the periaqueductal grey in the cat: a retrograde horseradish peroxidase study. *Neurosci. Lett.* 51, 271–275.
- Braundmeier, A.G., and Fazleabas, A.T. (2009). The non-human primate model of endometriosis: research and implications for fecundity. *Mol. Hum. Reprod.* 15, 577–586.
- Breivik, H., Collett, B., Ventafridda, V., Cohen, R., and Gallacher, D. (2006a). Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain* 10, 287–333.
- Breivik, H., Collett, B., Ventafridda, V., Cohen, R., and Gallacher, D. (2006b). Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain* 10, 287–333.
- Breivik, H., Eisenberg, E., O'Brien, T., and OPENMinds (2013). The individual and societal burden of chronic pain in Europe: the case for strategic prioritisation and action to improve knowledge and availability of appropriate care. *BMC Public Health* 13, 1229.
- Brennan, J.E., Calder, A.A., Leitch, C.R., Greer, I.A., Chou, M.M., and MacKenzie, I.Z. (1997). Recombinant human relaxin as a cervical ripening agent. *Br J Obstet Gynaecol* 104, 775–780.
- Brenner, D.S., Golden, J.P., and Gereau, R.W. (2012). A novel behavioral assay for measuring cold sensation in mice. *PLoS ONE* 7, e39765.
- Brodin, E., Ernberg, M., and Olgart, L. (2016). Neurobiology: General considerations—from acute to chronic pain. *Nor. Tannlegeforen. Tid* 126, 28–33.
- Brodkin, J., Frank, D., Grippo, R., Hausfater, M., Gulinello, M., Achterholt, N., and Gutzen, C. (2014). Validation and implementation of a novel high-throughput behavioral phenotyping instrument for mice. *J. Neurosci. Methods* 224, 48–57.
- van den Broek, M.F., van den Berg, W.B., van de Putte, L.B., and Severijnen, A.J. (1988). Streptococcal cell wall-induced arthritis and flare-up reaction in mice induced by homologous or heterologous cell walls. *Am J Pathol* 133, 139–149.
- Brown, C. (2015). Comparative evolutionary approach to pain perception in fishes. 7.
- Brown, D.C., Agnello, K., and Iadarola, M.J. (2015). Intrathecal resiniferatoxin in a dog model: efficacy in bone cancer pain. *Pain* 156, 1018–1024.
- Buck, S.H., Deshmukh, P.P., Yamamura, H.I., and Burks, T.F. (1981). Thermal analgesia and substance P depletion induced by capsaicin in guinea-pigs. *Neuroscience* 6, 2217–2222.

- Burma, N.E., Leduc-Pessah, H., Fan, C.Y., and Trang, T. (2017a). Animal models of chronic pain: Advances and challenges for clinical translation. *J. Neurosci. Res.* *95*, 1242–1256.
- Burma, N.E., Leduc-Pessah, H., Fan, C.Y., and Trang, T. (2017b). Animal models of chronic pain: Advances and challenges for clinical translation. *J. Neurosci. Res.* *95*, 1242–1256.
- Burnstock, G. (2016). Purinergic Mechanisms and Pain. *Adv. Pharmacol.* *75*, 91–137.
- Burnstock, G., and Knight, G.E. (2018). The potential of P2X7 receptors as a therapeutic target, including inflammation and tumour progression. *Purinergic Signal.* *14*, 1–18.
- Buzzi, M.G., Carter, W.B., Shimizu, T., Heath, H., and Moskowitz, M.A. (1991). Dihydroergotamine and sumatriptan attenuate levels of CGRP in plasma in rat superior sagittal sinus during electrical stimulation of the trigeminal ganglion. *Neuropharmacology* *30*, 1193–1200.
- Caldwell, J.C., and Tracey, W.D. (2010). Alternatives to mammalian pain models 2: using *Drosophila* to identify novel genes involved in nociception. *Methods Mol. Biol.* *617*, 19–29.
- Callander, G.E., and Bathgate, R. a. D. (2010). Relaxin family peptide systems and the central nervous system. *Cell. Mol. Life Sci.* *67*, 2327–2341.
- Campbell, J.N., and Meyer, R.A. (2006). Mechanisms of Neuropathic Pain. *Neuron* *52*, 77–92.
- Campos, A.C., Fogaça, M.V., Aguiar, D.C., and Guimarães, F.S. (2013). Animal models of anxiety disorders and stress. *Braz J Psychiatry* *35 Suppl 2*, S101-111.
- Canavero, S., and Bonicalzi, V. (2007). Central pain syndrome: elucidation of genesis and treatment. *Expert Rev Neurother* *7*, 1485–1497.
- Caplazi, P., Baca, M., Barck, K., Carano, R. a. D., DeVoss, J., Lee, W.P., Bolon, B., and Diehl, L. (2015). Mouse Models of Rheumatoid Arthritis. *Vet. Pathol.* *52*, 819–826.
- Carlton, S.M., Lekan, H.A., Kim, S.H., and Chung, J.M. (1994). Behavioral manifestations of an experimental model for peripheral neuropathy produced by spinal nerve ligation in the primate. *Pain* *56*, 155–166.
- Carter, R.B., and Francis, W.R. (1991). Capsaicin desensitization to plasma extravasation evoked by antidromic C-fiber stimulation is not associated with antinociception in the rat. *Neurosci. Lett.* *127*, 43–45.
- Cash, J.L., White, G.E., and Greaves, D.R. (2009). Chapter 17. Zymosan-induced peritonitis as a simple experimental system for the study of inflammation. *Meth. Enzymol.* *461*, 379–396.
- Catanzaro, A., Di Salvo, A., Steagall, P.V., Zampini, D., Polisca, A., and Della Rocca, G. (2016). Preliminary study on attitudes, opinions and knowledge of Italian veterinarians with regard to abdominal visceral pain in dogs. *Vet Anaesth Analg* *43*, 361–370.

- Caterina, M.J., Leffler, A., Malmberg, A.B., Martin, W.J., Trafton, J., Petersen-Zeitz, K.R., Koltzenburg, M., Basbaum, A.I., and Julius, D. (2000). Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 288, 306–313.
- Chae, J.-P., Park, M.S., Hwang, Y.-S., Min, B.-H., Kim, S.-H., Lee, H.-S., and Park, M.-J. (2015). Evaluation of developmental toxicity and teratogenicity of diclofenac using *Xenopus* embryos. *Chemosphere* 120, 52–58.
- Chan, L.J., Hossain, M.A., Samuel, C.S., Separovic, F., and Wade, J.D. (2011). The relaxin peptide family--structure, function and clinical applications. *Protein Pept. Lett.* 18, 220–229.
- Chang, A., and Quick, J. (2020). Capsaicin. In *StatPearls*, (Treasure Island (FL): StatPearls Publishing), p.
- Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M., and Yaksh, T.L. (1994). Quantitative assessment of tactile allodynia in the rat paw. *J. Neurosci. Methods* 53, 55–63.
- Chen, J., Kuei, C., Sutton, S.W., Bonaventure, P., Nepomuceno, D., Eriste, E., Sillard, R., Lovenberg, T.W., and Liu, C. (2005). Pharmacological characterization of relaxin-3/INSL7 receptors GPCR135 and GPCR142 from different mammalian species. *The Journal of Pharmacology and Experimental Therapeutics* 312, 83–95.
- Chen, T., Koga, K., Descalzi, G., Qiu, S., Wang, J., Zhang, L.-S., Zhang, Z.-J., He, X.-B., Qin, X., Xu, F.-Q., et al. (2014). Postsynaptic potentiation of corticospinal projecting neurons in the anterior cingulate cortex after nerve injury. *Mol Pain* 10, 33.
- Chen, T., Taniguchi, W., Chen, Q.-Y., Tozaki-Saitoh, H., Song, Q., Liu, R.-H., Koga, K., Matsuda, T., Kaito-Sugimura, Y., Wang, J., et al. (2018). Top-down descending facilitation of spinal sensory excitatory transmission from the anterior cingulate cortex. *Nat Commun* 9, 1886.
- Chen, T.-Y., Li, X., Hung, C.-H., Bahudhanapati, H., Tan, J., Kass, D.J., and Zhang, Y. (2020). The relaxin family peptide receptor 1 (RXFP1): An emerging player in human health and disease. *Mol Genet Genomic Med* 8, e1194.
- Chen, X., Feng, X., and Guang, S. (2016). Targeted genome engineering in *Caenorhabditis elegans*. *Cell Biosci* 6, 60.
- Cheng, L., Duan, B., Huang, T., Zhang, Y., Chen, Y., Britz, O., Garcia-Campmany, L., Ren, X., Vong, L., Lowell, B.B., et al. (2017a). Identification of spinal circuits involved in touch-evoked dynamic mechanical pain. *Nat Neurosci* 20, 804–814.
- Cheng, L., Duan, B., Huang, T., Zhang, Y., Chen, Y., Britz, O., Garcia-Campmany, L., Ren, X., Vong, L., Lowell, B.B., et al. (2017b). Identification of spinal circuits involved in touch-evoked dynamic mechanical pain. *Nat. Neurosci.* 20, 804–814.

- Chessell, I.P., Hatcher, J.P., Bountra, C., Michel, A.D., Hughes, J.P., Green, P., Egerton, J., Murfin, M., Richardson, J., Peck, W.L., et al. (2005). Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. *Pain* 114, 386–396.
- Choi, Y., Yoon, Y.W., Na, H.S., Kim, S.H., and Chung, J.M. (1994). Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. *Pain* 59, 369–376.
- Christensen, M.D., Everhart, A.W., Pickelman, J.T., and Hulsebosch, C.E. (1996). Mechanical and thermal allodynia in chronic central pain following spinal cord injury. *Pain* 68, 97–107.
- Christensen, S.L., Hansen, R.B., Storm, M.A., Olesen, J., Hansen, T.F., Ossipov, M., Izzarzugaza, J.M.G., Porreca, F., and Kristensen, D.M. (2020). Von Frey testing revisited: Provision of an online algorithm for improved accuracy of 50% thresholds. *Eur J Pain* 24, 783–790.
- Ciaramella, A. (2019). Psychopharmacology of chronic pain. *Handb Clin Neurol* 165, 317–337.
- Cockayne, D.A., Hamilton, S.G., Zhu, Q.M., Dunn, P.M., Zhong, Y., Novakovic, S., Malmberg, A.B., Cain, G., Berson, A., Kassotakis, L., et al. (2000). Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. *Nature* 407, 1011–1015.
- Colburn, R.W., Lubin, M.L., Stone, D.J., Wang, Y., Lawrence, D., D’Andrea, M.R., Brandt, M.R., Liu, Y., Flores, C.M., and Qin, N. (2007). Attenuated cold sensitivity in TRPM8 null mice. *Neuron* 54, 379–386.
- Cordero-Erausquin, M., Inquimbert, P., Schlichter, R., and Hugel, S. (2016). Neuronal networks and nociceptive processing in the dorsal horn of the spinal cord. *Neuroscience* 338, 230–247.
- Costa, B., Trovato, A.E., Colleoni, M., Giagnoni, G., Zarini, E., and Croci, T. (2005). Effect of the cannabinoid CB1 receptor antagonist, SR141716, on nociceptive response and nerve demyelination in rodents with chronic constriction injury of the sciatic nerve. *Pain* 116, 52–61.
- Costa, F.V., Rosa, L.V., Quadros, V.A., Santos, A.R.S., Kalueff, A.V., and Rosemberg, D.B. (2019). Understanding nociception-related phenotypes in adult zebrafish: Behavioral and pharmacological characterization using a new acetic acid model. *Behav. Brain Res.* 359, 570–578.
- Costigan, M., Scholz, J., and Woolf, C.J. (2009a). Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu. Rev. Neurosci.* 32, 1–32.
- Costigan, M., Scholz, J., and Woolf, C.J. (2009b). Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu. Rev. Neurosci.* 32, 1–32.
- Coull, J.A.M., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., Gravel, C., Salter, M.W., and De Koninck, Y. (2005). BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 438, 1017–1021.
- Courtenay, J.S., Dallman, M.J., Dayan, A.D., Martin, A., and Mosedale, B. (1980). Immunisation against heterologous type II collagen induces arthritis in mice. *Nature* 283, 666–668.

- Cragg, B., Ji, G., and Neugebauer, V. (2016). Differential contributions of vasopressin V1A and oxytocin receptors in the amygdala to pain-related behaviors in rats. *Mol Pain* *12*.
- Crawley, J., and Goodwin, F.K. (1980). Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol. Biochem. Behav.* *13*, 167–170.
- Crofford, L.J. (2015). Chronic Pain: Where the Body Meets the Brain. *Trans. Am. Clin. Climatol. Assoc.* *126*, 167–183.
- Cruccu, G., Nurmikko, T.J., Ernault, E., Riaz, F.K., McBride, W.T., and Haanpää, M. (2018). Superiority of capsaicin 8% patch versus oral pregabalin on dynamic mechanical allodynia in patients with peripheral neuropathic pain. *Eur J Pain* *22*, 700–706.
- Cui, Y., Toyoda, H., Sako, T., Onoe, K., Hayashinaka, E., Wada, Y., Yokoyama, C., Onoe, H., Kataoka, Y., and Watanabe, Y. (2015). A voxel-based analysis of brain activity in high-order trigeminal pathway in the rat induced by cortical spreading depression. *Neuroimage* *108*, 17–22.
- Curtright, A., Rosser, M., Goh, S., Keown, B., Wagner, E., Sharifi, J., Raible, D.W., and Dhaka, A. (2015). Modeling Nociception in Zebrafish: A Way Forward for Unbiased Analgesic Discovery. *PLoS One* *10*.
- D'Amour, F.E., and Smith, D.L. (1941). A method for determining loss of pain sensation.
- Danquah, W., Meyer-Schwesinger, C., Rissiek, B., Pinto, C., Serracant-Prat, A., Amadi, M., Iacenda, D., Knop, J.-H., Hammel, A., Bergmann, P., et al. (2016). Nanobodies that block gating of the P2X7 ion channel ameliorate inflammation. *Sci Transl Med* *8*, 366ra162.
- Davidson, E., Coppey, L., Lu, B., Arballo, V., Calcutt, N.A., Gerard, C., and Yorek, M. (2009). The roles of streptozotocin neurotoxicity and neutral endopeptidase in murine experimental diabetic neuropathy. *Exp Diabetes Res* *2009*, 431980.
- Davis, K.D., Taylor, S.J., Crawley, A.P., Wood, M.L., and Mikulis, D.J. (1997). Functional MRI of pain- and attention-related activations in the human cingulate cortex. *J. Neurophysiol.* *77*, 3370–3380.
- Deacon, R.M.J. (2006). Burrowing in rodents: a sensitive method for detecting behavioral dysfunction. *Nat Protoc* *1*, 118–121.
- Deakin, A.G., Buckley, J., AlZu'bi, H.S., Cossins, A.R., Spencer, J.W., Al'Nuaimy, W., Young, I.S., Thomson, J.S., and Sneddon, L.U. (2019). Automated monitoring of behaviour in zebrafish after invasive procedures. *Sci Rep* *9*, 1–13.
- Decosterd, I., and Woolf, C.J. (2000). Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain* *87*, 149–158.
- Demin, K.A., Meshalkina, D.A., Kysil, E.V., Antonova, K.A., Volgin, A.D., Yakovlev, O.A., Alekseeva, P.A., Firuleva, M.M., Lakstygal, A.M., de Abreu, M.S., et al. (2018). Zebrafish models

relevant to studying central opioid and endocannabinoid systems. *Prog. Neuropsychopharmacol. Biol. Psychiatry* *86*, 301–312.

Deuis, J.R., Dvorakova, L.S., and Vetter, I. (2017a). Methods Used to Evaluate Pain Behaviors in Rodents. *Front Mol Neurosci* *10*.

Deuis, J.R., Dvorakova, L.S., and Vetter, I. (2017b). Methods Used to Evaluate Pain Behaviors in Rodents. *Front Mol Neurosci* *10*, 284.

Deuis, J.R., Dvorakova, L.S., and Vetter, I. (2017c). Methods Used to Evaluate Pain Behaviors in Rodents. *Front Mol Neurosci* *10*, 284.

Diepenhorst, N.A., Petrie, E.J., Chen, C.Z., Wang, A., Hossain, M.A., Bathgate, R.A.D., and Gooley, P.R. (2014). Investigation of Interactions at the Extracellular Loops of the Relaxin Family Peptide Receptor 1 (RXFP1). *J Biol Chem* *289*, 34938–34952.

Dixon, W.J. (1980). Efficient analysis of experimental observations. *Annu. Rev. Pharmacol. Toxicol.* *20*, 441–462.

Dobremez, E., Bouali-Benazzouz, R., Fossat, P., Monteils, L., Dulluc, J., Nagy, F., and Landry, M. (2005). Distribution and regulation of L-type calcium channels in deep dorsal horn neurons after sciatic nerve injury in rats. *Eur. J. Neurosci.* *21*, 3321–3333.

Doherty, N.S., Poubelle, P., Borgeat, P., Beaver, T.H., Westrich, G.L., and Schrader, N.L. (1985). Intraperitoneal injection of zymosan in mice induces pain, inflammation and the synthesis of peptidoleukotrienes and prostaglandin E₂. *Prostaglandins* *30*, 769–789.

Dolensek, N., Gehrlach, D.A., Klein, A.S., and Gogolla, N. (2020). Facial expressions of emotion states and their neuronal correlates in mice. *Science* *368*, 89–94.

Dolique, T., Landry, M., and Nagy, F. (2010). Spinal cord: dorsal horn. In *Handbook of Brain Microcircuits*, (Oxford University Press), pp. 237–248.

Dray, A. (2008). Neuropathic pain: emerging treatments. *Br J Anaesth* *101*, 48–58.

Drewes, A.M., Schipper, K.P., Dimcevski, G., Petersen, P., Gregersen, H., Funch-Jensen, P., and Arendt-Nielsen, L. (2003). Gut pain and hyperalgesia induced by capsaicin: a human experimental model. *Pain* *104*, 333–341.

Drewes, A.M., Olesen, A.E., Farmer, A.D., Szigethy, E., Rebours, V., and Olesen, S.S. (2020). Gastrointestinal pain. *Nat Rev Dis Primers* *6*, 1.

Dschietzig, T., Bartsch, C., Wessler, S., Baumann, G., and Stangl, K. (2009a). Autoregulation of human relaxin-2 gene expression critically involves relaxin and glucocorticoid receptor binding to glucocorticoid response half-sites in the relaxin-2 promoter. *Regul Pept* *155*, 163–173.

- Dschietzig, T., Bartsch, C., Baumann, G., and Stangl, K. (2009b). RXFP1-inactive relaxin activates human glucocorticoid receptor: further investigations into the relaxin-GR pathway. *Regul Pept* *154*, 77–84.
- D'Souza, W.N., Ng, G.Y., Youngblood, B.D., Tsuji, W., and Lehto, S.G. (2011a). A review of current animal models of osteoarthritis pain. *Curr Pharm Biotechnol* *12*, 1596–1612.
- D'Souza, W.N., Ng, G.Y., Youngblood, B.D., Tsuji, W., and Lehto, S.G. (2011b). A review of current animal models of osteoarthritis pain. *Curr Pharm Biotechnol* *12*, 1596–1612.
- Duan, B., Cheng, L., Bourane, S., Britz, O., Padilla, C., Garcia-Campmany, L., Krashes, M., Knowlton, W., Velasquez, T., Ren, X., et al. (2014). Identification of spinal circuits transmitting and gating mechanical pain. *Cell* *159*, 1417–1432.
- Dubin, A.E., and Patapoutian, A. (2010). Nociceptors: the sensors of the pain pathway. *J. Clin. Invest.* *120*, 3760–3772.
- Dubuisson, D., and Dennis, S.G. (1977). The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain* *4*, 161–174.
- Dum, R.P., Levinthal, D.J., and Strick, P.L. (2009). The Spinothalamic System Targets Motor and Sensory Areas in the Cerebral Cortex of Monkeys. *J. Neurosci.* *29*, 14223–14235.
- Eliava, M., Melchior, M., Knobloch-Bollmann, H.S., Wahis, J., da Silva Gouveia, M., Tang, Y., Ciobanu, A.C., Triana Del Rio, R., Roth, L.C., Althammer, F., et al. (2016). A New Population of Parvocellular Oxytocin Neurons Controlling Magnocellular Neuron Activity and Inflammatory Pain Processing. *Neuron* *89*, 1291–1304.
- Ellis, L.D., Berrue, F., Morash, M., Achenbach, J.C., Hill, J., and McDougall, J.J. (2018). Comparison of cannabinoids with known analgesics using a novel high throughput zebrafish larval model of nociception. *Behav. Brain Res.* *337*, 151–159.
- Ellison, D.L. (2017). Physiology of Pain. *Crit Care Nurs Clin North Am* *29*, 397–406.
- Elramah, S., López-González, M.J., Bastide, M., Dixmérias, F., Roca-Lapirot, O., Wielanek-Bachelet, A.-C., Vital, A., Leste-Lasserre, T., Brochard, A., Landry, M., et al. (2017). Spinal miRNA-124 regulates synaptopodin and nociception in an animal model of bone cancer pain. *Sci Rep* *7*, 10949.
- Enomoto, H., Fujikoshi, S., Funai, J., Sasaki, N., Ossipov, M.H., Tsuji, T., Alev, L., and Ushida, T. (2017). Assessment of direct analgesic effect of duloxetine for chronic low back pain: post hoc path analysis of double-blind, placebo-controlled studies. *J Pain Res* *10*, 1357–1368.
- Enomoto, M., Mantyh, P.W., Murrell, J., Innes, J.F., and Lascelles, B.D.X. (2019). Anti-nerve growth factor monoclonal antibodies for the control of pain in dogs and cats. *Vet. Rec.* *184*, 23.

- Eto, K., Wake, H., Watanabe, M., Ishibashi, H., Noda, M., Yanagawa, Y., and Nabekura, J. (2011). Inter-regional Contribution of Enhanced Activity of the Primary Somatosensory Cortex to the Anterior Cingulate Cortex Accelerates Chronic Pain Behavior. *J. Neurosci.* *31*, 7631–7636.
- Evans, B.A., John, M., Fowler, K.J., Summers, R.J., Cronk, M., Shine, J., and Tregear, G.W. (1993). The mouse relaxin gene: nucleotide sequence and expression. *Journal of Molecular Endocrinology* *10*, 15–23.
- Falk, S., and Dickenson, A.H. (2014). Pain and nociception: mechanisms of cancer-induced bone pain. *J. Clin. Oncol.* *32*, 1647–1654.
- Falk, S., Appel, C.K., Bennedbæk, H.B., Al-Dihaissy, T., Unger, A., Dinkel, K., and Heegaard, A.-M. (2019). Chronic high dose P2X7 receptor inhibition exacerbates cancer-induced bone pain. *Eur. J. Pharmacol.* *845*, 48–55.
- Fang, H., and Beier, F. (2014). Mouse models of osteoarthritis: modelling risk factors and assessing outcomes. *Nat Rev Rheumatol* *10*, 413–421.
- Fehrenbacher, J.C., Vasko, M.R., and Duarte, D.B. (2012). Models of Inflammation: Carrageenan- or Complete Freund's Adjuvant-Induced Edema and Hypersensitivity in the Rat. *Curr Protoc Pharmacol* *0 5*, Unit5.4.
- Feng, S., Agoulnik, I.U., Truong, A., Li, Z., Creighton, C.J., Kaftanovskaya, E.M., Pereira, R., Han, H.D., Lopez-Berestein, G., Klonisch, T., et al. (2010). Suppression of relaxin receptor RXFP1 decreases prostate cancer growth and metastasis. *Endocr Relat Cancer* *17*, 1021–1033.
- Fernandes, E.S., Russell, F.A., Spina, D., McDougall, J.J., Graepel, R., Gentry, C., Staniland, A.A., Mountford, D.M., Keeble, J.E., Malcangio, M., et al. (2011). A distinct role for transient receptor potential ankyrin 1, in addition to transient receptor potential vanilloid 1, in tumor necrosis factor α -induced inflammatory hyperalgesia and Freund's complete adjuvant-induced monoarthritis. *Arthritis Rheum.* *63*, 819–829.
- Ferrari, L.F., Bogen, O., Reichling, D.B., and Levine, J.D. (2015). Accounting for the delay in the transition from acute to chronic pain: axonal and nuclear mechanisms. *J. Neurosci.* *35*, 495–507.
- Ferrari, L.F., Levine, J.D., and Green, P.G. (2016). Mechanisms mediating nitroglycerin-induced delayed-onset hyperalgesia in the rat. *Neuroscience* *317*, 121–129.
- Fields, H.L., Rowbotham, M., and Baron, R. (1998). Postherpetic neuralgia: irritable nociceptors and deafferentation. *Neurobiol. Dis.* *5*, 209–227.
- Filingeri, D. (2016). Neurophysiology of Skin Thermal Sensations. *Compr Physiol* *6*, 1429.
- Finnerup, N.B., Johannesen, I.L., Fuglsang-Frederiksen, A., Bach, F.W., and Jensen, T.S. (2003). Sensory function in spinal cord injury patients with and without central pain. *Brain* *126*, 57–70.

- Finnerup, N.B., Sindrup, S.H., and Jensen, T.S. (2010). The evidence for pharmacological treatment of neuropathic pain. *Pain* *150*, 573–581.
- Fischer, B.D., Adeyemo, A., O’Leary, M.E., and Bottaro, A. (2017). Animal models of rheumatoid pain: experimental systems and insights. *Arthritis Res. Ther.* *19*, 146.
- Fontana, B.D., Mezzomo, N.J., Kalueff, A.V., and Rosenberg, D.B. (2018). The developing utility of zebrafish models of neurological and neuropsychiatric disorders: A critical review. *Exp. Neurol.* *299*, 157–171.
- Ford, B., Holmes, C.J., Mainville, L., and Jones, B.E. (1995). GABAergic neurons in the rat pontomesencephalic tegmentum: codistribution with cholinergic and other tegmental neurons projecting to the posterior lateral hypothalamus. *The Journal of Comparative Neurology* *363*, 177–196.
- Fossat, P., Dobremez, E., Bouali-Benazzouz, R., Favereaux, A., Bertrand, S.S., Kilk, K., Léger, C., Cazalets, J.-R., Langel, U., Landry, M., et al. (2010). Knockdown of L calcium channel subtypes: differential effects in neuropathic pain. *J. Neurosci.* *30*, 1073–1085.
- Frias, B., and Merighi, A. (2016). Capsaicin, Nociception and Pain. *Molecules* *21*.
- Fricke, B., Muller, A., and René, F. (2008). Evaluation tools and animal models of peripheral neuropathies. *Neurodegener Dis* *5*, 72–108.
- Fuentes, I.M., and Christianson, J.A. (2018). The Influence of Early Life Experience on Visceral Pain. *Front Syst Neurosci* *12*, 2.
- Gagnon, M., Bergeron, M.J., Lavertu, G., Castonguay, A., Tripathy, S., Bonin, R.P., Perez-Sanchez, J., Boudreau, D., Wang, B., Dumas, L., et al. (2013). Chloride extrusion enhancers as novel therapeutics for neurological diseases. *Nat. Med.* *19*, 1524–1528.
- Gamse, R., Holzer, P., and Lembeck, F. (1980). Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. *Br. J. Pharmacol.* *68*, 207–213.
- Gamse, R., Petsche, U., Lembeck, F., and Jancsó, G. (1982). Capsaicin applied to peripheral nerve inhibits axoplasmic transport of substance P and somatostatin. *Brain Res.* *239*, 447–462.
- Ganella, D.E., Callander, G.E., Ma, S., Bye, C.R., Gundlach, A.L., and Bathgate, R. a. D. (2013). Modulation of feeding by chronic rAAV expression of a relaxin-3 peptide agonist in rat hypothalamus. *Gene Ther.* *20*, 703–716.
- Gao, Y.-J., Ren, W.-H., Zhang, Y.-Q., and Zhao, Z.-Q. (2004). Contributions of the anterior cingulate cortex and amygdala to pain- and fear-conditioned place avoidance in rats. *PAIN* *110*, 343–353.

García-Martínez, C., Humet, M., Planells-Cases, R., Gomis, A., Caprini, M., Viana, F., De La Pena, E., Sanchez-Baeza, F., Carbonell, T., De Felipe, C., et al. (2002). Attenuation of thermal nociception and hyperalgesia by VR1 blockers. *Proc. Natl. Acad. Sci. U.S.A.* *99*, 2374–2379.

Gaviria, M., Haton, H., Sandillon, F., and Privat, A. (2002). A mouse model of acute ischemic spinal cord injury. *J. Neurotrauma* *19*, 205–221.

Gearing, D.P., Virtue, E.R., Gearing, R.P., and Drew, A.C. (2013). A fully caninised anti-NGF monoclonal antibody for pain relief in dogs. *BMC Vet. Res.* *9*, 226.

Gerber, B., Yarali, A., Diegelmann, S., Wotjak, C.T., Pauli, P., and Fendt, M. (2014). Pain-relief learning in flies, rats, and man: basic research and applied perspectives. *Learn. Mem.* *21*, 232–252.

Ghilardi, J.R., Röhrich, H., Lindsay, T.H., Sevcik, M.A., Schwei, M.J., Kubota, K., Halvorson, K.G., Poblete, J., Chaplan, S.R., Dubin, A.E., et al. (2005a). Selective blockade of the capsaicin receptor TRPV1 attenuates bone cancer pain. *J. Neurosci.* *25*, 3126–3131.

Ghilardi, J.R., Röhrich, H., Lindsay, T.H., Sevcik, M.A., Schwei, M.J., Kubota, K., Halvorson, K.G., Poblete, J., Chaplan, S.R., Dubin, A.E., et al. (2005b). Selective blockade of the capsaicin receptor TRPV1 attenuates bone cancer pain. *J. Neurosci.* *25*, 3126–3131.

Giacomotto, J., and Ségalat, L. (2010). High-throughput screening and small animal models, where are we? *Br J Pharmacol* *160*, 204–216.

Giamberardino, M.A., Valente, R., de Bigontina, P., and Vecchiet, L. (1995). Artificial ureteral calculosis in rats: behavioural characterization of visceral pain episodes and their relationship with referred lumbar muscle hyperalgesia. *Pain* *61*, 459–469.

Gierthmühlen, J., Maier, C., Baron, R., Tölle, T., Treede, R.-D., Birbaumer, N., Hüge, V., Koroschetz, J., Krumova, E.K., Lauchart, M., et al. (2012). Sensory signs in complex regional pain syndrome and peripheral nerve injury. *Pain* *153*, 765–774.

Gilchrist, L.S., Cain, D.M., Harding-Rose, C., Kov, A.N., Wendelschafer-Crabb, G., Kennedy, W.R., and Simone, D.A. (2005). Re-organization of P2X3 receptor localization on epidermal nerve fibers in a murine model of cancer pain. *Brain Res.* *1044*, 197–205.

Gingras, J., Smith, S., Matson, D.J., Johnson, D., Nye, K., Couture, L., Feric, E., Yin, R., Moyer, B.D., Peterson, M.L., et al. (2014). Global Nav1.7 knockout mice recapitulate the phenotype of human congenital indifference to pain. *PLoS ONE* *9*, e105895.

Glasson, S.S., Blanchet, T.J., and Morris, E.A. (2007). The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. *Osteoarthr. Cartil.* *15*, 1061–1069.

Glauser, D.A., Chen, W.C., Agin, R., Macinnis, B.L., Hellman, A.B., Garrity, P.A., Tan, M.-W., and Goodman, M.B. (2011). Heat avoidance is regulated by transient receptor potential (TRP) channels and a neuropeptide signaling pathway in *Caenorhabditis elegans*. *Genetics* *188*, 91–103.

- Goll, Y., Atlan, G., and Citri, A. (2015). Attention: the claustrum. *Trends Neurosci* 38, 486–495.
- Gomis, A., Miralles, A., Schmidt, R.F., and Belmonte, C. (2007). Nociceptive nerve activity in an experimental model of knee joint osteoarthritis of the guinea pig: effect of intra-articular hyaluronan application. *Pain* 130, 126–136.
- Gonzalez-Nunez, V., and Rodríguez, R.E. (2009). The zebrafish: a model to study the endogenous mechanisms of pain. *ILAR J* 50, 373–386.
- Goto, M., Swanson, L.W., and Canteras, N.S. (2001). Connections of the nucleus incertus. *The Journal of Comparative Neurology* 438, 86–122.
- Goyal, N., Rana, A., Ahlawat, A., Bijjem, K.R.V., and Kumar, P. (2014). Animal models of inflammatory bowel disease: a review. *Inflammopharmacology* 22, 219–233.
- de Grauw, J.C., and van Loon, J.P. a. M. (2016). Systematic pain assessment in horses. *Vet. J.* 209, 14–22.
- Greenwood-Van Meerveld, B., and Johnson, A.C. (2017). Stress-Induced Chronic Visceral Pain of Gastrointestinal Origin. *Front Syst Neurosci* 11, 86.
- Gregory, N.S., Harris, A.L., Robinson, C.R., Dougherty, P.M., Fuchs, P.N., and Sluka, K.A. (2013). An overview of animal models of pain: disease models and outcome measures. *J Pain* 14, 1255–1269.
- Groenewald, C.B., and Palermo, T.M. (2015). The price of pain: the economics of chronic adolescent pain. *Pain Manag* 5, 61–64.
- Gruen, M.E., Thomson, A.E., Griffith, E.H., Paradise, H., Gearing, D.P., and Lascelles, B.D.X. (2016). A Feline-Specific Anti-Nerve Growth Factor Antibody Improves Mobility in Cats with Degenerative Joint Disease-Associated Pain: A Pilot Proof of Concept Study. *J. Vet. Intern. Med.* 30, 1138–1148.
- Guedon, J.-M.G., Longo, G., Majuta, L.A., Thomspson, M.L., Fealk, M.N., and Mantyh, P.W. (2016). Dissociation between the relief of skeletal pain behaviors and skin hypersensitivity in a model of bone cancer pain. *Pain* 157, 1239–1247.
- Gunnensen, J.M., Crawford, R.J., and Tregear, G.W. (1995). Expression of the relaxin gene in rat tissues. *Mol Cell Endocrinol* 110, 55–64.
- Gunthorpe, M.J., and Chizh, B.A. (2009). Clinical development of TRPV1 antagonists: targeting a pivotal point in the pain pathway. *Drug Discov. Today* 14, 56–67.
- Guo, N., and Gu, X. (2014). Sciatic Nerve Neuropathy in Cynomolgus Monkey *Macaca Fascicularis*: Altered Leg Usage and Peripheral Nerve Firing. *Journal of Neurology & Neurophysiology* 05.

- Haanpää, M., Cruccu, G., Nurmikko, T.J., McBride, W.T., Docu Axelarad, A., Bosilkov, A., Chambers, C., Ernault, E., and Abdulahad, A.K. (2016). Capsaicin 8% patch versus oral pregabalin in patients with peripheral neuropathic pain. *Eur J Pain* 20, 316–328.
- Hahn, A.F. (1996). Experimental allergic neuritis (EAN) as a model for the immune-mediated demyelinating neuropathies. *Rev. Neurol. (Paris)* 152, 328–332.
- Halls, M.L., and Cooper, D.M.F. (2010). Sub-picomolar relaxin signalling by a pre-assembled RXFP1, AKAP79, AC2, β -arrestin 2, PDE4D3 complex. *The EMBO Journal* 29, 2772.
- Halls, M.L., Bathgate, R.A.D., and Summers, R.J. (2006). Relaxin family peptide receptors RXFP1 and RXFP2 modulate cAMP signaling by distinct mechanisms. *Mol. Pharmacol.* 70, 214–226.
- Halls, M.L., van der Westhuizen, E.T., Bathgate, R. a. D., and Summers, R.J. (2007). Relaxin family peptide receptors--former orphans reunite with their parent ligands to activate multiple signalling pathways. *Br. J. Pharmacol.* 150, 677–691.
- Halls, M.L., Hewitson, T.D., Moore, X.-L., Du, X.-J., Bathgate, R.A.D., and Summers, R.J. (2009). Relaxin activates multiple cAMP signaling pathway profiles in different target cells. *Ann N Y Acad Sci* 1160, 108–111.
- Hamalainen, M.M., Subieta, A., Arpey, C., and Brennan, T.J. (2009). Differential effect of capsaicin treatment on pain related behaviors after plantar incision. *J Pain* 10, 637–645.
- Hamilton, S.G., Warburton, J., Bhattacharjee, A., Ward, J., and McMahon, S.B. (2000). ATP in human skin elicits a dose-related pain response which is potentiated under conditions of hyperalgesia. *Brain* 123 (Pt 6), 1238–1246.
- Han, Y., and Yu, L.-C. (2009). Involvement of oxytocin and its receptor in nociceptive modulation in the central nucleus of amygdala of rats. *Neurosci Lett* 454, 101–104.
- Han, L., Luo, J., Bai, S., Jia, Y., Chen, X., Zhao, Y., Chen, L., Zhu, X., Li, Y., Jiang, Y., et al. (2017). Combined Assessment of Relaxin and B-Type Natriuretic Peptide Improves Diagnostic Value in Patients With Congestive Heart Failure. *Am. J. Med. Sci.* 354, 480–485.
- Han, P., Zhao, J., Liu, S.-B., Yang, C.-J., Wang, Y.-Q., Wu, G.-C., Xu, D.-M., and Mi, W.-L. (2013). Interleukin-33 mediates formalin-induced inflammatory pain in mice. *Neuroscience* 241, 59–66.
- Han, P., Liu, S., Zhang, M., Zhao, J., Wang, Y., Wu, G., and Mi, W. (2015a). Inhibition of Spinal Interleukin-33/ST2 Signaling and Downstream ERK and JNK Pathways in Electroacupuncture Analgesia in Formalin Mice. *PLoS ONE* 10, e0129576.
- Han, S., Soleiman, M.T., Soden, M.E., Zweifel, L.S., and Palmiter, R.D. (2015b). Elucidating an Affective Pain Circuit that Creates a Threat Memory. *Cell* 162, 363–374.

- Handa, J., Sekiguchi, M., Krupkova, O., and Konno, S.-I. (2016). The effect of serotonin-noradrenaline reuptake inhibitor duloxetine on the intervertebral disk-related radiculopathy in rats. *Eur Spine J* 25, 877–887.
- Hargreaves, K., Dubner, R., Brown, F., Flores, C., and Joris, J. (1988). A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 32, 77–88.
- Harriott, A.M., Strother, L.C., Vila-Pueyo, M., and Holland, P.R. (2019). Animal models of migraine and experimental techniques used to examine trigeminal sensory processing. *J Headache Pain* 20, 91.
- Harvey, V.L., and Dickenson, A.H. (2009). Behavioural and electrophysiological characterisation of experimentally induced osteoarthritis and neuropathy in C57Bl/6 mice. *Mol Pain* 5, 18.
- Hashizume, H., Kawakami, M., Yoshida, M., Okada, M., Enyo, Y., and Inomata, Y. (2007). Sarpogrelate hydrochloride, a 5-HT_{2A} receptor antagonist, attenuates neurogenic pain induced by nucleus pulposus in rats. *Spine* 32, 315–320.
- Haugaard-Kedström, L.M., Shabanpoor, F., Hossain, M.A., Clark, R.J., Ryan, P.J., Craik, D.J., Gundlach, A.L., Wade, J.D., Bathgate, R.A.D., and Rosengren, K.J. (2011). Design, synthesis, and characterization of a single-chain peptide antagonist for the relaxin-3 receptor RXFP3. *J. Am. Chem. Soc.* 133, 4965–4974.
- Haugaard-Kedström, L.M., Lee, H.S., Jones, M.V., Song, A., Rathod, V., Hossain, M.A., Bathgate, R.A.D., and Rosengren, K.J. (2018). Binding conformation and determinants of a single-chain peptide antagonist at the relaxin-3 receptor RXFP3. *J Biol Chem* 293, 15765–15776.
- van Hecke, O., Torrance, N., and Smith, B.H. (2013). Chronic pain epidemiology and its clinical relevance. *Br J Anaesth* 111, 13–18.
- Heinricher, M.M., Tavares, I., Leith, J.L., and Lumb, B.M. (2009). Descending control of nociception: Specificity, recruitment and plasticity. *Brain Res Rev* 60, 214–225.
- Henze, D.A., and Urban, M.O. (2010). Large Animal Models for Pain Therapeutic Development. In *Translational Pain Research: From Mouse to Man*, L. Kruger, and A.R. Light, eds. (Boca Raton (FL): CRC Press/Taylor & Francis), p.
- Herzberg, U., and Sagen, J. (2001). Peripheral nerve exposure to HIV viral envelope protein gp120 induces neuropathic pain and spinal gliosis. *J. Neuroimmunol.* 116, 29–39.
- Hilfiger, L., Zhao, Q., Kerspern, D., Inquimbert, P., Andry, V., Goumon, Y., Darbon, P., Hibert, M., and Charlet, A. (2020). A Nonpeptide Oxytocin Receptor Agonist for a Durable Relief of Inflammatory Pain. *Sci Rep* 10, 3017.
- Hill, R. (2000). NK1 (substance P) receptor antagonists--why are they not analgesic in humans? *Trends Pharmacol. Sci.* 21, 244–246.

Hisaw, F.L. (1926). Experimental relaxation of the pubic ligament of the guinea pig. *Proceedings of the Society for Experimental Biology and Medicine* 23, 661–663.

Hoare, B.L., Bruell, S., Sethi, A., Gooley, P.R., Lew, M.J., Hossain, M.A., Inoue, A., Scott, D.J., and Bathgate, R.A.D. (2018). Multi-Component Mechanism of H2 Relaxin Binding to RXFP1 through NanoBRET Kinetic Analysis. *IScience* 11, 93–113.

Hoher, B., Ziebig, R., Krause, R., Asmus, G., Neumayer, H.-H., Liefeldt, L., and Stasch, J.-P. (2004). Relaxin is an independent risk factor predicting death in male patients with end-stage kidney disease. *Circulation* 109, 2266–2268.

Hojo, K., Hossain, M.A., Tailhades, J., Shabanpoor, F., Wong, L.L.L., Ong-Pålsson, E.E.K., Kastman, H.E., Ma, S., Gundlach, A.L., Rosengren, K.J., et al. (2016). Development of a Single-Chain Peptide Agonist of the Relaxin-3 Receptor Using Hydrocarbon Stapling. *J Med Chem* 59, 7445–7456.

Hökfelt, T., Barde, S., Xu, Z.-Q.D., Kuteeva, E., Rüegg, J., Le Maitre, E., Risling, M., Kehr, J., Ihnatko, R., Theodorsson, E., et al. (2018). Neuropeptide and Small Transmitter Coexistence: Fundamental Studies and Relevance to Mental Illness. *Front Neural Circuits* 12, 106.

Holden, E., Calvo, G., Collins, M., Bell, A., Reid, J., Scott, E.M., and Nolan, A.M. (2014). Evaluation of facial expression in acute pain in cats. *J Small Anim Pract* 55, 615–621.

Honore, P., Kage, K., Mikusa, J., Watt, A.T., Johnston, J.F., Wyatt, J.R., Faltynek, C.R., Jarvis, M.F., and Lynch, K. (2002). Analgesic profile of intrathecal P2X(3) antisense oligonucleotide treatment in chronic inflammatory and neuropathic pain states in rats. *Pain* 99, 11–19.

Hootman, J.M., Helmick, C.G., Barbour, K.E., Theis, K.A., and Boring, M.A. (2016). Updated Projected Prevalence of Self-Reported Doctor-Diagnosed Arthritis and Arthritis-Attributable Activity Limitation Among US Adults, 2015–2040. *Arthritis Rheumatol* 68, 1582–1587.

Hopkins, E.J., Bathgate, R.A., and Gooley, P.R. (2005). The Human LGR7 Low-Density Lipoprotein Class A Module Requires Calcium for Structure. *Annals of the New York Academy of Sciences* 1041, 27–34.

Hopkins, E.J., Layfield, S., Ferraro, T., Bathgate, R.A.D., and Gooley, P.R. (2007). The NMR solution structure of the relaxin (RXFP1) receptor lipoprotein receptor class A module and identification of key residues in the N-terminal region of the module that mediate receptor activation. *J Biol Chem* 282, 4172–4184.

Hossain, M.A., Samuel, C.S., Binder, C., Hewitson, T.D., Tregear, G.W., Wade, J.D., and Bathgate, R.A.D. (2010). The chemically synthesized human relaxin-2 analog, B-R13/17K H2, is an RXFP1 antagonist. *Amino Acids* 39, 409–416.

Hossain, M.A., Kocan, M., Yao, S.T., Royce, S.G., Nair, V.B., Siwek, C., Patil, N.A., Harrison, I.P., Rosengren, K.J., Selemidis, S., et al. (2016). A single-chain derivative of the relaxin hormone

is a functionally selective agonist of the G protein-coupled receptor, RXFP1. *Chem Sci* 7, 3805–3819.

Hou, T., Xiang, H., Yu, L., Su, W., Shu, Y., Li, H., Zhu, H., Lin, L., Hu, X., Liang, S., et al. (2019). Electroacupuncture inhibits visceral pain via adenosine receptors in mice with inflammatory bowel disease. *Purinergic Signal*. 15, 193–204.

Hoyer, D., and Bartfai, T. (2012). Neuropeptides and neuropeptide receptors: drug targets, and peptide and non-peptide ligands: a tribute to Prof. Dieter Seebach. *Chem. Biodivers.* 9, 2367–2387.

Hsu, S.Y.T. (2003). New insights into the evolution of the relaxin-LGR signaling system. *Trends in Endocrinology and Metabolism: TEM* 14, 303–309.

Hsu, S.Y., Nakabayashi, K., Nishi, S., Kumagai, J., Kudo, M., Sherwood, O.D., and Hsueh, A.J.W. (2002). Activation of orphan receptors by the hormone relaxin. *Science* 295, 671–674.

Huang, J., Gadotti, V.M., Chen, L., Souza, I.A., Huang, S., Wang, D., Ramakrishnan, C., Deisseroth, K., Zhang, Z., and Zamponi, G.W. (2019). A neuronal circuit for activating descending modulation of neuropathic pain. *Nature Neuroscience* 22, 1659–1668.

Huang, W., Calvo, M., Pheby, T., Bennett, D.L.H., and Rice, A.S.C. (2017). A rodent model of HIV protease inhibitor indinavir induced peripheral neuropathy. *Pain* 158, 75–85.

Hudson, P., John, M., Crawford, R., Haralambidis, J., Scanlon, D., Gorman, J., Tregear, G., Shine, J., and Niall, H. (1984). Relaxin gene expression in human ovaries and the predicted structure of a human preprorelaxin by analysis of cDNA clones. *The EMBO Journal* 3, 2333–2339.

Hunskar, S., and Hole, K. (1987a). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain* 30, 103–114.

Hunskar, S., and Hole, K. (1987b). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain* 30, 103–114.

Ibrahim, A., Hirschfeld, S., Cohen, M.H., Griebel, D.J., Williams, G.A., and Pazdur, R. (2004). FDA drug approval summaries: oxaliplatin. *Oncologist* 9, 8–12.

Ilie, M.A., Caruntu, C., Tampa, M., Georgescu, S.-R., Matei, C., Negrei, C., Ion, R.-M., Constantin, C., Neagu, M., and Boda, D. (2019). Capsaicin: Physicochemical properties, cutaneous reactions and potential applications in painful and inflammatory conditions. *Exp Ther Med* 18, 916–925.

Ishikawa, G., Koya, Y., Tanaka, H., and Nagakura, Y. (2015). Long-term analgesic effect of a single dose of anti-NGF antibody on pain during motion without notable suppression of joint edema and lesion in a rat model of osteoarthritis. *Osteoarthr. Cartil.* 23, 925–932.

- Isola, M., Ferrari, V., Miolo, A., Stabile, F., Bernardini, D., Carnier, P., and Busetto, R. (2011). Nerve growth factor concentrations in the synovial fluid from healthy dogs and dogs with secondary osteoarthritis. *Vet Comp Orthop Traumatol* 24, 279–284.
- Janak, P.H., and Tye, K.M. (2015). From circuits to behaviour in the amygdala. *Nature* 517, 284–292.
- Jarvis, M.F., Burgard, E.C., McGaraughty, S., Honore, P., Lynch, K., Brennan, T.J., Subieta, A., Van Biesen, T., Cartmell, J., Bianchi, B., et al. (2002). A-317491, a novel potent and selective non-nucleotide antagonist of P2X3 and P2X2/3 receptors, reduces chronic inflammatory and neuropathic pain in the rat. *Proc. Natl. Acad. Sci. U.S.A.* 99, 17179–17184.
- Jayasinghe, C.D., and Jayawardena, U.A. (2019). Toxicity Assessment of Herbal Medicine Using Zebrafish Embryos: A Systematic Review (Hindawi).
- Jeibmann, A., and Paulus, W. (2009). *Drosophila melanogaster* as a model organism of brain diseases. *Int J Mol Sci* 10, 407–440.
- Jensen, D.D., Lieu, T., Halls, M.L., Veldhuis, N.A., Imlach, W.L., Mai, Q.N., Poole, D.P., Quach, T., Aurelio, L., Conner, J., et al. (2017). Neurokinin 1 receptor signaling in endosomes mediates sustained nociception and is a viable therapeutic target for prolonged pain relief. *Sci Transl Med* 9.
- Ji, B., Chauffour, A., Andries, K., and Jarlier, V. (2006). Bactericidal activities of R207910 and other newer antimicrobial agents against *Mycobacterium leprae* in mice. *Antimicrob. Agents Chemother.* 50, 1558–1560.
- Ji, R.-R., Xu, Z.-Z., and Gao, Y.-J. (2014). Emerging targets in neuroinflammation-driven chronic pain. *Nat Rev Drug Discov* 13, 533–548.
- Jimenez-Andrade, J.M., Ghilardi, J.R., Castañeda-Corral, G., Kuskowski, M.A., and Mantyh, P.W. (2011). Preventive or late administration of anti-NGF therapy attenuates tumor-induced nerve sprouting, neuroma formation, and cancer pain. *Pain* 152, 2564–2574.
- Johansen, J.P., Fields, H.L., and Manning, B.H. (2001). The affective component of pain in rodents: Direct evidence for a contribution of the anterior cingulate cortex. *PNAS* 98, 8077–8082.
- John, M.J., Borjesson, B.W., Walsh, J.R., and Niall, H.D. (1981). Limited sequence homology between porcine and rat relaxins: implications for physiological studies. *Endocrinology* 108, 726–729.
- Johnson, M.R., Okokon, E., Collins, W.P., Sharma, V., and Lightman, S.L. (1991). The Effect of Human Chorionic Gonadotropin and Pregnancy on the Circulating Level of Relaxin. *J Clin Endocrinol Metab* 72, 1042–1047.

- Jongen-Rêlo, A.L., and Amaral, D.G. (1998). Evidence for a GABAergic projection from the central nucleus of the amygdala to the brainstem of the macaque monkey: a combined retrograde tracing and in situ hybridization study. *Eur. J. Neurosci.* *10*, 2924–2933.
- Jorgensen, E.M., and Mango, S.E. (2002). The art and design of genetic screens: *caenorhabditis elegans*. *Nat. Rev. Genet.* *3*, 356–369.
- Julius, D., and Basbaum, A.I. (2001). Molecular mechanisms of nociception. *Nature* *413*, 203–210.
- Kahn-Kirby, A.H., and Bargmann, C.I. (2006). TRP channels in *C. elegans*. *Annu. Rev. Physiol.* *68*, 719–736.
- Kalbhenn, D.A. (1987). Chemical model of osteoarthritis--a pharmacological evaluation. *J. Rheumatol.* *14 Spec No*, 130–131.
- Kato, K., Sekiguchi, M., Kikuchi, S., and Konno, S. (2015). The effect of a 5-HT_{2A} receptor antagonist on pain-related behavior, endogenous 5-hydroxytryptamine production, and the expression 5-HT_{2A} receptors in dorsal root ganglia in a rat lumbar disc herniation model. *Spine* *40*, 357–362.
- Katz, R.J. (1982). Animal model of depression: pharmacological sensitivity of a hedonic deficit. *Pharmacol. Biochem. Behav.* *16*, 965–968.
- Kayser, V., and Christensen, D. (2000). Antinociceptive effect of systemic gabapentin in mononeuropathic rats, depends on stimulus characteristics and level of test integration. *Pain* *88*, 53–60.
- Kc, R., Li, X., Kroin, J.S., Liu, Z., Chen, D., Xiao, G., Levine, B., Li, J., Hamilton, J.L., van Wijnen, A.J., et al. (2016). PKC δ null mutations in a mouse model of osteoarthritis alter osteoarthritic pain independently of joint pathology by augmenting NGF/TrkA-induced axonal outgrowth. *Ann. Rheum. Dis.* *75*, 2133–2141.
- Kee, T., Sanda, P., Gupta, N., Stopfer, M., and Bazhenov, M. (2015). Feed-Forward versus Feedback Inhibition in a Basic Olfactory Circuit. *PLoS Comput Biol* *11*.
- Keeble, J., Russell, F., Curtis, B., Starr, A., Pinter, E., and Brain, S.D. (2005). Involvement of transient receptor potential vanilloid 1 in the vascular and hyperalgesic components of joint inflammation. *Arthritis Rheum.* *52*, 3248–3256.
- Kelly, K.M., Sanga, P., Zaki, N., Wang, S., Haeussler, J., Louie, J., and Thippawong, J. (2019). Safety and efficacy of fulranumab in osteoarthritis of the hip and knee: results from four early terminated phase III randomized studies. *Curr Med Res Opin* *35*, 2117–2127.
- Kepler, L.D., McDiarmid, T.A., and Rankin, C.H. (2020). Habituation in high-throughput genetic model organisms as a tool to investigate the mechanisms of neurodevelopmental disorders. *Neurobiol Learn Mem* *171*, 107208.

- Keswani, S.C., Jack, C., Zhou, C., and Höke, A. (2006). Establishment of a rodent model of HIV-associated sensory neuropathy. *J. Neurosci.* *26*, 10299–10304.
- Key, B. (2015). Fish do not feel pain and its implications for understanding phenomenal consciousness. *Biol Philos* *30*, 149–165.
- Khakh, B.S., and North, R.A. (2006). P2X receptors as cell-surface ATP sensors in health and disease. *Nature* *442*, 527–532.
- Khuong, T.M., and Neely, G.G. (2013). Conserved systems and functional genomic assessment of nociception. *FEBS J.* *280*, 5298–5306.
- Khuong, T.M., Wang, Q.-P., Manion, J., Oyston, L.J., Lau, M.-T., Towler, H., Lin, Y.Q., and Neely, G.G. (2019). Nerve injury drives a heightened state of vigilance and neuropathic sensitization in *Drosophila*. *Sci Adv* *5*, eaaw4099.
- Kim, S.H., and Chung, J.M. (1992). An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain* *50*, 355–363.
- King, T., Vera-Portocarrero, L., Gutierrez, T., Vanderah, T.W., Dussor, G., Lai, J., Fields, H.L., and Porreca, F. (2009). Unmasking the tonic-aversive state in neuropathic pain. *Nat. Neurosci.* *12*, 1364–1366.
- Kiso, T., Watabiki, T., Tsukamoto, M., Okabe, M., Kagami, M., Nishimura, K., Aoki, T., and Matsuoka, N. (2008). Pharmacological characterization and gene expression profiling of an L5/L6 spinal nerve ligation model for neuropathic pain in mice. *Neuroscience* *153*, 492–500.
- Kleczkowska, P., and Lipkowski, A.W. (2013). Neurotensin and neurotensin receptors: characteristic, structure-activity relationship and pain modulation--a review. *Eur J Pharmacol* *716*, 54–60.
- Klinck, M.P., Mogil, J.S., Moreau, M., Lascelles, B.D.X., Flecknell, P.A., Poitte, T., and Troncy, E. (2017). Translational pain assessment: could natural animal models be the missing link? *Pain* *158*, 1633–1646.
- Knight, B., Katz, D.R., Isenberg, D.A., Ibrahim, M.A., Le Page, S., Hutchings, P., Schwartz, R.S., and Cooke, A. (1992). Induction of adjuvant arthritis in mice. *Clin Exp Immunol* *90*, 459–465.
- Knights, C.B., Gentry, C., and Bevan, S. (2012). Partial medial meniscectomy produces osteoarthritis pain-related behaviour in female C57BL/6 mice. *Pain* *153*, 281–292.
- Knyihár-Csillik, E., Tajti, J., Samsam, M., Sáros, G., Slezák, S., and Vécsei, L. (1997). Effect of a serotonin agonist (sumatriptan) on the peptidergic innervation of the rat cerebral dura mater and on the expression of c-fos in the caudal trigeminal nucleus in an experimental migraine model. *J. Neurosci. Res.* *48*, 449–464.

- Koehler, P.J., and Endtz, L.J. (1986). The Brown-Séquard syndrome. True or false? *Arch. Neurol.* *43*, 921–924.
- Koga, K., Li, X., Chen, T., Steenland, H.W., Descalzi, G., and Zhuo, M. (2010). In vivo whole-cell patch-clamp recording of sensory synaptic responses of cingulate pyramidal neurons to noxious mechanical stimuli in adult mice. *Mol Pain* *6*, 62.
- Kong, R.C.K., Shilling, P.J., Lobb, D.K., Gooley, P.R., and Bathgate, R.A.D. (2010). Membrane receptors: structure and function of the relaxin family peptide receptors. *Mol. Cell. Endocrinol.* *320*, 1–15.
- Konno, S., Oda, N., Ochiai, T., and Alev, L. (2016). Randomized, Double-blind, Placebo-controlled Phase III Trial of Duloxetine Monotherapy in Japanese Patients With Chronic Low Back Pain. *Spine* *41*, 1709–1717.
- Koyama, S., Katayama, Y., Maejima, S., Hirayama, T., Fujii, M., and Tsubokawa, T. (1993). Thalamic neuronal hyperactivity following transection of the spinothalamic tract in the cat: involvement of N-methyl-D-aspartate receptor. *Brain Res.* *612*, 345–350.
- Kremer, M., Becker, L.J., Barrot, M., and Yalcin, I. (2020a). How to study anxiety and depression in rodent models of chronic pain? *European Journal of Neuroscience* *n/a*.
- Kremer, M., Becker, L.J., Barrot, M., and Yalcin, I. (2020b). How to study anxiety and depression in rodent models of chronic pain? *Eur. J. Neurosci.*
- Kroenke, K., Bair, M.J., Damush, T.M., Wu, J., Hoke, S., Sutherland, J., and Tu, W. (2009). Optimized antidepressant therapy and pain self-management in primary care patients with depression and musculoskeletal pain: a randomized controlled trial. *JAMA* *301*, 2099–2110.
- Kuan, Y.-H., and Shyu, B.-C. (2016). Nociceptive transmission and modulation via P2X receptors in central pain syndrome. *Mol Brain* *9*, 58.
- Kubota, Y., Hattori, R., and Yui, Y. (1994). Three distinct subpopulations of GABAergic neurons in rat frontal agranular cortex. *Brain Research* *649*, 159–173.
- Kumar, J.R., Rajkumar, R., Jayakody, T., Marwari, S., Hong, J.M., Ma, S., Gundlach, A.L., Lai, M.K.P., and Dawe, G.S. (2017). Relaxin’ the brain: a case for targeting the nucleus incertus network and relaxin-3/RXFP3 system in neuropsychiatric disorders. *Br. J. Pharmacol.* *174*, 1061–1076.
- Kuner, R., and Flor, H. (2016). Structural plasticity and reorganisation in chronic pain. *Nat. Rev. Neurosci.* *18*, 20–30.
- Kupers, R.C., Chen, C.C., and Bushnell, M.C. (1997). A model of transient hyperalgesia in the behaving monkey induced by topical application of capsaicin. *Pain* *72*, 269–275.

- Kwon, M., Altin, M., Duenas, H., and Alev, L. (2014). The role of descending inhibitory pathways on chronic pain modulation and clinical implications. *Pain Pract* *14*, 656–667.
- LaBuda, C.J., and Little, P.J. (2005). Pharmacological evaluation of the selective spinal nerve ligation model of neuropathic pain in the rat. *J. Neurosci. Methods* *144*, 175–181.
- Laffray, S., Bouali-Benazzouz, R., Papon, M.-A., Favereaux, A., Jiang, Y., Holm, T., Spriet, C., Desbarats, P., Fossat, P., Le Feuvre, Y., et al. (2012). Impairment of GABAB receptor dimer by endogenous 14-3-3 ζ in chronic pain conditions. *EMBO J.* *31*, 3239–3251.
- Laird, J., Martinez-Caro, L., Garcia-Nicas, E., and Cervero, F. (2001a). A new model of visceral pain and referred hyperalgesia in the mouse. *Pain* *92*, 335–342.
- Laird, J.M., Martinez-Caro, L., Garcia-Nicas, E., and Cervero, F. (2001b). A new model of visceral pain and referred hyperalgesia in the mouse. *Pain* *92*, 335–342.
- Lam, M., Royce, S.G., Samuel, C.S., and Bourke, J.E. (2018). Serelaxin as a novel therapeutic opposing fibrosis and contraction in lung diseases. *Pharmacol Ther* *187*, 61–70.
- Lambert, D.G. (2009). Capsaicin receptor antagonists: a promising new addition to the pain clinic. *Br J Anaesth* *102*, 153–155.
- Langford, D.J., Bailey, A.L., Chanda, M.L., Clarke, S.E., Drummond, T.E., Echols, S., Glick, S., Ingrao, J., Klassen-Ross, T., Lacroix-Fralish, M.L., et al. (2010). Coding of facial expressions of pain in the laboratory mouse. *Nat. Methods* *7*, 447–449.
- Lapointe, T.K., Basso, L., Iftinca, M.C., Flynn, R., Chapman, K., Dietrich, G., Vergnolle, N., and Altier, C. (2015). TRPV1 sensitization mediates postinflammatory visceral pain following acute colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* *309*, G87-99.
- Larauche, M., Mulak, A., and Taché, Y. (2012). Stress and visceral pain: from animal models to clinical therapies. *Exp. Neurol.* *233*, 49–67.
- Lascelles, B.D.X., Brown, D.C., Maixner, W., and Mogil, J.S. (2018). Spontaneous painful disease in companion animals can facilitate the development of chronic pain therapies for humans. *Osteoarthr. Cartil.* *26*, 175–183.
- Latremoliere, A., and Woolf, C.J. (2009). Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. *The Journal of Pain: Official Journal of the American Pain Society* *10*, 895.
- Le Bars, D., Gozariu, M., and Cadden, S.W. (2001). Animal models of nociception. *Pharmacol. Rev.* *53*, 597–652.
- LeDoux, J.E. (2000). Emotion circuits in the brain. *Annu. Rev. Neurosci.* *23*, 155–184.

- Lee, J.H., Koh, S.Q., Guadagna, S., Francis, P.T., Esiri, M.M., Chen, C.P., Wong, P.T.-H., Dawe, G.S., and Lai, M.K.P. (2016). Altered relaxin family receptors RXFP1 and RXFP3 in the neocortex of depressed Alzheimer's disease patients. *Psychopharmacology (Berl.)* 233, 591–598.
- Lee-Liu, D., Méndez-Olivos, E.E., Muñoz, R., and Larrain, J. (2017). The African clawed frog *Xenopus laevis*: A model organism to study regeneration of the central nervous system. *Neurosci. Lett.* 652, 82–93.
- Leite-Almeida, H., Pinto-Ribeiro, F., and Almeida, A. (2015). Animal Models for the Study of Comorbid Pain and Psychiatric Disorders. *Mod Trends Pharmacopsychiatry* 30, 1–21.
- Lembeck, F., and Donnerer, J. (1981). Time course of capsaicin-induced functional impairments in comparison with changes in neuronal substance P content. *Naunyn Schmiedebergs Arch. Pharmacol.* 316, 240–243.
- Leon-Cabrera, S., Solís-Lozano, L., Suárez-Álvarez, K., González-Chávez, A., Béjar, Y.L., Robles-Díaz, G., and Escobedo, G. (2013). Hyperleptinemia is associated with parameters of low-grade systemic inflammation and metabolic dysfunction in obese human beings. *Front Integr Neurosci* 7.
- Leung, K., Mohammadi, A., Ryu, W.S., and Nemenman, I. (2016). Stereotypical Escape Behavior in *Caenorhabditis elegans* Allows Quantification of Effective Heat Stimulus Level. *PLoS Comput Biol* 12.
- Li, S.-Y., Huo, M.-L., Wu, X.-Y., Huang, Y.-Q., Wang, L., Zhang, X., Jiang, Y.-M., Zhang, M.-L., Wang, L.-L., and Yu, L.-C. (2017). Involvement of galanin and galanin receptor 1 in nociceptive modulation in the central nucleus of amygdala in normal and neuropathic rats. *Scientific Reports* 7, 15317.
- Liepe, K., Geidel, H., Haase, M., Hakenberg, O.W., Runge, R., and Kotzerke, J. (2005). New model for the induction of osteoblastic bone metastases in rat. *Anticancer Res.* 25, 1067–1073.
- Lindsay, T.H., Jonas, B.M., Sevcik, M.A., Kubota, K., Halvorson, K.G., Ghilardi, J.R., Kuskowski, M.A., Stelow, E.B., Mukherjee, P., Gendler, S.J., et al. (2005). Pancreatic cancer pain and its correlation with changes in tumor vasculature, macrophage infiltration, neuronal innervation, body weight and disease progression. *Pain* 119, 233–246.
- Lister, R.G. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology (Berl.)* 92, 180–185.
- Liu, M.-G., and Chen, J. (2014). Preclinical research on pain comorbidity with affective disorders and cognitive deficits: Challenges and perspectives. *Prog. Neurobiol.* 116, 13–32.
- Liu, C., Chen, J., Sutton, S., Roland, B., Kuei, C., Farmer, N., Sillard, R., and Lovenberg, T.W. (2003). Identification of relaxin-3/INSL7 as a ligand for GPCR142. *The Journal of Biological Chemistry* 278, 50765–50770.

- Liu, Y., Li, Y., Liu, Q., Wu, Z., Cui, J., Zhu, K., Zhao, H., Zhou, C., and Shi, B. (2019). Role of GM-CSF in a mouse model of experimental autoimmune prostatitis. *Am. J. Physiol. Renal Physiol.* *317*, F23–F29.
- Lomax, A.E., Pradhananga, S., Sessenwein, J.L., and O'Malley, D. (2019). Bacterial modulation of visceral sensation: mediators and mechanisms. *Am. J. Physiol. Gastrointest. Liver Physiol.* *317*, G363–G372.
- López-González, M.J., Soula, A., Landry, M., and Favereaux, A. (2018). Oxaliplatin treatment impairs extension of sensory neuron neurites in vitro through miR-204 overexpression. *Neurotoxicology* *68*, 91–100.
- Lopez-Luna, J., Al-Jubouri, Q., Al-Nuaimy, W., and Sneddon, L.U. (2017). Reduction in activity by noxious chemical stimulation is ameliorated by immersion in analgesic drugs in zebrafish. *J. Exp. Biol.* *220*, 1451–1458.
- Lorenzo, L.-E., Godin, A.G., Ferrini, F., Bachand, K., Plasencia-Fernandez, I., Labrecque, S., Girard, A.A., Boudreau, D., Kianicka, I., Gagnon, M., et al. (2020). Enhancing neuronal chloride extrusion rescues $\alpha 2/\alpha 3$ GABAA-mediated analgesia in neuropathic pain. *Nat Commun* *11*, 869.
- Louwies, T., Ligon, C.O., Johnson, A.C., and Greenwood-Van Meerveld, B. (2019). Targeting epigenetic mechanisms for chronic visceral pain: A valid approach for the development of novel therapeutics. *Neurogastroenterol. Motil.* *31*, e13500.
- Lu, W.-Y., Xiong, Z.-G., Lei, S., Orser, B.A., Dudek, E., Browning, M.D., and MacDonald, J.F. (1999). G-protein-coupled receptors act via protein kinase C and Src to regulate NMDA receptors. *Nature Neuroscience* *2*, 331–338.
- Lucas, J.M., Ji, Y., and Masri, R. (2011). Motor cortex stimulation reduces hyperalgesia in an animal model of central pain. *Pain* *152*, 1398–1407.
- Lukács, M., Haanes, K.A., Majláth, Z., Tajti, J., Vécsei, L., Warfvinge, K., and Edvinsson, L. (2015). Dural administration of inflammatory soup or Complete Freund's Adjuvant induces activation and inflammatory response in the rat trigeminal ganglion. *J Headache Pain* *16*, 564.
- Lynn, B., Ye, W., and Cotcell, B. (1992). The actions of capsaicin applied topically to the skin of the rat on C-fibre afferents, antidromic vasodilatation and substance P levels. *Br. J. Pharmacol.* *107*, 400–406.
- M Keppel Hesselink, J. (2016). Arguments to develop TRPV1 antagonist in neuropathic pain. Lessons for drug development. *Clin Res Trials* *3*.
- Ma, S., and Gundlach, A.L. (2007). Relaxin-family peptide and receptor systems in brain: insights from recent anatomical and functional studies. *Adv. Exp. Med. Biol.* *612*, 119–137.

- Ma, S., and Gundlach, A.L. (2015). Ascending control of arousal and motivation: role of nucleus incertus and its peptide neuromodulators in behavioural responses to stress. *J. Neuroendocrinol.* *27*, 457–467.
- Ma, W., and Peschanski, M. (1988). Spinal and trigeminal projections to the parabrachial nucleus in the rat: electron-microscopic evidence of a spino-ponto-amygdalian somatosensory pathway. *Somatosens Res* *5*, 247–257.
- Ma, S., Roozendaal, B., Burazin, T.C.D., Tregear, G.W., McGaugh, J.L., and Gundlach, A.L. (2005). Relaxin receptor activation in the basolateral amygdala impairs memory consolidation. *Eur. J. Neurosci.* *22*, 2117–2122.
- Ma, S., Shen, P.-J., Burazin, T.C.D., Tregear, G.W., and Gundlach, A.L. (2006). Comparative localization of leucine-rich repeat-containing G-protein-coupled receptor-7 (RXFP1) mRNA and [33P]-relaxin binding sites in rat brain: restricted somatic co-expression a clue to relaxin action? *Neuroscience* *141*, 329–344.
- Ma, S., Bonaventure, P., Ferraro, T., Shen, P.-J., Burazin, T.C.D., Bathgate, R. a. D., Liu, C., Tregear, G.W., Sutton, S.W., and Gundlach, A.L. (2007). Relaxin-3 in GABA projection neurons of nucleus incertus suggests widespread influence on forebrain circuits via G-protein-coupled receptor-135 in the rat. *Neuroscience* *144*, 165–190.
- Ma, S., Olucha-Bordonau, F.E., Hossain, M.A., Lin, F., Kuei, C., Liu, C., Wade, J.D., Sutton, S.W., Nuñez, A., and Gundlach, A.L. (2009). Modulation of hippocampal theta oscillations and spatial memory by relaxin-3 neurons of the nucleus incertus. *Learn. Mem.* *16*, 730–742.
- Ma, S., Smith, C.M., Blasiak, A., and Gundlach, A.L. (2017). Distribution, physiology and pharmacology of relaxin-3/RXFP3 systems in brain. *British Journal of Pharmacology* *174*, 1034–1048.
- MacEwen, E.G. (1990). Spontaneous tumors in dogs and cats: models for the study of cancer biology and treatment. *Cancer Metastasis Rev.* *9*, 125–136.
- MacLennan, A.H., Green, R.C., Grant, P., and Nicolson, R. (1986). Ripening of the human cervix and induction of labor with intracervical purified porcine relaxin. *Obstet Gynecol* *68*, 598–601.
- Malafoglia, V., Colasanti, M., Raffaelli, W., Balciunas, D., Giordano, A., and Bellipanni, G. (2014). Extreme Thermal Noxious Stimuli Induce Pain Responses in Zebrafish Larvae. *J Cell Physiol* *229*, 300–308.
- Malfait, A.M., Little, C.B., and McDougall, J.J. (2013). A commentary on modelling osteoarthritis pain in small animals. *Osteoarthr. Cartil.* *21*, 1316–1326.
- Malmberg, A.B., and Basbaum, A.I. (1998). Partial sciatic nerve injury in the mouse as a model of neuropathic pain: behavioral and neuroanatomical correlates. *Pain* *76*, 215–222.

- Manda, P., Kushwaha, A.S., Kundu, S., Shivakumar, H.N., Jo, S.B., and Murthy, S.N. (2016). Delivery of ziconotide to cerebrospinal fluid via intranasal pathway for the treatment of chronic pain. *J Control Release* 224, 69–76.
- Mansfield, C., and Beths, T. (2015). Management of acute pancreatitis in dogs: a critical appraisal with focus on feeding and analgesia. *J Small Anim Pract* 56, 27–39.
- Mantyh, P.W. (1983). Connections of midbrain periaqueductal gray in the monkey. II. Descending efferent projections. *J. Neurophysiol.* 49, 582–594.
- Mapplebeck, J.C.S., Dalgarno, R., Tu, Y., Moriarty, O., Beggs, S., Kwok, C.H.T., Halievski, K., Assi, S., Mogil, J.S., Trang, T., et al. (2018). Microglial P2X4R-evoked pain hypersensitivity is sexually dimorphic in rats. *Pain* 159, 1752–1763.
- Markaki, M., and Tavernarakis, N. (2020). *Caenorhabditis elegans* as a model system for human diseases. *Curr. Opin. Biotechnol.* 63, 118–125.
- Marmioli, P., Riva, B., Pozzi, E., Ballarini, E., Lim, D., Chiorazzi, A., Meregalli, C., Distasi, C., Renn, C.L., Semperboni, S., et al. (2017). Susceptibility of different mouse strains to oxaliplatin peripheral neurotoxicity: Phenotypic and genotypic insights. *PLoS ONE* 12, e0186250.
- Martin, L.J., Acland, E.L., Cho, C., Gandhi, W., Chen, D., Corley, E., Kadoura, B., Levy, T., Mirali, S., Tohyama, S., et al. (2019). Male-Specific Conditioned Pain Hypersensitivity in Mice and Humans. *Curr. Biol.* 29, 192-201.e4.
- Masri, R., Quiton, R.L., Lucas, J.M., Murray, P.D., Thompson, S.M., and Keller, A. (2009). Zona incerta: a role in central pain. *J. Neurophysiol.* 102, 181–191.
- Matsumoto, M., Kamohara, M., Sugimoto, T., Hidaka, K., Takasaki, J., Saito, T., Okada, M., Yamaguchi, T., and Furuichi, K. (2000). The novel G-protein coupled receptor SALPR shares sequence similarity with somatostatin and angiotensin receptors. *Gene* 248, 183–189.
- Matsumura, Y., Yamashita, T., Sasaki, A., Nakata, E., Kohno, K., Masuda, T., Tozaki-Saitoh, H., Imai, T., Kuraishi, Y., Tsuda, M., et al. (2016). A novel P2X4 receptor-selective antagonist produces anti-allodynic effect in a mouse model of herpetic pain. *Sci Rep* 6, 32461.
- Mayer, S., Spickschen, J., Stein, K.V., Crevenna, R., Dorner, T.E., and Simon, J. (2019). The societal costs of chronic pain and its determinants: The case of Austria. *PLoS ONE* 14, e0213889.
- McCoy, A.M. (2015). Animal Models of Osteoarthritis: Comparisons and Key Considerations. *Vet. Pathol.* 52, 803–818.
- McDonald, G.A., Sarkar, P., Rennke, H., Unemori, E., Kalluri, R., and Sukhatme, V.P. (2003). Relaxin increases ubiquitin-dependent degradation of fibronectin in vitro and ameliorates renal fibrosis in vivo. *American Journal of Physiology - Renal Physiology* 285, F59–F67.

- McDougall, J.J., Karimian, S.M., and Ferrell, W.R. (1995). Prolonged alteration of vasoconstrictor and vasodilator responses in rat knee joints by adjuvant monoarthritis. *Exp. Physiol.* *80*, 349–357.
- McDowell, G.C., and Pope, J.E. (2016). Intrathecal Ziconotide: Dosing and Administration Strategies in Patients With Refractory Chronic Pain. *Neuromodulation* *19*, 522–532.
- McGivern, J.G. (2007). Ziconotide: a review of its pharmacology and use in the treatment of pain. *Neuropsychiatr Dis Treat* *3*, 69–85.
- McGowan, B.M., Stanley, S.A., Smith, K.L., Minnion, J.S., Donovan, J., Thompson, E.L., Patterson, M., Connolly, M.M., Abbott, C.R., Small, C.J., et al. (2006). Effects of acute and chronic relaxin-3 on food intake and energy expenditure in rats.
- McGowan, B.M., Minnion, J.S., Murphy, K.G., Roy, D., Stanley, S.A., Dhillon, W.S., Gardiner, J.V., Ghatei, M.A., and Bloom, S.R. (2014). Relaxin-3 stimulates the neuro-endocrine stress axis via corticotrophin-releasing hormone. *The Journal of Endocrinology* *221*, 337–346.
- McGowan, B.M.C., Stanley, S.A., Smith, K.L., White, N.E., Connolly, M.M., Thompson, E.L., Gardiner, J.V., Murphy, K.G., Ghatei, M.A., and Bloom, S.R. (2005). Central relaxin-3 administration causes hyperphagia in male Wistar rats. *Endocrinology* *146*, 3295–3300.
- McIlwraith, C.W., Frisbie, D.D., and Kawcak, C.E. (2012). The horse as a model of naturally occurring osteoarthritis. *Bone Joint Res* *1*, 297–309.
- McMahon, S.B., and Bevan, S. (2005). Inflammatory mediators and modulators of pain. *Wall and Melzack's Textbook of Pain* E-Dition 49–72.
- McNamara, C.R., Mandel-Brehm, J., Bautista, D.M., Siemens, J., Deranian, K.L., Zhao, M., Hayward, N.J., Chong, J.A., Julius, D., Moran, M.M., et al. (2007). TRPA1 mediates formalin-induced pain. *Proc. Natl. Acad. Sci. U.S.A.* *104*, 13525–13530.
- McNamee, K.E., Burleigh, A., Gompels, L.L., Feldmann, M., Allen, S.J., Williams, R.O., Dawbarn, D., Vincent, T.L., and Inglis, J.J. (2010). Treatment of murine osteoarthritis with TrkAd5 reveals a pivotal role for nerve growth factor in non-inflammatory joint pain. *Pain* *149*, 386–392.
- Medalla, M., and Barbas, H. (2012). The Anterior Cingulate Cortex May Enhance Inhibition of Lateral Prefrontal Cortex Via m2 Cholinergic Receptors at Dual Synaptic Sites. *J Neurosci* *32*, 15611–15625.
- Medhurst, S.J., Walker, K., Bowes, M., Kidd, B.L., Glatt, M., Muller, M., Hattenberger, M., Vaxelaire, J., O'Reilly, T., Wotherspoon, G., et al. (2002). A rat model of bone cancer pain. *Pain* *96*, 129–140.
- Medina, G., Ji, G., Grégoire, S., and Neugebauer, V. (2014). Nasal application of neuropeptide S inhibits arthritis pain-related behaviors through an action in the amygdala. *Mol Pain* *10*, 32.

- Meller, S.T., and Gebhart, G.F. (1997). Intraplantar zymosan as a reliable, quantifiable model of thermal and mechanical hyperalgesia in the rat. *European Journal of Pain* *1*, 43–52.
- Melo-Carrillo, A., and Lopez-Avila, A. (2013). A chronic animal model of migraine, induced by repeated meningeal nociception, characterized by a behavioral and pharmacological approach. *Cephalalgia* *33*, 1096–1105.
- Melzack, R., and Wall, P.D. (1965). Pain mechanisms: a new theory. *Science* *150*, 971–979.
- Mert, T., Sahin, E., Yaman, S., and Sahin, M. (2018). Pain-Relieving Effectiveness of Co-Treatment with Local Tramadol and Systemic Minocycline in Carrageenan-Induced Inflammatory Pain Model. *Inflammation* *41*, 1238–1249.
- Milinkeviciute, G., Gentile, C., and Neely, G.G. (2012). *Drosophila* as a tool for studying the conserved genetics of pain. *Clin. Genet.* *82*, 359–366.
- Millan, M.J. (2002). Descending control of pain. *Prog. Neurobiol.* *66*, 355–474.
- Millhr, R.R., Berk, A.M., and Springer, A.D. (1974). Acquisition and retention of active avoidance in *Xenopus laevis*. *Bull. Psychon. Soc.* *3*, 139–141.
- Miquel, S., Martín, R., Lashermes, A., Gillet, M., Meleine, M., Gelot, A., Eschalier, A., Ardid, D., Bermúdez-Humarán, L.G., Sokol, H., et al. (2016). Anti-nociceptive effect of *Faecalibacterium prausnitzii* in non-inflammatory IBS-like models. *Sci Rep* *6*, 19399.
- Mirauccourt, L.S., Moisset, X., Dallel, R., and Voisin, D.L. (2009). Glycine inhibitory dysfunction induces a selectively dynamic, morphine-resistant, and neurokinin 1 receptor- independent mechanical allodynia. *J. Neurosci.* *29*, 2519–2527.
- Mishra, A., Guo, Y., Zhang, L., More, S., Weng, T., Chintagari, N.R., Huang, C., Liang, Y., Pushparaj, S., Gou, D., et al. (2016). A Critical Role for P2X7 Receptor-Induced VCAM-1 Shedding and Neutrophil Infiltration during Acute Lung Injury. *J. Immunol.* *197*, 2828–2837.
- Miyagi, M., Ishikawa, T., Kamoda, H., Suzuki, M., Inoue, G., Sakuma, Y., Oikawa, Y., Orita, S., Uchida, K., Takahashi, K., et al. (2017). Efficacy of nerve growth factor antibody in a knee osteoarthritis pain model in mice. *BMC Musculoskelet Disord* *18*, 428.
- Moehring, F., Halder, P., Seal, R.P., and Stucky, C.L. (2018). Uncovering the Cells and Circuits of Touch in Normal and Pathological Settings. *Neuron* *100*, 349–360.
- Mogil, J.S. (2009). Animal models of pain: progress and challenges. *Nat. Rev. Neurosci.* *10*, 283–294.
- Mogil, J.S., Davis, K.D., and Derbyshire, S.W. (2010). The necessity of animal models in pain research. *Pain* *151*, 12–17.

- Monteiro, B.P., de Lorimier, L.-P., Moreau, M., Beauchamp, G., Blair, J., Lussier, B., Pelletier, J.-P., and Troncy, E. (2018). Pain characterization and response to palliative care in dogs with naturally-occurring appendicular osteosarcoma: An open label clinical trial. *PLoS ONE* *13*, e0207200.
- Moreau, M., Lussier, B., Ballaz, L., and Troncy, E. (2014). Kinetic measurements of gait for osteoarthritis research in dogs and cats. *Can. Vet. J.* *55*, 1057–1065.
- Morgan, P.G., Kayser, E.-B., and Sedensky, M.M. (2007). *C. elegans* and volatile anesthetics (WormBook).
- Muley, M.M., Krustev, E., and McDougall, J.J. (2016). Preclinical Assessment of Inflammatory Pain. *CNS Neurosci Ther* *22*, 88–101.
- Munro, G., Jansen-Olesen, I., and Olesen, J. (2017). Animal models of pain and migraine in drug discovery. *Drug Discov. Today* *22*, 1103–1111.
- Na, H.S., Han, J.S., Ko, K.H., and Hong, S.K. (1994). A behavioral model for peripheral neuropathy produced in rat's tail by inferior caudal trunk injury. *Neurosci. Lett.* *177*, 50–52.
- Nagasaka, K., Takashima, I., Matsuda, K., and Higo, N. (2020). Brain activity changes in a monkey model of central post-stroke pain. *Experimental Neurology* *323*, 113096.
- Nakamura, H., Katayama, Y., and Kawakami, Y. (2010). Hippocampal CA1/subiculum-prefrontal cortical pathways induce plastic changes of nociceptive responses in cingulate and prelimbic areas. *BMC Neurosci* *11*, 100.
- Nandakumar, K.S., Svensson, L., and Holmdahl, R. (2003). Collagen Type II-Specific Monoclonal Antibody-Induced Arthritis in Mice. *Am J Pathol* *163*, 1827–1837.
- Narita, M., Kaneko, C., Miyoshi, K., Nagumo, Y., Kuzumaki, N., Nakajima, M., Nanjo, K., Matsuzawa, K., Yamazaki, M., and Suzuki, T. (2006a). Chronic pain induces anxiety with concomitant changes in opioidergic function in the amygdala. *Neuropsychopharmacology* *31*, 739–750.
- Narita, M., Kaneko, C., Miyoshi, K., Nagumo, Y., Kuzumaki, N., Nakajima, M., Nanjo, K., Matsuzawa, K., Yamazaki, M., and Suzuki, T. (2006b). Chronic pain induces anxiety with concomitant changes in opioidergic function in the amygdala. *Neuropsychopharmacology* *31*, 739–750.
- Naseri, K., Saghaei, E., Abbaszadeh, F., Afhami, M., Haeri, A., Rahimi, F., and Jorjani, M. (2013). Role of microglia and astrocyte in central pain syndrome following electrolytic lesion at the spinothalamic tract in rats. *J. Mol. Neurosci.* *49*, 470–479.
- Navratilova, E., Morimura, K., Xie, J.Y., Atcherley, C.W., Ossipov, M.H., and Porreca, F. (2016). Positive emotions and brain reward circuits in chronic pain. *J. Comp. Neurol.* *524*, 1646–1652.

- Neely, G.G., Hess, A., Costigan, M., Keene, A.C., Goulas, S., Langeslag, M., Griffin, R.S., Belfer, I., Dai, F., Smith, S., et al. (2010). A genome-wide *Drosophila* screen for heat nociception identifies $\alpha\delta\delta 3$ as an evolutionary-conserved pain gene. *Cell* *143*, 628–638.
- Neely, G.G., Keene, A.C., Duchek, P., Chang, E.C., Wang, Q.-P., Aksoy, Y.A., Rosenzweig, M., Costigan, M., Woolf, C.J., Garrity, P.A., et al. (2011). TrpA1 regulates thermal nociception in *Drosophila*. *PLoS ONE* *6*, e24343.
- Negus, S.S., Vanderah, T.W., Brandt, M.R., Bilsky, E.J., Becerra, L., and Borsook, D. (2006). Preclinical assessment of candidate analgesic drugs: recent advances and future challenges. *J. Pharmacol. Exp. Ther.* *319*, 507–514.
- Ness, T.J. (1999). Models of Visceral Nociception. *ILAR J* *40*, 119–128.
- Ness, T.J., and Gebhart, G.F. (1988). Colorectal distension as a noxious visceral stimulus: physiologic and pharmacologic characterization of pseudoaffective reflexes in the rat. *Brain Res.* *450*, 153–169.
- Neugebauer, V. (2015). 15. Amygdala pain mechanisms. *Handb Exp Pharmacol* *227*, 261–284.
- Neugebauer, V., and Li, W. (2002). Processing of nociceptive mechanical and thermal information in central amygdala neurons with knee-joint input. *J. Neurophysiol.* *87*, 103–112.
- Neugebauer, V., Li, W., Bird, G.C., and Han, J.S. (2004). The Amygdala and Persistent Pain. *Neuroscientist* *10*, 221–234.
- Neugebauer, V., Galhardo, V., Maione, S., and Mackey, S.C. (2009). Forebrain Pain Mechanisms. *Brain Res Rev* *60*, 226–242.
- Nielsen, C.K., Lewis, R.J., Alewood, D., Drinkwater, R., Palant, E., Patterson, M., Yaksh, T.L., McCumber, D., and Smith, M.T. (2005). Anti-allodynic efficacy of the chi-conopeptide, Xen2174, in rats with neuropathic pain. *Pain* *118*, 112–124.
- Nisar, A., Akhter, N., Singh, G., Masood, A., Malik, A., Banday, B., and Zargar, M.A. (2015). Modulation of T-helper cytokines and inflammatory mediators by *Atropa accuminata*. Royle in adjuvant induced arthritic tissues. *J Ethnopharmacol* *162*, 215–224.
- Nishimoto-Kakiuchi, A., Netsu, S., Okabayashi, S., Taniguchi, K., Tanimura, H., Kato, A., Suzuki, M., Sankai, T., and Konno, R. (2018). Spontaneous endometriosis in cynomolgus monkeys as a clinically relevant experimental model. *Hum. Reprod.* *33*, 1228–1236.
- Nistri, S., and Bani, D. (2003). Relaxin receptors and nitric oxide synthases: search for the missing link. *Reproductive Biology and Endocrinology* *1*, 5.
- Nkambeu, B., Salem, J.B., Leonelli, S., Marashi, F.A., and Beaudry, F. (2019). EGL-3 and EGL-21 are required to trigger nocifensive response of *Caenorhabditis elegans* to noxious heat. *Neuropeptides* *73*, 41–48.

- van Nooten, F., Treur, M., Pantiri, K., Stoker, M., and Charokopou, M. (2017). Capsaicin 8% Patch Versus Oral Neuropathic Pain Medications for the Treatment of Painful Diabetic Peripheral Neuropathy: A Systematic Literature Review and Network Meta-analysis. *Clin Ther* 39, 787-803.e18.
- Nozu, T., Miyagishi, S., Kumei, S., Nozu, R., Takakusaki, K., and Okumura, T. (2018). Lovastatin inhibits visceral allodynia and increased colonic permeability induced by lipopolysaccharide or repeated water avoidance stress in rats. *Eur. J. Pharmacol.* 818, 228–234.
- Nozu, T., Miyagishi, S., Kumei, S., Nozu, R., Takakusaki, K., and Okumura, T. (2019). Metformin inhibits visceral allodynia and increased gut permeability induced by stress in rats. *J. Gastroenterol. Hepatol.* 34, 186–193.
- Ochoa-Cortes, F., Liñán-Rico, A., Jacobson, K.A., and Christofi, F.L. (2014). Potential for developing purinergic drugs for gastrointestinal diseases. *Inflamm. Bowel Dis.* 20, 1259–1287.
- O'Connor, W.B., Cain, G.D., and Zarrow, M.X. (1966). Elongation of the interpubic ligament in the little brown bat (*Myotis lucifugus*). *Proc Soc Exp Biol Med* 123, 935–937.
- Ogren, S.O., and Berge, O.G. (1984). Test-dependent variations in the antinociceptive effect of p-chloroamphetamine-induced release of 5-hydroxytryptamine. *Neuropharmacology* 23, 915–924.
- Oh, J., Lee, S., Kim, A., Yoon, J., Jang, K., Lee, D.H., Cho, S., Lee, S.R., Yu, K.-S., and Chung, J.-Y. (2018). Safety, Tolerability, and Pharmacokinetic Characteristics of a Novel Nonopioid Analgesic, VVZ-149 Injections in Healthy Volunteers: A First-in-Class, First-in-Human Study. *J Clin Pharmacol* 58, 64–73.
- Olesen, J., Burstein, R., Ashina, M., and Tfelt-Hansen, P. (2009). Origin of pain in migraine: evidence for peripheral sensitisation. *Lancet Neurol* 8, 679–690.
- Olucha-Bordonau, F.E., Teruel, V., Barcia-González, J., Ruiz-Torner, A., Valverde-Navarro, A.A., and Martínez-Soriano, F. (2003). Cytoarchitecture and efferent projections of the nucleus incertus of the rat. *The Journal of Comparative Neurology* 464, 62–97.
- Olucha-Bordonau, F.E., Albert-Gascó, H., Ros-Bernal, F., Rytova, V., Ong-Pålsson, E.K.E., Ma, S., Sánchez-Pérez, A.M., and Gundlach, A.L. (2018). Modulation of forebrain function by nucleus incertus and relaxin-3/RXFP3 signaling. *CNS Neurosci Ther* 24, 694–702.
- O'Mahony, S.M., Tramullas, M., Fitzgerald, P., and Cryan, J.F. (2012). Rodent models of colorectal distension. *Curr Protoc Neurosci Chapter 9*, Unit 9.40.
- O'Malley, D., Julio-Pieper, M., O'Mahony, S.M., Dinan, T.G., and Cryan, J.F. (2014). Differential visceral pain sensitivity and colonic morphology in four common laboratory rat strains. *Exp. Physiol.* 99, 359–367.
- Omi, E.C., Zhao, S., Shanks, R.D., and Sherwood, O.D. (1997). Evidence that systemic relaxin promotes moderate water consumption during late pregnancy in rats. *J Endocrinol* 153, 33–40.

- Orr, P.M., Shank, B.C., and Black, A.C. (2017). The Role of Pain Classification Systems in Pain Management. *Critical Care Nursing Clinics of North America* 29, 407–418.
- Osheroff, P.L., and Ho, W.H. (1993). Expression of relaxin mRNA and relaxin receptors in postnatal and adult rat brains and hearts. Localization and developmental patterns. *J. Biol. Chem.* 268, 15193–15199.
- Osheroff, P.L., and Phillips, H.S. (1991). Autoradiographic localization of relaxin binding sites in rat brain. *Proc. Natl. Acad. Sci. U.S.A.* 88, 6413–6417.
- Ossipov, M.H., Dussor, G.O., and Porreca, F. (2010). Central modulation of pain. *J. Clin. Invest.* 120, 3779–3787.
- Ossipov, M.H., Morimura, K., and Porreca, F. (2014a). Descending pain modulation and chronification of pain. *Curr Opin Support Palliat Care* 8, 143–151.
- Ossipov, M.H., Morimura, K., and Porreca, F. (2014b). Descending pain modulation and chronification of pain. *Curr Opin Support Palliat Care* 8, 143–151.
- Otterness, I.G., and Moore, P.F. (1988). Carrageenan foot edema test. *Meth. Enzymol.* 162, 320–327.
- Ozaki, N., Bielefeldt, K., Sengupta, J.N., and Gebhart, G.F. (2002). Models of gastric hyperalgesia in the rat. *Am. J. Physiol. Gastrointest. Liver Physiol.* 283, G666–676.
- Palecek, J., Dougherty, P.M., Kim, S.H., Palecková, V., Lekan, H., Chung, J.M., Carlton, S.M., and Willis, W.D. (1992). Responses of spinothalamic tract neurons to mechanical and thermal stimuli in an experimental model of peripheral neuropathy in primates. *J. Neurophysiol.* 68, 1951–1966.
- Panksepp, J.B., and Lahvis, G.P. (2011). Rodent empathy and affective neuroscience. *Neurosci Biobehav Rev* 35, 1864–1875.
- Pape, H.-C., and Pare, D. (2010). Plastic Synaptic Networks of the Amygdala for the Acquisition, Expression, and Extinction of Conditioned Fear. *Physiol Rev* 90, 419–463.
- Parikh Ashish, Patel Divyang, McTiernan Charles F., Xiang Wenyu, Haney Jamie, Yang Lei, Lin Bo, Kaplan Aaron D., Bett Glenna C.L., Rasmusson Randall L., et al. (2013). Relaxin Suppresses Atrial Fibrillation by Reversing Fibrosis and Myocyte Hypertrophy and Increasing Conduction Velocity and Sodium Current in Spontaneously Hypertensive Rat Hearts. *Circulation Research* 113, 313–321.
- Patel, R., Montagut-Bordas, C., and Dickenson, A.H. (2018). Calcium channel modulation as a target in chronic pain control. *Br. J. Pharmacol.* 175, 2173–2184.

- Patil, N.A., Rosengren, K.J., Separovic, F., Wade, J.D., Bathgate, R.A.D., and Hossain, M.A. (2017). Relaxin family peptides: structure-activity relationship studies. *Br. J. Pharmacol.* *174*, 950–961.
- Peirs, C., Williams, S.-P.G., Zhao, X., Walsh, C.E., Gedeon, J.Y., Cagle, N.E., Goldring, A.C., Hioki, H., Liu, Z., Marell, P.S., et al. (2015). Dorsal Horn Circuits for Persistent Mechanical Pain. *Neuron* *87*, 797–812.
- Pelletier, J.P., Boileau, C., Altman, R.D., and Martel-Pelletier, J. (2010). Experimental models of osteoarthritis: Usefulness in the development of disease-modifying osteoarthritis drugs/agents. *Therapy* *7*, 621–634.
- Pellow, S., Chopin, P., File, S.E., and Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods* *14*, 149–167.
- Pertovaara, A. (2006). Noradrenergic pain modulation. *Prog. Neurobiol.* *80*, 53–83.
- Petitjean, H., Pawlowski, S.A., Fraine, S.L., Sharif, B., Hamad, D., Fatima, T., Berg, J., Brown, C.M., Jan, L.-Y., Ribeiro-da-Silva, A., et al. (2015). Dorsal Horn Parvalbumin Neurons Are Gate-Keepers of Touch-Evoked Pain after Nerve Injury. *Cell Reports* *13*, 1246–1257.
- Pezalla, P.D. (1983). Morphine-induced analgesia and explosive motor behavior in an amphibian. *Brain Res.* *273*, 297–305.
- Pharmaceuticals, G. Glenmark’s novel monoclonal antibody GBR 900 for treatment of chronic pain entering human trials.
- Phillips, C.J. (2006). Economic burden of chronic pain. *Expert Rev Pharmacoecon Outcomes Res* *6*, 591–601.
- Phillips, T.J.C., Brown, M., Ramirez, J.D., Perkins, J., Woldeamanuel, Y.W., Williams, A.C. de C., Orengo, C., Bennett, D.L.H., Bodi, I., Cox, S., et al. (2014). Sensory, psychological, and metabolic dysfunction in HIV-associated peripheral neuropathy: A cross-sectional deep profiling study. *Pain* *155*, 1846–1860.
- Piel, M.J., Kroin, J.S., van Wijnen, A.J., Kc, R., and Im, H.-J. (2014). Pain assessment in animal models of osteoarthritis. *Gene* *537*, 184–188.
- Pintalhão, M., Castro-Chaves, P., Vasques-Novoa, F., Gonçalves, F., Mendonça, L., Fontes-Carvalho, R., Lourenço, P., Almeida, P., Leite-Moreira, A., and Bettencourt, P. (2017). Relaxin serum levels in acute heart failure are associated with pulmonary hypertension and right heart overload. *Eur J Heart Fail* *19*, 218–225.
- Pitcher, G.M., Ritchie, J., and Henry, J.L. (1999). Paw withdrawal threshold in the von Frey hair test is influenced by the surface on which the rat stands. *J. Neurosci. Methods* *87*, 185–193.

- Poisbeau, P., Grinevich, V., and Charlet, A. (2018). Oxytocin Signaling in Pain: Cellular, Circuit, System, and Behavioral Levels. *Curr Top Behav Neurosci* 35, 193–211.
- Polepalli, J.S., Gooch, H., and Sah, P. (2020). Diversity of interneurons in the lateral and basal amygdala. *Npj Science of Learning* 5, 1–9.
- Pomrenze, M.B., Millan, E.Z., Hopf, F.W., Keiflin, R., Maiya, R., Blasio, A., Dadgar, J., Kharazia, V., De Guglielmo, G., Crawford, E., et al. (2015). A Transgenic Rat for Investigating the Anatomy and Function of Corticotrophin Releasing Factor Circuits. *Front Neurosci* 9, 487.
- Porro, C.A., Cettolo, V., Francescato, M.P., and Baraldi, P. (1998). Temporal and Intensity Coding of Pain in Human Cortex. *Journal of Neurophysiology* 80, 3312–3320.
- Porsolt, R.D., Bertin, A., and Jalfre, M. (1977a). Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* 229, 327–336.
- Porsolt, R.D., Le Pichon, M., and Jalfre, M. (1977b). Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266, 730–732.
- Pradhan, A.A., Smith, M.L., McGuire, B., Tarash, I., Evans, C.J., and Charles, A. (2014). Characterization of a novel model of chronic migraine. *Pain* 155, 269–274.
- Pratt, K.G., and Khakhhalin, A.S. (2013). Modeling human neurodevelopmental disorders in the *Xenopus tadpole*: from mechanisms to therapeutic targets. *Dis Model Mech* 6, 1057–1065.
- Praveen, P., Kocan, M., Valkovic, A., Bathgate, R., and Hossain, M.A. (2019). Single chain peptide agonists of relaxin receptors. *Molecular and Cellular Endocrinology* 487, 34–39.
- Pusceddu, M.M., and Gareau, M.G. (2018). Visceral pain: gut microbiota, a new hope? *J. Biomed. Sci.* 25, 73.
- Qaseem, A., Wilt, T.J., McLean, R.M., Forciea, M.A., and Clinical Guidelines Committee of the American College of Physicians (2017). Noninvasive Treatments for Acute, Subacute, and Chronic Low Back Pain: A Clinical Practice Guideline From the American College of Physicians. *Ann. Intern. Med.* 166, 514–530.
- Qu, X., Chen, L., Sun, L., Chen, C., Gao, Z., Huang, W., and Zhou, H. (2019). Serum relaxin level predicts recurrence of atrial fibrillation after radiofrequency catheter ablation. *Heart Vessels* 34, 1543–1551.
- Queneau, P., Serrie, A., Trèves, R., and Bontoux, D. (2018). Les douleurs chroniques en France. Recommandations de l'Académie nationale de médecine pour une meilleure prise en charge des malades. *Douleurs : Évaluation - Diagnostic - Traitement* 19, 265–272.
- Quick, M.L., Wong, L., Mukherjee, S., Done, J.D., Schaeffer, A.J., and Thumbikat, P. (2013). Th1-Th17 cells contribute to the development of uropathogenic *Escherichia coli*-induced chronic pelvic pain. *PLoS ONE* 8, e60987.

- Quiton, R.L., Masri, R., Thompson, S.M., and Keller, A. (2010). Abnormal activity of primary somatosensory cortex in central pain syndrome. *J. Neurophysiol.* *104*, 1717–1725.
- Raboisson, P., and Dallel, R. (2004). The orofacial formalin test. *Neurosci Biobehav Rev* *28*, 219–226.
- Radat, F., Margot-Duclot, A., and Attal, N. (2013). Psychiatric co-morbidities in patients with chronic peripheral neuropathic pain: a multicentre cohort study. *Eur J Pain* *17*, 1547–1557.
- Rainville, P., Duncan, G.H., Price, D.D., Carrier, B., and Bushnell, M.C. (1997). Pain affect encoded in human anterior cingulate but not somatosensory cortex. *Science* *277*, 968–971.
- Ralston, H.J. (2005). Pain and the primate thalamus. *Prog. Brain Res.* *149*, 1–10.
- Ramachandran, R., Bhatt, D.K., Ploug, K.B., Hay-Schmidt, A., Jansen-Olesen, I., Gupta, S., and Olesen, J. (2014). Nitric oxide synthase, calcitonin gene-related peptide and NK-1 receptor mechanisms are involved in GTN-induced neuronal activation. *Cephalalgia* *34*, 136–147.
- Rambukkana, A., Zanazzi, G., Tapinos, N., and Salzer, J.L. (2002). Contact-dependent demyelination by *Mycobacterium leprae* in the absence of immune cells. *Science* *296*, 927–931.
- Ramsey, I.S., Delling, M., and Clapham, D.E. (2006). An introduction to TRP channels. *Annu. Rev. Physiol.* *68*, 619–647.
- Randall, L.O., and Selitto, J.J. (1957). A method for measurement of analgesic activity on inflamed tissue. *Arch Int Pharmacodyn Ther* *111*, 409–419.
- Raputova, J., Srotova, I., Vlckova, E., Sommer, C., Üçeyler, N., Birklein, F., Rittner, H.L., Reborn, C., Adamova, B., Kovalova, I., et al. (2017). Sensory phenotype and risk factors for painful diabetic neuropathy: a cross-sectional observational study. *Pain* *158*, 2340–2353.
- Reddi, D., Curran, N., and Stephens, R. (2013). An introduction to pain pathways and mechanisms. *Br J Hosp Med* *74*, C188–C191.
- Rein, K., Zöckler, M., Mader, M.T., Grübel, C., and Heisenberg, M. (2002). The *Drosophila* standard brain. *Curr. Biol.* *12*, 227–231.
- Ren, K., and Dubner, R. (1999). Inflammatory Models of Pain and Hyperalgesia. *ILAR J* *40*, 111–118.
- Renn, C.L., and Dorsey, S.G. (2005). The physiology and processing of pain: a review. *AACN Clin Issues* *16*, 277–290; quiz 413–415.
- Renn, C.L., Carozzi, V.A., Rhee, P., Gallop, D., Dorsey, S.G., and Cavaletti, G. (2011). Multimodal assessment of painful peripheral neuropathy induced by chronic oxaliplatin-based chemotherapy in mice. *Mol Pain* *7*, 29.

- Rice, A.S.C., Finnerup, N.B., Kemp, H.I., Currie, G.L., and Baron, R. (2018). Sensory profiling in animal models of neuropathic pain: a call for back-translation. *Pain* 159, 819–824.
- Roca-Lapirot, O., Fossat, P., Ma, S., Egron, K., Trigilio, G., López-González, M.-J., Covita, J., Bouali-Benazzouz, R., Favereaux, A., Gundlach, A.L., et al. (2019). Acquisition of analgesic properties by the cholecystokinin (CCK)/CCK2 receptor system within the amygdala in a persistent inflammatory pain condition. *Pain* 160, 345–357.
- Rosen, S.F., Ham, B., Haichin, M., Walters, I.C., Tohyama, S., Sotocinal, S.G., and Mogil, J.S. (2019). Increased pain sensitivity and decreased opioid analgesia in T-cell-deficient mice and implications for sex differences. *Pain* 160, 358–366.
- Rothman, G.R., Blackiston, D.J., and Levin, M. (2016). Color and intensity discrimination in *Xenopus laevis* tadpoles. *Anim Cogn* 19, 911–919.
- Roughan, J.V., Bertrand, H.G.M.J., and Isles, H.M. (2016). Meloxicam prevents COX-2-mediated post-surgical inflammation but not pain following laparotomy in mice. *Eur J Pain* 20, 231–240.
- Ryan, P.J., Büchler, E., Shabanpoor, F., Hossain, M.A., Wade, J.D., Lawrence, A.J., and Gundlach, A.L. (2013). Central relaxin-3 receptor (RXFP3) activation decreases anxiety- and depressive-like behaviours in the rat. *Behav. Brain Res.* 244, 142–151.
- Sah, P., Faber, E.S.L., Lopez De Armentia, M., and Power, J. (2003). The Amygdaloid Complex: Anatomy and Physiology. *Physiological Reviews* 83, 803–834.
- Salomon, B., Rhee, L., Bour-Jordan, H., Hsin, H., Montag, A., Soliven, B., Arcella, J., Girvin, A.M., Padilla, J., Miller, S.D., et al. (2001). Development of spontaneous autoimmune peripheral polyneuropathy in B7-2-deficient NOD mice. *J. Exp. Med.* 194, 677–684.
- Samuel, C.S., Tian, H., Zhao, L., and Amento, E.P. (2003). Relaxin Is a Key Mediator of Prostate Growth and Male Reproductive Tract Development. *Laboratory Investigation* 83, 1055–1067.
- Sandkühler, J. (2009). Models and Mechanisms of Hyperalgesia and Allodynia. *Physiological Reviews* 89, 707–758.
- Sanga, P., Katz, N., Polverejan, E., Wang, S., Kelly, K.M., Haeussler, J., and Thippawong, J. (2017). Long-Term Safety and Efficacy of Fulranumab in Patients With Moderate-to-Severe Osteoarthritis Pain: A Phase II Randomized, Double-Blind, Placebo-Controlled Extension Study. *Arthritis & Rheumatology (Hoboken, N.J.)* 69, 763–773.
- Sanoja, R., Tortorici, V., Fernandez, C., Price, T.J., and Cervero, F. (2010). Role of RVM neurons in capsaicin-evoked visceral nociception and referred hyperalgesia. *Eur J Pain* 14, 120.e1-120.e9.
- Santos, F.N., Pereira, C.W., Sánchez-Pérez, A.M., Otero-García, M., Ma, S., Gundlach, A.L., and Olucha-Bordonau, F.E. (2016). Comparative Distribution of Relaxin-3 Inputs and Calcium-Binding Protein-Positive Neurons in Rat Amygdala. *Front Neuroanat* 10, 36.

- Santos-Nogueira, E., Redondo Castro, E., Mancuso, R., and Navarro, X. (2012). Randall-Selitto Test: A New Approach for the Detection of Neuropathic Pain after Spinal Cord Injury. *J Neurotrauma* 29, 898–904.
- Sapio, M.R., Neubert, J.K., LaPaglia, D.M., Maric, D., Keller, J.M., Raithel, S.J., Rohrs, E.L., Anderson, E.M., Butman, J.A., Caudle, R.M., et al. (2018). Pain control through selective chemotaxotomy of centrally projecting TRPV1+ sensory neurons. *J. Clin. Invest.* 128, 1657–1670.
- Sassen, W.A., and Köster, R.W. (2015). A molecular toolbox for genetic manipulation of zebrafish (Dove Press).
- Schachtschneider, K.M., Schwind, R.M., Newson, J., Kinachtchouk, N., Rizko, M., Mendoza-Elias, N., Grippo, P., Principe, D.R., Park, A., Overgaard, N.H., et al. (2017). The Oncopig Cancer Model: An Innovative Large Animal Translational Oncology Platform. *Front Oncol* 7, 190.
- Schneider, L.E., Henley, K.Y., Turner, O.A., Pat, B., Niedzielko, T.L., and Floyd, C.L. (2017). Application of the Rat Grimace Scale as a Marker of Supraspinal Pain Sensation after Cervical Spinal Cord Injury. *J. Neurotrauma* 34, 2982–2993.
- Schnitzer, T.J., Ekman, E.F., Spierings, E.L.H., Greenberg, H.S., Smith, M.D., Brown, M.T., West, C.R., and Verburg, K.M. (2015). Efficacy and safety of tanezumab monotherapy or combined with non-steroidal anti-inflammatory drugs in the treatment of knee or hip osteoarthritis pain. *Ann. Rheum. Dis.* 74, 1202–1211.
- Schroeder, C.I., and Craik, D.J. (2012). Therapeutic potential of conopeptides. *Future Med Chem* 4, 1243–1255.
- Schwabe, C., McDonald, J.K., and Steinetz, B.G. (1976). Primary structure of the A chain of porcine relaxin. *Biochemical and Biophysical Research Communications* 70, 397–405.
- Schwartz, E.S., and Gebhart, G.F. (2014). Visceral pain. *Curr Top Behav Neurosci* 20, 171–197.
- Schwei, M.J., Honore, P., Rogers, S.D., Salak-Johnson, J.L., Finke, M.P., Ramnaraine, M.L., Clohisy, D.R., and Mantyh, P.W. (1999). Neurochemical and cellular reorganization of the spinal cord in a murine model of bone cancer pain. *J. Neurosci.* 19, 10886–10897.
- Scott, D.J., Layfield, S., Riesewijk, A., Morita, H., Tregear, G.W., and Bathgate, R. a. D. (2004). Identification and characterization of the mouse and rat relaxin receptors as the novel orthologues of human leucine-rich repeat-containing G-protein-coupled receptor 7. *Clin Exp Pharmacol Physiol* 31, 828–832.
- Scott, D.J., Layfield, S., Yan, Y., Sudo, S., Hsueh, A.J.W., Tregear, G.W., and Bathgate, R.A.D. (2006). Characterization of novel splice variants of LGR7 and LGR8 reveals that receptor signaling is mediated by their unique low density lipoprotein class A modules. *J Biol Chem* 281, 34942–34954.

- Sellmeijer, J., Mathis, V., Hugel, S., Li, X.-H., Song, Q., Chen, Q.-Y., Barthas, F., Lutz, P.-E., Karatas, M., Luthi, A., et al. (2018). Hyperactivity of Anterior Cingulate Cortex Areas 24a/24b Drives Chronic Pain-Induced Anxiodepressive-like Consequences. *J. Neurosci.* *38*, 3102–3115.
- Seltzer, Z., Dubner, R., and Shir, Y. (1990). A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. *Pain* *43*, 205–218.
- Sethi, A., Bruell, S., Patil, N., Hossain, M.A., Scott, D.J., Petrie, E.J., Bathgate, R.A.D., and Gooley, P.R. (2016). The complex binding mode of the peptide hormone H2 relaxin to its receptor RXFP1. *Nat Commun* *7*, 11344.
- Sevcik, M.A., Ghilardi, J.R., Peters, C.M., Lindsay, T.H., Halvorson, K.G., Jonas, B.M., Kubota, K., Kuskowski, M.A., Boustany, L., Shelton, D.L., et al. (2005). Anti-NGF therapy profoundly reduces bone cancer pain and the accompanying increase in markers of peripheral and central sensitization. *Pain* *115*, 128–141.
- Shabanpoor, F., Akhter Hossain, M., Ryan, P.J., Belgi, A., Layfield, S., Kocan, M., Zhang, S., Samuel, C.S., Gundlach, A.L., Bathgate, R.A.D., et al. (2012). Minimization of human relaxin-3 leading to high-affinity analogues with increased selectivity for relaxin-family peptide 3 receptor (RXFP3) over RXFP1. *J Med Chem* *55*, 1671–1681.
- Shepherd, J.K., Grewal, S.S., Fletcher, A., Bill, D.J., and Dourish, C.T. (1994). Behavioural and pharmacological characterisation of the elevated “zero-maze” as an animal model of anxiety. *Psychopharmacology (Berl.)* *116*, 56–64.
- Sherwood, O.D. (2004). Relaxin’s physiological roles and other diverse actions. *Endocr Rev* *25*, 205–234.
- Shyu, B.-C., and Vogt, B.A. (2009). Short-term synaptic plasticity in the nociceptive thalamic-anterior cingulate pathway. *Mol Pain* *5*, 51.
- Siddall, P., Xu, C.L., and Cousins, M. (1995). Allodynia following traumatic spinal cord injury in the rat. *Neuroreport* *6*, 1241–1244.
- Sikes, R.W., and Vogt, B.A. (1992). Nociceptive neurons in area 24 of rabbit cingulate cortex. *Journal of Neurophysiology* *68*, 1720–1732.
- Simjee, S.U., Jawed, H., Quadri, J., and Saeed, S.A. (2007). Quantitative gait analysis as a method to assess mechanical hyperalgesia modulated by disease-modifying antirheumatoid drugs in the adjuvant-induced arthritic rat. *Arthritis Res Ther* *9*, R91.
- Simmons, J.K., Hildreth, B.E., Supsavhad, W., Elshafae, S.M., Hassan, B.B., Dirksen, W.P., Toribio, R.E., and Rosol, T.J. (2015). Animal Models of Bone Metastasis. *Vet. Pathol.* *52*, 827–841.

- Simpson, L.A., Eng, J.J., Hsieh, J.T.C., Wolfe, D.L., and Spinal Cord Injury Rehabilitation Evidence Scire Research Team (2012). The health and life priorities of individuals with spinal cord injury: a systematic review. *J. Neurotrauma* 29, 1548–1555.
- Skljarevski, V., Desai, D., Liu-Seifert, H., Zhang, Q., Chappell, A.S., Detke, M.J., Iyengar, S., Atkinson, J.H., and Backonja, M. (2010a). Efficacy and safety of duloxetine in patients with chronic low back pain. *Spine* 35, E578-585.
- Skljarevski, V., Zhang, S., Desai, D., Alaka, K.J., Palacios, S., Miazgowski, T., and Patrick, K. (2010b). Duloxetine versus placebo in patients with chronic low back pain: a 12-week, fixed-dose, randomized, double-blind trial. *J Pain* 11, 1282–1290.
- Slatkin, N., Zaki, N., Sanga, P., Wang, S., Louie, J., Kelly, K., and Thippawong, J. (2016). (382) Efficacy, safety, and tolerability of Fulranumab as adjunctive therapy for cancer-related pain: a randomized, double-blind, placebo-controlled, multicenter study. *The Journal of Pain* 17, S70–S71.
- Slosky, L.M., Largent-Milnes, T.M., and Vanderah, T.W. (2015). Use of Animal Models in Understanding Cancer-induced Bone Pain. *Cancer Growth Metastasis* 8, 47–62.
- Smith, E.S.J., and Lewin, G.R. (2009). Nociceptors: a phylogenetic view. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* 195, 1089–1106.
- Smith, C.M., Shen, P.-J., Banerjee, A., Bonaventure, P., Ma, S., Bathgate, R.A.D., Sutton, S.W., and Gundlach, A.L. (2010). Distribution of relaxin-3 and RXFP3 within arousal, stress, affective, and cognitive circuits of mouse brain. *The Journal of Comparative Neurology* 518, 4016–4045.
- Smith, C.M., Ryan, P.J., Hosken, I.T., Ma, S., and Gundlach, A.L. (2011). Relaxin-3 systems in the brain—The first 10 years. *Journal of Chemical Neuroanatomy* 42, 262–275.
- Smith, C.M., Walker, A.W., Hosken, I.T., Chua, B.E., Zhang, C., Haidar, M., and Gundlach, A.L. (2014). Relaxin-3/RXFP3 networks: an emerging target for the treatment of depression and other neuropsychiatric diseases? *Front Pharmacol* 5, 46.
- Smith, D.J., Hawranko, A.A., Monroe, P.J., Gully, D., Urban, M.O., Craig, C.R., Smith, J.P., and Smith, D.L. (1997). Dose-dependent pain-facilitatory and -inhibitory actions of neurotensin are revealed by SR 48692, a nonpeptide neurotensin antagonist: influence on the antinociceptive effect of morphine. *J Pharmacol Exp Ther* 282, 899–908.
- Smith, J.A., Kitt, M.M., Morice, A.H., Birring, S.S., McGarvey, L.P., Sher, M.R., Li, Y.-P., Wu, W.-C., Xu, Z.J., Muccino, D.R., et al. (2020). Gefapixant, a P2X3 receptor antagonist, for the treatment of refractory or unexplained chronic cough: a randomised, double-blind, controlled, parallel-group, phase 2b trial. *Lancet Respir Med* 8, 775–785.
- Sneddon, L.U. (2018). Comparative Physiology of Nociception and Pain. *Physiology (Bethesda)* 33, 63–73.

- Sneddon, L.U. (2019). Evolution of nociception and pain: evidence from fish models. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 374, 20190290.
- Sneddon, L.U., Elwood, R.W., Adamo, S.A., and Leach, M.C. (2014). Defining and assessing animal pain. *Animal Behaviour* 97, 201–212.
- Sneddon, L.U., Halsey, L.G., and Bury, N.R. (2017). Considering aspects of the 3Rs principles within experimental animal biology. *J. Exp. Biol.* 220, 3007–3016.
- Sopata, M., Katz, N., Carey, W., Smith, M.D., Keller, D., Verburg, K.M., West, C.R., Wolfram, G., and Brown, M.T. (2015). Efficacy and safety of tanezumab in the treatment of pain from bone metastases. *Pain* 156, 1703–1713.
- Sotocinal, S.G., Sorge, R.E., Zaloum, A., Tuttle, A.H., Martin, L.J., Wieskopf, J.S., Mapplebeck, J.C.S., Wei, P., Zhan, S., Zhang, S., et al. (2011). The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain* 7, 55.
- Souslova, V., Cesare, P., Ding, Y., Akopian, A.N., Stanfa, L., Suzuki, R., Carpenter, K., Dickenson, A., Boyce, S., Hill, R., et al. (2000). Warm-coding deficits and aberrant inflammatory pain in mice lacking P2X3 receptors. *Nature* 407, 1015–1017.
- St Johnston, D. (2013). Using mutants, knockdowns, and transgenesis to investigate gene function in *Drosophila*. *Wiley Interdiscip Rev Dev Biol* 2, 587–613.
- Stadig, S., Lascelles, B.D.X., Nyman, G., and Bergh, A. (2019). Evaluation and comparison of pain questionnaires for clinical screening of osteoarthritis in cats. *Vet. Rec.* 185, 757.
- Starkey, M.L., Davies, M., Yip, P.K., Carter, L.M., Wong, D.J.N., McMahon, S.B., and Bradbury, E.J. (2009). Expression of the regeneration-associated protein SPRR1A in primary sensory neurons and spinal cord of the adult mouse following peripheral and central injury. *J Comp Neurol* 513, 51–68.
- Steel, Z., Marnane, C., Iranpour, C., Chey, T., Jackson, J.W., Patel, V., and Silove, D. (2014). The global prevalence of common mental disorders: a systematic review and meta-analysis 1980–2013. *Int J Epidemiol* 43, 476–493.
- Steru, L., Chermat, R., Thierry, B., and Simon, P. (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl.)* 85, 367–370.
- Stevens, C.W. (2011). Analgesia in amphibians: preclinical studies and clinical applications. *Vet Clin North Am Exot Anim Pract* 14, 33–44.
- Stevens, C.W. (2015). Bioinformatics and evolution of vertebrate nociceptin and opioid receptors. *Vitam. Horm.* 97, 57–94.

- Stoop, R., Buma, P., van der Kraan, P.M., Hollander, A.P., Billingham, R.C., Meijers, T.H., Poole, A.R., and van den Berg, W.B. (2001). Type II collagen degradation in articular cartilage fibrillation after anterior cruciate ligament transection in rats. *Osteoarthr. Cartil.* *9*, 308–315.
- Sudo, S., Kumagai, J., Nishi, S., Layfield, S., Ferraro, T., Bathgate, R.A.D., and Hsueh, A.J.W. (2003). H3 relaxin is a specific ligand for LGR7 and activates the receptor by interacting with both the ectodomain and the exoloop 2. *The Journal of Biological Chemistry* *278*, 7855–7862.
- Summerlee, A.J., Hornsby, D.J., and Ramsey, D.G. (1998). The dipsogenic effects of rat relaxin: The effect of photoperiod and the potential role of relaxin on drinking in pregnancy. *Endocrinology* *139*, 2322–2328.
- Sun Junhui, Hao Weidong, Fillmore Natasha, Ma Hanley, Springer Danielle, Yu Zu-Xi, Sadowska Agnieszka, Garcia Andrew, Chen Ruoyan, Muniz-Medina Vanessa, et al. (2019). Human Relaxin-2 Fusion Protein Treatment Prevents and Reverses Isoproterenol-Induced Hypertrophy and Fibrosis in Mouse Heart. *Journal of the American Heart Association* *8*, e013465.
- Sung, B., Loh, H.H., and Wei, L. (2000). Association of kappa opioid receptor mRNA upregulation in dorsal root ganglia with mechanical allodynia in mice following nerve injury. *Neurosci. Lett.* *291*, 163–166.
- Sunn, N., Egli, M., Burazin, T.C.D., Burns, P., Colvill, L., Davern, P., Denton, D.A., Oldfield, B.J., Weisinger, R.S., Rauch, M., et al. (2002). Circulating relaxin acts on subfornical organ neurons to stimulate water drinking in the rat. *Proc Natl Acad Sci U S A* *99*, 1701–1706.
- Sutton, S.W., Bonaventure, P., Kuei, C., Nepomuceno, D., Wu, J., Zhu, J., Lovenberg, T.W., and Liu, C. (2005). G-protein-coupled receptor (GPCR)-142 does not contribute to relaxin-3 binding in the mouse brain: further support that relaxin-3 is the physiological ligand for GPCR135. *Neuroendocrinology* *82*, 139–150.
- Sutton, S.W., Shelton, J., Smith, C., Williams, J., Yun, S., Motley, T., Kuei, C., Bonaventure, P., Gundlach, A., Liu, C., et al. (2009). Metabolic and neuroendocrine responses to RXFP3 modulation in the central nervous system. *Ann N Y Acad Sci* *1160*, 242–249.
- Suurväli, J., Boudinot, P., Kanellopoulos, J., and Rützel Boudinot, S. (2017). P2X4: A fast and sensitive purinergic receptor. *Biomed J* *40*, 245–256.
- Swierczek, N.A., Giles, A.C., Rankin, C.H., and Kerr, R.A. (2011). High-throughput behavioral analysis in *C. elegans*. *Nat. Methods* *8*, 592–598.
- Takasusuki, T., and Yaksh, T.L. (2011). Regulation of Spinal Substance P Release by Intrathecal Calcium Channel Blockade. *Anesthesiology* *115*, 153–164.
- Tan, L.L., Pelzer, P., Heintz, C., Tang, W., Gangadharan, V., Flor, H., Sprengel, R., Kuner, T., and Kuner, R. (2017). A pathway from midcingulate cortex to posterior insula gates nociceptive hypersensitivity. *Nat Neurosci* *20*, 1591–1601.

- Tan, Y.Y., Wade, J.D., Tregear, G.W., and Summers, R.J. (1999). Quantitative autoradiographic studies of relaxin binding in rat atria, uterus and cerebral cortex: characterization and effects of oestrogen treatment. *Br J Pharmacol* *127*, 91–98.
- Tanaka, M., Iijima, N., Miyamoto, Y., Fukusumi, S., Itoh, Y., Ozawa, H., and Ibata, Y. (2005). Neurons expressing relaxin 3/INSL 7 in the nucleus incertus respond to stress. *The European Journal of Neuroscience* *21*, 1659–1670.
- Tandon, P., Conlon, F., Furlow, J.D., and Horb, M.E. (2017). Expanding the genetic toolkit in *Xenopus*: Approaches and opportunities for human disease modeling. *Dev. Biol.* *426*, 325–335.
- Tang, Y., Peng, H., Liao, Q., Gan, L., Zhang, R., Huang, L., Ding, Z., Yang, H., Yan, X., Gu, Y., et al. (2016). Study of breakthrough cancer pain in an animal model induced by endothelin-1. *Neurosci. Lett.* *617*, 108–115.
- Tappe-Theodor, A., and Kuner, R. (2014). Studying ongoing and spontaneous pain in rodents--challenges and opportunities. *Eur. J. Neurosci.* *39*, 1881–1890.
- Taylor, J.C., Dewberry, L.S., Totsch, S.K., Yessick, L.R., DeBerry, J.J., Watts, S.A., and Sorge, R.E. (2017). A novel zebrafish-based model of nociception. *Physiol. Behav.* *174*, 83–88.
- Teeple, E., Jay, G.D., Elsaid, K.A., and Fleming, B.C. (2013a). Animal models of osteoarthritis: challenges of model selection and analysis. *AAPS J* *15*, 438–446.
- Teeple, E., Jay, G.D., Elsaid, K.A., and Fleming, B.C. (2013b). Animal models of osteoarthritis: challenges of model selection and analysis. *AAPS J* *15*, 438–446.
- Teerlink, J.R., Cotter, G., Davison, B.A., Felker, G.M., Filippatos, G., Greenberg, B.H., Ponikowski, P., Unemori, E., Voors, A.A., Adams, K.F., et al. (2013). Serelaxin, recombinant human relaxin-2, for treatment of acute heart failure (RELAX-AHF): a randomised, placebo-controlled trial. *The Lancet* *381*, 29–39.
- van Thiel, I. a. M., Botschuijver, S., de Jonge, W.J., and Seppen, J. (2020). Painful interactions: Microbial compounds and visceral pain. *Biochim Biophys Acta Mol Basis Dis* *1866*, 165534.
- Thompson, J.M., and Neugebauer, V. (2017). Amygdala Plasticity and Pain. *Pain Res Manag* *2017*, 8296501.
- Tirabassi, R.S., Flanagan, J.F., Wu, T., Kislauskis, E.H., Birckbichler, P.J., and Guberski, D.L. (2004). The BBZDR/Wor rat model for investigating the complications of type 2 diabetes mellitus. *ILAR J* *45*, 292–302.
- Tjølsen, A., Berge, O.-G., Hunskaar, S., Rosland, J.H., and Hole, K. (1992). The formalin test: an evaluation of the method. *Pain* *51*, 5–17.
- Tobin, D.M., and Bargmann, C.I. (2004). Invertebrate nociception: behaviors, neurons and molecules. *J. Neurobiol.* *61*, 161–174.

- Toyka, K.V. (1999). Eighty three years of the Guillain-Barré syndrome: clinical and immunopathologic aspects, current and future treatments. *Rev. Neurol. (Paris)* *155*, 849–856.
- Tracey, W.D., Wilson, R.I., Laurent, G., and Benzer, S. (2003). *painless*, a *Drosophila* gene essential for nociception. *Cell* *113*, 261–273.
- Trang, T., Beggs, S., Wan, X., and Salter, M.W. (2009). P2X4-receptor-mediated synthesis and release of brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated protein kinase activation. *J. Neurosci.* *29*, 3518–3528.
- Treede, R.-D., Jensen, T.S., Campbell, J.N., Cruccu, G., Dostrovsky, J.O., Griffin, J.W., Hansson, P., Hughes, R., Nurmikko, T., and Serra, J. (2008). Neuropathic pain: redefinition and a grading system for clinical and research purposes. *Neurology* *70*, 1630–1635.
- Treede, R.-D., Rief, W., Barke, A., Aziz, Q., Bennett, M.I., Benoliel, R., Cohen, M., Evers, S., Finnerup, N.B., First, M.B., et al. (2015). A classification of chronic pain for ICD-11. *Pain* *156*, 1003.
- Treede, R.-D., Rief, W., Barke, A., Aziz, Q., Bennett, M.I., Benoliel, R., Cohen, M., Evers, S., Finnerup, N.B., First, M.B., et al. (2019). Chronic pain as a symptom or a disease: the IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11). *PAIN* *160*, 19–27.
- Trimble, N., Johnson, A.C., Foster, A., and Greenwood-van Meerveld, B. (2007). Corticotropin-releasing factor receptor 1-deficient mice show decreased anxiety and colonic sensitivity. *Neurogastroenterol. Motil.* *19*, 754–760.
- Tsuda, M. (2017). P2 receptors, microglial cytokines and chemokines, and neuropathic pain. *J. Neurosci. Res.* *95*, 1319–1329.
- Tsuda, M., Shigemoto-Mogami, Y., Koizumi, S., Mizokoshi, A., Kohsaka, S., Salter, M.W., and Inoue, K. (2003). P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* *424*, 778–783.
- Tucker, L.B., and McCabe, J.T. (2017). Behavior of Male and Female C57BL/6J Mice Is More Consistent with Repeated Trials in the Elevated Zero Maze than in the Elevated Plus Maze. *Front Behav Neurosci* *11*, 13.
- Uematsu, T., Sakai, A., Ito, H., and Suzuki, H. (2011). Intra-articular administration of tachykinin NK₁ receptor antagonists reduces hyperalgesia and cartilage destruction in the inflammatory joint in rats with adjuvant-induced arthritis. *Eur. J. Pharmacol.* *668*, 163–168.
- Ulmann, L., Hatcher, J.P., Hughes, J.P., Chaumont, S., Green, P.J., Conquet, F., Buell, G.N., Reeve, A.J., Chessell, I.P., and Rassendren, F. (2008). Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. *J. Neurosci.* *28*, 11263–11268.

- Urban, M.O., Coutinho, S.V., and Gebhart, G.F. (1999). Biphasic Modulation of Visceral Nociception by Neurotensin in Rat Rostral Ventromedial Medulla. *J Pharmacol Exp Ther* *290*, 207–213.
- Vanegas, H., and Schaible, H.-G. (2004). Descending control of persistent pain: inhibitory or facilitatory? *Brain Res. Brain Res. Rev.* *46*, 295–309.
- Vaz, R., Hofmeister, W., and Lindstrand, A. (2019). Zebrafish Models of Neurodevelopmental Disorders: Limitations and Benefits of Current Tools and Techniques. *Int J Mol Sci* *20*.
- Veinante, P., Yalcin, I., and Barrot, M. (2013). The amygdala between sensation and affect: a role in pain. *J Mol Psychiatry* *1*, 9.
- Venken, K.J.T., and Bellen, H.J. (2014). Chemical mutagens, transposons, and transgenes to interrogate gene function in *Drosophila melanogaster*. *Methods* *68*, 15–28.
- Ventzel, L., Madsen, C.S., Karlsson, P., Tankisi, H., Isak, B., Fuglsang-Frederiksen, A., Jensen, A.B., Jensen, A.R., Jensen, T.S., and Finnerup, N.B. (2018). Chronic Pain and Neuropathy Following Adjuvant Chemotherapy. *Pain Med* *19*, 1813–1824.
- Vera-Portocarrero, L.P., and Westlund, K.N. (2004). Attenuation of nociception in a model of acute pancreatitis by an NK-1 antagonist. *Pharmacol. Biochem. Behav.* *77*, 631–640.
- Vertes, R.P. (2005). Hippocampal theta rhythm: a tag for short-term memory. *Hippocampus* *15*, 923–935.
- Visser, K., and Meert, T. (2005). A behavioral and pharmacological validation of the acetone spray test in gerbils with a chronic constriction injury. *Anesth. Analg.* *101*, 457–464, table of contents.
- Vuralli, D., Wattiez, A.-S., Russo, A.F., and Bolay, H. (2019a). Behavioral and cognitive animal models in headache research. *J Headache Pain* *20*, 11.
- Vuralli, D., Wattiez, A.-S., Russo, A.F., and Bolay, H. (2019b). Behavioral and cognitive animal models in headache research. *J Headache Pain* *20*, 11.
- Vuralli, D., Wattiez, A.-S., Russo, A.F., and Bolay, H. (2019c). Behavioral and cognitive animal models in headache research. *J Headache Pain* *20*, 11.
- Wacnik, P.W., Eikmeier, L.J., Ruggles, T.R., Ramnaraine, M.L., Walcheck, B.K., Beitz, A.J., and Wilcox, G.L. (2001). Functional interactions between tumor and peripheral nerve: morphology, algogen identification, and behavioral characterization of a new murine model of cancer pain. *J. Neurosci.* *21*, 9355–9366.
- Waeber, C., and Moskowitz, M.A. (2005). Migraine as an inflammatory disorder. *Neurology* *64*, S9-15.

- Wagner, T., Poole, C., and Roth-Daniek, A. (2013). The Capsaicin 8% Patch for Neuropathic Pain in Clinical Practice: A Retrospective Analysis. *Pain Med* *14*, 1202–1211.
- Waksman, B.H., and Adams, R.D. (1956). A comparative study of experimental allergic neuritis in the rabbit, guinea pig, and mouse. *J. Neuropathol. Exp. Neurol.* *15*, 293–334.
- Walensky, L.D., and Bird, G.H. (2014). Hydrocarbon-Stapled Peptides: Principles, Practice, and Progress. *J. Med. Chem.* *57*, 6275–6288.
- Walicke, P.A., Hefti, F., Bales, R., Lu, S.-P., Ruckle, J.L., Brown, M.T., West, C.R., and Shelton, D.L. (2018). First-in-human randomized clinical trials of the safety and efficacy of tanezumab for treatment of chronic knee osteoarthritis pain or acute bunionectomy pain. *Pain Rep* *3*, e653.
- Walker, A.K., Kavelaars, A., Heijnen, C.J., and Dantzer, R. (2014). Neuroinflammation and Comorbidity of Pain and Depression. *Pharmacol Rev* *66*, 80–101.
- Wallace, V.C.J., Norbury, T.A., and Rice, A.S.C. (2005). Ultrasound vocalisation by rodents does not correlate with behavioural measures of persistent pain. *Eur J Pain* *9*, 445–452.
- Walters, E.T. (2018). Nociceptive Biology of Molluscs and Arthropods: Evolutionary Clues About Functions and Mechanisms Potentially Related to Pain. *Front Physiol* *9*.
- Walton, M.B., Cowderoy, E., Lascelles, D., and Innes, J.F. (2013). Evaluation of construct and criterion validity for the “Liverpool Osteoarthritis in Dogs” (LOAD) clinical metrology instrument and comparison to two other instruments. *PLoS ONE* *8*, e58125.
- Wang, G., and Thompson, S.M. (2008). Maladaptive homeostatic plasticity in a rodent model of central pain syndrome: thalamic hyperexcitability after spinothalamic tract lesions. *J. Neurosci.* *28*, 11959–11969.
- Wang, B., Chandrasekera, P.C., and Pippin, J.J. (2014). Leptin- and leptin receptor-deficient rodent models: relevance for human type 2 diabetes. *Curr Diabetes Rev* *10*, 131–145.
- Wang, D., Stoveken, H.M., Zucca, S., Dao, M., Orlandi, C., Song, C., Masuho, I., Johnston, C., Opperman, K.J., Giles, A.C., et al. (2019). Genetic behavioral screen identifies an orphan anti-opioid system. *Science* *365*, 1267–1273.
- Wang, J.-W., Lundeberg, T., and Yu, L.-C. (2003). Antinociceptive role of oxytocin in the nucleus raphe magnus of rats, an involvement of mu-opioid receptor. *Regul Pept* *115*, 153–159.
- Wang, Q., Ng, L., Harris, J.A., Feng, D., Li, Y., Royall, J.J., Oh, S.W., Bernard, A., Sunkin, S.M., Koch, C., et al. (2017). Organization of the connections between claustrum and cortex in the mouse. *J Comp Neurol* *525*, 1317–1346.
- Watson, B.D., Dietrich, W.D., Busto, R., Wachtel, M.S., and Ginsberg, M.D. (1985). Induction of reproducible brain infarction by photochemically initiated thrombosis. *Ann. Neurol.* *17*, 497–504.

- Way, J.C., and Chalfie, M. (1989). The *mec-3* gene of *Caenorhabditis elegans* requires its own product for maintained expression and is expressed in three neuronal cell types. *Genes Dev.* *3*, 1823–1833.
- Webb, M.P., Helander, E.M., Menard, B.L., Urman, R.D., and Kaye, A.D. (2018). Tanezumab: a selective humanized mAb for chronic lower back pain. *Ther Clin Risk Manag* *14*, 361–367.
- Webster, R.P., Anderson, G.I., and Gearing, D.P. (2014). Canine Brief Pain Inventory scores for dogs with osteoarthritis before and after administration of a monoclonal antibody against nerve growth factor. *Am. J. Vet. Res.* *75*, 532–535.
- Weiss, G. (1989). Relaxin in the male. *Biol Reprod* *40*, 197–200.
- Wermeling, D.P., and Berger, J.R. (2006). Ziconotide infusion for severe chronic pain: case series of patients with neuropathic pain. *Pharmacotherapy* *26*, 395–402.
- van der Westhuizen, E.T., Christopoulos, A., Sexton, P.M., Wade, J.D., and Summers, R.J. (2010). H2 relaxin is a biased ligand relative to H3 relaxin at the relaxin family peptide receptor 3 (RXFP3). *Mol Pharmacol* *77*, 759–772.
- Wheeler, G.N., and Brändli, A.W. (2009). Simple vertebrate models for chemical genetics and drug discovery screens: lessons from zebrafish and *Xenopus*. *Dev. Dyn.* *238*, 1287–1308.
- White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* *314*, 1–340.
- Wie, C.S., and Derian, A. (2020). Ziconotide. In *StatPearls*, (Treasure Island (FL): StatPearls Publishing), p.
- Wilkinson, T.N., Speed, T.P., Tregear, G.W., and Bathgate, R.A.D. (2005a). Coevolution of the relaxin-like peptides and their receptors. *Annals of the New York Academy of Sciences* *1041*, 534–539.
- Wilkinson, T.N., Speed, T.P., Tregear, G.W., and Bathgate, R.A.D. (2005b). Evolution of the relaxin-like peptide family. *BMC Evol. Biol.* *5*, 14.
- Williams, C.J., James, L.E., Bertelsen, M.F., and Wang, T. (2019). Analgesia for non-mammalian vertebrates. *Current Opinion in Physiology* *11*, 75–84.
- Williams, L.S., Jones, W.J., Shen, J., Robinson, R.L., Weinberger, M., and Kroenke, K. (2003). Prevalence and impact of depression and pain in neurology outpatients. *J. Neurol. Neurosurg. Psychiatry* *74*, 1587–1589.
- Winter, C.A., Risley, E.A., and Nuss, G.W. (1962). Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proc. Soc. Exp. Biol. Med.* *111*, 544–547.

- Wittenburg, N., and Baumeister, R. (1999). Thermal avoidance in *Caenorhabditis elegans*: an approach to the study of nociception. *Proc. Natl. Acad. Sci. U.S.A.* *96*, 10477–10482.
- Wong, L.L.L., Scott, D.J., Hossain, M.A., Kaas, Q., Rosengren, K.J., and Bathgate, R.A.D. (2018). Distinct but overlapping binding sites of agonist and antagonist at the relaxin family peptide 3 (RXFP3) receptor. *J. Biol. Chem.* *293*, 15777–15789.
- Woolf, C.J. (1983a). Evidence for a central component of post-injury pain hypersensitivity. *Nature* *306*, 686–688.
- Woolf, C.J. (1983b). Evidence for a central component of post-injury pain hypersensitivity. *Nature* *306*, 686–688.
- Woolf, C.J. (2011). Central sensitization: implications for the diagnosis and treatment of pain. *Pain* *152*, S2-15.
- Woolf, C.J., and Salter, M.W. (2000a). Neuronal Plasticity: Increasing the Gain in Pain. *Science* *288*, 1765–1768.
- Woolf, C.J., and Salter, M.W. (2000b). Neuronal plasticity: increasing the gain in pain. *Science* *288*, 1765–1769.
- Woolfe, G., and Macdonald, A.D. (1944). The Evaluation of the Analgesic Action of Pethidine Hydrochloride (demerol). *J Pharmacol Exp Ther* *80*, 300–307.
- Wu, J.X., Xu, M.Y., Miao, X.R., Lu, Z.J., Yuan, X.M., Li, X.Q., and Yu, W.F. (2012). Functional up-regulation of P2X3 receptors in dorsal root ganglion in a rat model of bone cancer pain. *Eur J Pain* *16*, 1378–1388.
- Xing, H., Chen, M., Ling, J., Tan, W., and Gu, J.G. (2007). TRPM8 mechanism of cold allodynia after chronic nerve injury. *J. Neurosci.* *27*, 13680–13690.
- Xu, L., Nwosu, L.N., Burston, J.J., Millns, P.J., Sagar, D.R., Mapp, P.I., Meesawatsom, P., Li, L., Bennett, A.J., Walsh, D.A., et al. (2016). The anti-NGF antibody muMab 911 both prevents and reverses pain behaviour and subchondral osteoclast numbers in a rat model of osteoarthritis pain. *Osteoarthr. Cartil.* *24*, 1587–1595.
- Yaksh, T.L. (2002). Future advances in pain pharmacology: what does the present say about the future? *Proc. West. Pharmacol. Soc.* *45*, 211–218.
- Yaksh, T.L., Farb, D.H., Leeman, S.E., and Jessell, T.M. (1979). Intrathecal capsaicin depletes substance P in the rat spinal cord and produces prolonged thermal analgesia. *Science* *206*, 481–483.
- Yalcin, I., Megat, S., Barthas, F., Waltisperger, E., Kremer, M., Salvat, E., and Barrot, M. (2014a). The sciatic nerve cuffing model of neuropathic pain in mice. *J Vis Exp*.

- Yalcin, I., Barthas, F., and Barrot, M. (2014b). Emotional consequences of neuropathic pain: insight from preclinical studies. *Neurosci Biobehav Rev* 47, 154–164.
- Yalcin, I., Barthas, F., and Barrot, M. (2014c). Emotional consequences of neuropathic pain: insight from preclinical studies. *Neurosci Biobehav Rev* 47, 154–164.
- Yam, M.F., Loh, Y.C., Tan, C.S., Khadijah Adam, S., Abdul Manan, N., and Basir, R. (2018). General Pathways of Pain Sensation and the Major Neurotransmitters Involved in Pain Regulation. *Int J Mol Sci* 19.
- Yamamura, H., Iwata, K., Tsuboi, Y., Toda, K., Kitajima, K., Shimizu, N., Nomura, H., Hibiya, J., Fujita, S., and Sumino, R. (1996). Morphological and electrophysiological properties of ACCx nociceptive neurons in rats. *Brain Res.* 735, 83–92.
- Yang, Y., and Santamaria, P. (2006). Lessons on autoimmune diabetes from animal models. *Clin. Sci.* 110, 627–639.
- Yang, J., Liang, J.-Y., Li, P., Pan, Y.-J., Qiu, P.-Y., Zhang, J., Hao, F., and Wang, D.-X. (2011a). Oxytocin in the periaqueductal gray participates in pain modulation in the rat by influencing endogenous opiate peptides. *Peptides* 32, 1255–1261.
- Yang, J., Li, P., Liang, J.-Y., Pan, Y.-J., Yan, X.-Q., Yan, F.-L., Hao, F., Zhang, X.-Y., Zhang, J., Qiu, P.-Y., et al. (2011b). Oxytocin in the periaqueductal grey regulates nociception in the rat. *Regul Pept* 169, 39–42.
- Yang, M., Rainone, A., Shi, X.Q., Fournier, S., and Zhang, J. (2014). A new animal model of spontaneous autoimmune peripheral polyneuropathy: implications for Guillain-Barré syndrome. *Acta Neuropathol Commun* 2, 5.
- Yang, P., Kajiwara, R., Tonoki, A., and Itoh, M. (2018). Successive and discrete spaced conditioning in active avoidance learning in young and aged zebrafish. *Neurosci. Res.* 130, 1–7.
- Yegorov, S., Good-Avila, S.V., Parry, L., and Wilson, B.C. (2009). Relaxin family genes in humans and teleosts. *Annals of the New York Academy of Sciences* 1160, 42–44.
- Yoneda, T., Sasaki, A., and Mundy, G.R. (1994). Osteolytic bone metastasis in breast cancer. *Breast Cancer Res. Treat.* 32, 73–84.
- Yoshida, M., Takayanagi, Y., Inoue, K., Kimura, T., Young, L.J., Onaka, T., and Nishimori, K. (2009). Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. *J Neurosci* 29, 2259–2271.
- Zagami, A.S., Goadsby, P.J., and Edvinsson, L. (1990). Stimulation of the superior sagittal sinus in the cat causes release of vasoactive peptides. *Neuropeptides* 16, 69–75.
- Zeng, S.L., Sudlow, L.C., and Berezin, M.Y. (2020). Using *Xenopus* oocytes in neurological disease drug discovery. *Expert Opin Drug Discov* 15, 39–52.

- Zhang, J., and Banerjee, B. (2015). Role of MicroRNA in Visceral Pain. *J Neurogastroenterol Motil* 21, 159–171.
- Zhang, W.Y., and Li Wan Po, A. (1994). The effectiveness of topically applied capsaicin. A meta-analysis. *Eur. J. Clin. Pharmacol.* 46, 517–522.
- Zhang, C., Chua, B.E., Yang, A., Shabanpoor, F., Hossain, M.A., Wade, J.D., Rosengren, K.J., Smith, C.M., and Gundlach, A.L. (2015). Central relaxin-3 receptor (RXFP3) activation reduces elevated, but not basal, anxiety-like behaviour in C57BL/6J mice. *Behavioural Brain Research* 292, 125–132.
- Zhang, L., Wang, R., Bai, T., Xiang, X., Qian, W., Song, J., and Hou, X. (2019a). EphrinB2/ephB2-mediated myenteric synaptic plasticity: mechanisms underlying the persistent muscle hypercontractility and pain in postinfectious IBS. *FASEB J.* 33, 13644–13659.
- Zhang, M.-L., Wang, H.-B., Fu, F.-H., and Yu, L.-C. (2017). Involvement of galanin and galanin receptor 2 in nociceptive modulation in anterior cingulate cortex of normal rats and rats with mononeuropathy. *Scientific Reports* 7, 45930.
- Zhang, Q., Liu, S.-H., Erikson, M., Lewis, M., and Unemori, E. (2002). Relaxin activates the MAP kinase pathway in human endometrial stromal cells. *J Cell Biochem* 85, 536–544.
- Zhang, Y., Gao, Y., Li, C.-Y., Dong, W., Li, M.-N., Liu, Y.-N., Dong, Y., and Xu, S.-L. (2019b). Galanin plays a role in antinociception via binding to galanin receptors in the nucleus accumbens of rats with neuropathic pain. *Neurosci Lett* 706, 93–98.
- Zhao, L., Roche, P.J., Gunnensen, J.M., Hammond, V.E., Tregear, G.W., Wintour, E.M., and Beck, F. (1999). Mice without a functional relaxin gene are unable to deliver milk to their pups. *Endocrinology* 140, 445–453.
- Zhao, Z.-Q., Chiechio, S., Sun, Y.-G., Zhang, K.-H., Zhao, C.-S., Scott, M., Johnson, R.L., Deneris, E.S., Renner, K.J., Gereau, R.W., et al. (2007). Mice lacking central serotonergic neurons show enhanced inflammatory pain and an impaired analgesic response to antidepressant drugs. *J. Neurosci.* 27, 6045–6053.
- Zheng, Z., Lauritzen, J.S., Perlman, E., Robinson, C.G., Nichols, M., Milkie, D., Torrens, O., Price, J., Fisher, C.B., Sharifi, N., et al. (2018). A Complete Electron Microscopy Volume of the Brain of Adult *Drosophila melanogaster*. *Cell* 174, 730-743.e22.
- Zhu, C., Xu, J., Lin, Y., Ju, P., Duan, D., Luo, Y., Ding, W., Huang, S., Chen, J., and Cui, D. (2018a). Loss of Microglia and Impaired Brain-Neurotrophic Factor Signaling Pathway in a Comorbid Model of Chronic Pain and Depression. *Front Psychiatry* 9, 442.
- Zhu, H., Xiao, X., Chai, Y., Li, D., Yan, X., and Tang, H. (2019). MiRNA-29a modulates visceral hyperalgesia in irritable bowel syndrome by targeting HTR7. *Biochem. Biophys. Res. Commun.* 511, 671–678.

Zhu, X., Liu, Z., Qin, Y., Niu, W., Wang, Q., Li, L., and Zhou, J. (2018b). Analgesic effects of electroacupuncture at ST25 and CV12 in a rat model of postinflammatory irritable bowel syndrome visceral pain. *Acupunct Med* 36, 240–246.

Zingg, B., Hintiryan, H., Gou, L., Song, M.Y., Bay, M., Bienkowski, M.S., Foster, N.N., Yamashita, S., Bowman, I., Toga, A.W., et al. (2014). Neural networks of the mouse neocortex. *Cell* 156, 1096–1111.

(1979). Pain terms: a list with definitions and notes on usage. Recommended by the IASP Subcommittee on Taxonomy. *Pain* 6, 249.

	Animal species	Procedure	Clinical relevance	References
<i>Inflammatory pain models</i>				
<i>Pain model induced by capsaicin</i>	Mice Rats	Intra-plantar injection of capsaicin	Mimic skin inflammation and inflammatory bowel disease	Kenins P., 1982 Muley et al., 2016
<i>Pain model induced by formalin</i>	Mice Rats	Injection (subcutaneous, intra-plantar) of diluted formaldehyde solution (0.5-5%)	Not specific to a clinical pathology.	Dubuisson et al., 1977 Muley et al., 2016
<i>Pain model induced by complete Freund's adjuvant (CFA)</i>	Mice Rats	Subcutaneous injection of CFA in the hind paw or intra-articular	Assess inflammatory pain in mice and rats Robust pain model for the study RA	Stein et al., 1988 Muley et al., 2016
<i>Pain model induced by carrageenan</i>	Mice Rats	Subcutaneously injection of carrageenan	Joint pain inflammation	(Winter et al., 1962)
<i>Pain model induced by zymosan</i>	Mice	Injection of cell walls of <i>Saccharomyces cerevisiae</i>	Acute inflammation	Doherty et al., 1985
<i>Osteoarthritis pain models</i>	Mice Rats Guinea-Pigs Dogs Cats	Chemical injections Joint surgery and ligament manipulation Streptococcus injection	Musculoskeletal disease and joint inflammation	Kalbhen, 1987 Stoop et al., 2001 Gomis et al., 2007 Knights et al., 2012 van den Broek et al., 1988 Mc Coy, 2015

<i>Rheumatoid arthritis models</i>	Mice	Collagen injection Collagen antibody injection	chronic autoimmune disorder	Courtenay et al., 1980 Nandakumar et al., 2003
<i>Streptococcal cell wall induced arthritis (SCW)</i>	Rats	Intra-articular injection of Streptococcus pyogenes	Mimic RA	van den Broek et al., 1988 Bessis et al., 2017
Centrally induced neuropathic pain models				
<i>Contusive Spinal cord injury</i>	Mice Rats	Weight drop on spinal cord	Clinically relevant model, but not specific to a pathology	Siddall et al., 1995
<i>Spinal hemi-section</i>	Rats	Hemi section of a thoracic segment usually cranial to L1 dorsal root entry	Mimic chronic central pain after spinal cord trauma	Koehler and Endtz, 1986
<i>Photochemical injury</i>	Rats	Ischemia & tissue necrosis of spinal cord	Reproduce a mechanical trauma of the spinal cord without surgery	Watson et al., 1986
Peripherally induced neuropathic pain models				
<i>Sciatic nerve total transection</i>	Mice Rats	Complete sciatic nerve transection	Mimic the clinical symptoms of “phantom limb”	Wall et al., 1979
<i>Chronic constriction injury</i>	Mice Rats	Four loose knots around sciatic nerve or the infra-orbital nerve	Mimic causalgia or complex regional pain syndrome	Bennett and Xie, 1988
<i>Cuffing of sciatic nerve</i>	Mice Rats	Implantation of a polyethylene tubing (cuff) around the common branch of the sciatic nerve	Study anxiety-depressive comorbidities associated with NP that lasts for weeks after loss of hypersensitivity to pain	Mosconi et al., 1996 Benbouzid et al., 2008
<i>Partial sciatic nerve injury</i>	Mice Rats	Partial ligation of dorsal third or half of sciatic nerve, peroneal or tibial branch or both	Mimic human causalgia symptoms (injury to the peripheral nerve)	Seltzer et al., 1990 Malmberg and Basbaum, 1998
<i>Spinal Nerve Ligation</i>	Mice Rats	Unilateral ligation of lumbar spinal nerves L5 and L6 distal to dorsal root ganglia	Mimic human causalgia symptoms (injury to the peripheral nerve)	Kim and Chung, 1992. Kiso et al., 2008
<i>Spared nerve injury</i>	Mice Rats	Lesion of two of the three terminal branches of the sciatic nerve	Not specific to a clinical pathology.	Decosterd and Woolf, 2000

Table 1: Classical inflammatory and neuropathic pain models in rodents with notes on companion animals.

	Anim al Species	Procedure	Cancer cell line	Pain Tests	References
<i>Bone Cancer Induced Pain (CIBP)</i>	Mice	Intraosseous Injection Cancer Cells into Femur Humerus Calcaneus bone of hindpaw	66.1 or 4T1- Luc2 mammary adenocarcinoma NTCT2472 Fibrosarcoma B16 melanoma C26 colon adenocarcinoma	Flinching Guarding von Frey Test Limb use Tail Flick Assay Dynamic weight bearing Palpation (light touching)	Schwei et al., 1999; Wacnik et al., 2001; Sabino et al., 2003; Vermeirsch et al., 2004; Grenald et al., 2017; Elmarah et al., 2017; Appel et al., 2019
		Transgenic mice	n.a.	Flinching Guarding	Ghilardi et al., 2005
	Rat	Intraosseous Injection Cancer Cells into Tibia Femur	MRMT-1 murine mammary carcinoma Walker 256 rat mammary carcinoma MLL rat prostate adenocarcinoma	von Frey Test Limb use Weight- bearing test	Medhurst et al., 2002; Wu et al., 2012; Zhu et al., 2018; Wang et al., 2018; Falk et al., 2018
	Dog	Naturally- Occurring Bone Cancer	n.a.	Lameness Canine BPI QST VAL QoL	Brown et al., 2015; Sapio et al., 2018; Monteiro et al., 2018
Breakthrough Cancer pain (BTcP)	Mice	Injection of Cancer Cells into Femur +ET-1 injection into tumor cells	Lewis Lung carcinoma	von Frey Test Limb use	Tang et al., 2016;
<i>Metastasis Bone Cancer Pain</i>	Mice/ rat	Injection of Cancer Cells into: Tail vein Intracardiac Intraosseous Orthotopic	ACE-1 canine prostate carcinoma R3327 Mat LyLu cells rat prostatic adenocarcinoma	Flinching Guarding Thermal Paw stimulator Posture changes, movement limitations	Liepe et al., 2005; Halvorson et al., 2005
Breast Cancer Pain	Mice	Injection of cancer cells into mammary fat pad	4T1 murine breast cancer	von Frey Test Acetone drop grimace scale	De Almeida et al., 2019
Pancreatic Cancer Pain	Mice	Injection of cancer cells into pancreas	SW1990 human pancreatic cancer	Abdominal withdrawal threshold Hunching VMR	Wang et al., 2017
		Transgenic mice	n.a.	Hunching Vocalization	Lindsay et al., 2005

Peritoneal carcinomatosis	Mice	Injection of cancer cells into abdominal cavity	⁶⁰ As6Luc derived from HSC60 human gastric scirrhous carcinoma	von Frey test licking Abdominal withdrawal threshold Hunching	Suzuki et al., 2012
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Table 2: Animal Models of Cancer Pain. Canine BPI: Canine Brief Pain Inventory; ET-1: Endothelin-1 ; n.a: not applicable; QoL: Quality of Life; QST: quantitative sensory testing;VAL; Visual analog scale; VMR: Visceromotor responses

	Animal Species	Procedure	Clinical relevance	Pain/Noiceptive tests	References
<i>Abdominal Viscera</i>	Mice	Intraperitoneal injection of chemicals Colorectal distension (CRD)	Visceral Pain	Writhing Test Responses VMR	Alonso-Castro et al., 2017 Kamp et al., 2002
	Rat	Colorectal distension		Passive avoidance behavioral paradigm AWR	Ness and Gebhart, 1988; Ness and Gebhart, 1991;
	Dog	Colorectal distension		VMR	Lyubashina et al., 2016
<i>Digestive Tract</i>	Mice	Intracolorectal injection of chemicals	IBD	“up and down” method von Frey Test	Hou et al., 2019
		Oral ingestion of chemicals	IBD Ulcerative colitis	Intracolonical administration of allyl isithiocyanate (mustard oil) (0,5%) VMR	Lapointe et al., 2015
	Rat	Intracolorectal injection of chemicals	IBD	VMR AWR	Huang et al., 2019; Parisio et al., 2020
<i>Colon-Bowel</i>	Mice	Castor-oil induced diarrhea	<i>IBS-D</i>	Intracolonical administration of allyl isithiocyanate (mustard oil) Writhing Test	Sobczak et al., 2014
		Repeated WAS NMS PRS	<i>IBS</i>	AWR VMR	Zhu et al., 2019 Miquel et al., 2016
	Rat	Colorectal injection of inflamogens or irritants Intragastric Parasite infection Subcutaneous LPS injection	PI-IBS Ulcerative colitis	AWR VMR	Zhu et al., 2018 Zhang et al., 2019 Nozu et al., 2019
		Repeated WAS	IBS		Nozu et al., 2019
<i>Stomach</i>		Hollow stomach distention	Gastric hyperalgesia	VMR Passive Avoidance	Ozaki et al, 2002

		Gastric injection/Oral injection of irritant chemical		Behavior paradigm	
Ureter	Rat	Intraureteral injection of dental cement	Ureteric calculi (Kidney stones)	vocalization to electrical stimulation	Giamberardino et al., 1995
	Mice	oral injection of DBTC	Chronic pancreatitis	von Frey Test animal posture gait disturbances	Oz, 2016
Pancreas	Rat	High fat (65%)/ethanol (6%) diet (10 weeks)	Chronic pancreatitis	von Frey Test up-down method Hot plate	McIlwrath and Westlund, 2015
		Intraductal infusion of a bile salt + Intraperitoneal injection of aCCK analogue Intravenous injection of chemical	Acute pancreatitis	Exploratory activity von Frey Test abdominal withdrawal to heat stimulus	Zhang et al., 2004 Vera-Portocarrero and Westlund, 2004
	Dog	Naturally-Occurring	Pancreatitis	Glasgow Composite Pain Scale	Mansfield and Beths, 2015 Catanzaro et al., 2016
Prostate	Mice	Infection with <i>E.coli</i> bacteria isolate from patient EAP induction	Chronic prostatitis/ chronic pelvic pain syndrome (CP/CPPS)	von Frey Test	Quick et al., 2013 Liu et al., 2019
Biliary system	Mice	Injection of solvents into bile duct	Cholangiopathy	Grimace face Parameters activity	Berntsen et al., 2017
	Rat	Bile duct resection	Cholestasis	Tail-flick latency	Hasanein, 2010
Referred visceral pain	Mice Rats	Intracolonic capsaicin instillation	Prolonged referred hyperalgesia	Von frey test	(Laird et al., 2001b) Sanoja et al., 2010

Table 3: Visceral pain models. AWR: Abdominal Withdrawal Reflex; CRD : Colorectal distension; EAP: Experimental autoimmune prostatitis; IBD: Inflammatory Bowel Disease; IBS: Irritable Bowel syndrome; IBS-D: Irritable Bowel syndrome - diarrhea-predominant; NMS : Neonatal Maternal Separation; PRS: Partial restraint stress; VMR: Visceromotor responses, WAR: Water avoidance stress

	Nociception	Nociceptors	TRP-like channels	Neuromodulators	Opioid system	References
<i>C.elegans</i>	Thermal, chemical and mechanical	ASH pair of neurons DRG polymodal nociceptors	Osm-9 and ocr-2 (=TRPV)	SubstanceP, calcitonin, Glutamate FLP18/FLP-21/NRP-1	μ-opioid like receptor	Bargmann and Kaplan, 1998; Kahn-Kirby and Bargmann, 2006; Venkatachalam et al., 2014; Wittenburg and Baumesiter, 1999; Nkambeu et al., 2018; Glauser et al., 2011; Hills et al., 2016
<i>Drosophila</i>	Thermal, chemical and mechanical	Multidendritic (Md) class IV and class III neurons	Painless, TRPA1, pyrexia (≈ TRPA) Pkd2, NompC, and Trpm Inactive (≈ TRPV)	NRP1 Neuropeptide F Amnesiac	μ-opioid like receptor	Hwang et al., 2007; Walters, 2019; Tracey et al., 2003; Neely et al., 2011; Lee et al., 2005; Turner et al., 2016; Kwon et al., 2010; Hu et al., 2017; Aldrich et al., 2010; Kuong and Neely, 2013
<i>Zebrafish</i>	Thermal, chemical and mechanical	Polymodal nociceptors Un-myelinated C-fibre and Myelinated Aδ-fibres	10 TRP channels (TRPV, TRPM, TRPA)	Substance P, Glutamate,	14 opioid (μ,δ,κ and opioid-like) receptors	Williams et al., 2019; Sneddon, 2019; Saito and Shangai, 2006; Demin et al., 2018; Stevens, 2015
<i>Xenopus</i>	Thermal, chemical and mechanical	Polymodal nociceptors Un-myelinated C-fibre and myelinated Aδ-fibres	14 TRP channels (TRPV, TRPM, TRPA)	Substance P, CGRP, Glutamate	4 opioid (μ,δ,κ and opioid-like) receptors	Williams et al., 2019; Stevens, 2011; Saito and Shangai, 2006; Stevens et al., 2009

Table 4: The nociceptive pathways in invertebrates (*C.elegans* and *Drosophila*) and lower vertebrates (*Xenopus* and *Zebrafish*). Some examples of neurotransmitters or TRP channels are given; only TRPM, TRPA and TRPV channels are indicated for *Xenopus* and *Zebrafish*, *demonstrated in amphibian class, †demonstrated in fish class.

	Age	Thermal assay	Mechanical assay	Chemical assay	Chronic pain-like assay	Neuropathic pain-like assay	References
<i>C. elegans</i>		Heated metal pen tip or laser diode Thermal barrier assay Noxious heat thermogradient assay and four quadrants assay	Harsh touch assay	Octanol avoidance assay Thrashing assay	N.T.	N.T.	Mills et al., 2016; Cohen et al., 2014; Tobin and Bargmann, 2004; Wittenburg and Baumeister, 1999; Glauser et al., 2011; Nkambeu et al., 2019; Wang et al., 2019
<i>Drosophila</i>	Larvae	Soldering iron heated to 46°C Heated Water Droplet Cold probe contact	von Frey fibres assay	HCl (quick exposure or incubation)	N.T.	N.T.	Khuong et al., 2019; Calvo et al., 2019; Im and Galko, 2012; Milinkeviciute et al., 2012; Lopez-Bellido et al., 2019; Heather et al., 2017
	Adult	Water-tight chamber surrounded by hot water 'Jump' reflex assay 'Hotplate' assay The light-driven heat avoidance test Thermal challenge (warm water bath)	N.T.	Feeding session Direct contact with food/liquid laced with noxious compound	'Sunburn' UV radiation	Nerve injury (Middle leg amputation)	
<i>Zebrafish</i>	Embryo/Larvae	Temperature aversion assay Cold and warm water tank	N.T.	Acetic Acid exposure	Acetic acid test (exposure)	Extreme Thermal exposure	Malafovia et al., 2014; Curtright et al., 2015; Taylor et al., 2017; Deakin et al., 2019; Ellis et al., 2018; Yang et al., 2018; Costa et al., 2019; Sneddon, 2018
	Adult	N.T.	Electric shock	Acetic acid test (exposure or subcutaneous injection) Algogen injections	Acetic acid test (exposure or subcutaneous injection)	N.T.	
<i>Xenopus</i>	Adult	Hargreaves test	Pinch by a bulldog clamp in the dactyls of the forelimbs Pull of the ovaries Electric shock	Acetic acid test (exposure)	Acetic acid test (exposure)	N.T.	Stevens, 2011; Miller et al., 1974

Table 5: Examples of assays to study nociception performed in *C. elegans*, *Drosophila*, Zebrafish and *Xenopus*

Annexe 2:

Brain region	RLN3-LI	RXFP3 mRNA	RXFP3 binding sites
Rhinencephalon			
<i>Olfactory bulb</i>			
anterior olfactory n.	+	+	+
ependyma and olfactory ventricle	+	-	-
external plexiform layer	-	+	-
glomerular layer	-	-	+
internal granule cell layer	-	-	-
Telencephalon			
accumbens n., shell	+/-	+	-
dorsal tenia tecta	+++	+	+
dorsal transition zone	+++	-	+
substantia innominata	+++	++	+
ventral pallidum	-	+	-
ventral tenia tecta	+	-	+
Amygdala			
amygdalohippocampal area	-	++++	++++
amygdalopiriform transition area	-	++	++++
amygdalostriatal transition area	+	+/-	-
anterior amygdaloid area	-	+	-
anterior cortical amygdaloid n.	-	+/-	+
basolateral n.	+/-	+++	+++
basomedial n.	+	+++	++++
central n.	+	+++	++
cortex-amygdala transition area	-	+/-	+
lateral n.	+/-	+++	+++
medial n.	+	++++	++++
posterolat cortical amygdaloid n.	-	+	+
posteromed cortical amygdaloid n.	-	+++	++
Bed nucleus of the stria terminalis			
intraamygdaloid division	+	++	+++
lateral division	+	+++	++
medial division	+	+++	+++
ventral division	-	+	+/-
Cerebral cortex			
auditory cortex	-	+	+
cingulate cortex	+++	+	-
claustrum	+	+/-	-
dorsal peduncular cortex	++	+/-	-
ectorhinal cortex	-	+	+
endopiriform n. dorsal	+	+	+/-++
endopiriform n. ventral	-	+	+++
entorhinal cortex	+	+	+
frontal association cortex	-	+/-	+/-
infralimbic cortex	+	+	-
insular cortex	-	+	+
orbital cortex, medial	+/-++	+	+/-
perirhinal cortex	-	+	+
piriform cortex	-	++	+/-++
posterior parietal associative area	-	+	+
prelimbic cortex	+	+/-	+/-
primary motor cortex	+/-	+	+
retrosplenial cortex	+++	+	-
secondary motor cortex	+	+	+
somatosensory cortex	-	+	+
subiculum	+	+	+/-
temporal cortex	-	+	+
visual cortex	+	+	+
Hippocampal formation			
alveus	++	-	+/-
CA1 field	+	++	++
CA2 field	+	+	++
CA3 field	+/-++	++	++
DG, molecular layer	+/-	-	+
DG, polymorph layer	++	+++	+++

Table 9: Evaluation qualitative (partie 1) de la distribution régionale des fibres rln-3 (rln3-like immunoreactivity), des ARNm de RXFP-3 et de la densité relative des sites de fixations de RXFP- dans le cerveau de souris C57Bl6/J (Smith et al., 2010)

Brain region	RLN3-LI	RXFP3 mRNA	RXFP3 binding sites
DG, granular layer	+/-	++	++
dorsal commissure, anterior	+	-	-
dorsal fornix, anterior	++	+/-	-
fimbria	+	+	+/-
oriens layer	+++	+++	+++
pyramidal cell layer	+	+++	++
<i>Nucleus diagonal band</i>			
horizontal limb	+++	++	+/-
vertical limb	+++	+++	+/-
<i>Septum</i>			
lambdoid septal zone	++++	++	-
lateral septal n., dorsal	++	+	-
lateral septal n., intermediate	++++	++	+/-
lateral septal n., ventral	+	+++	+
medial septal n.	++++	+++	+/-
septofimbrial n.	+++	++	+/-
septohippocampal n.	+++	++	+/-
triangular septal n.	+	+/-	+
<i>Diencephalon</i>			
<i>Hypothalamus</i>			
anterior hypothalamic n.	+/-	+	+/-
arcuate n.	-	+	-
dorsomedial hypothalamic n.	+	+	+
lateral hypothalamic area	+++	++	+
lateroanterior hypothalamic n.	+/-	+	-
paraventricular hypothalamic n.	+	++++	+++
periventricular hypothalamic n.	+/-	+++	++
posterior hypothalamic area	++++	++	+
preoptic area, lateral	++	++	++
preoptic area, medial	+/-	+	+/-
sub-paraventricular zone	+	-	-
suprachiasmatic n.	+	+	-
supraoptic n.	+++	+++	++
tuberomammillary n.	+	+	+
ventromedial hypothalamic n.	+/-++	+	+
<i>Mammillary body</i>			
lateral mammillary n.	++	+/-	-
medial mammillary	+	-	-
pre-mammillary n.	+	+	+
supramammillary n.	+++	++	+/-
<i>Thalamus</i>			
anterodorsal thalamic n.	-	+	++++
centromedial n.	+/-	+/-	+/-
habenula	+	+++	+++
intermediodorsal thalamic n.	+	-	-
Lateral posterior thalamic n.	+	+	-
n. access optic tract, dorsal terminal	++++	+	-
n. access optic tract, lateral terminal	++++	+	-
parafascicular thalamic n.	-	+	++
paraventricular thalamic n.	+	+/-	+/-
peripeduncular n.	++++	++++	+/-
periventricular fiber system	-	++	-
posterior intralaminar thalamic n.	+++	+++	+/-
precommissural n.	+	++	-
reuniens thalamic n.	+	-	+/-
rhomboid thalamic n.	+	-	+/-
subparafascicular thalamic n.	-	+++	++
zona incerta	++	+	+
<i>Geniculate system</i>			
dorsolateral geniculate n.	+	-	-
intergeniculate leaflet	++	+/-	-
med geniculate n.	+	-	-
subgeniculate n.	++	+	-
supragenulate n.	++	+	-
ventrolateral geniculate n.	+++	++	-

Table 10 : Evaluation qualitative (partie 2) de la distribution régionale des fibres *rln-3* (*rln3*-like immunoreactivity), des ARNm de RXFP-3 et de la densité relative des sites de fixations de RXFP- dans le cerveau de souris C57Bl6/J (Smith et al., 2010)

Brain region	RLN3-LI	RXFP3 mRNA	RXFP3 binding sites
Mesencephalon			
caudal linear n. raphé	++++	++	+/-
cuneiform n.	-	+	-
dorsal raphé n.	++++	+++	+
inferior colliculus	+	++	-
inferior colliculus, dorsal cortex	-	+++	+++
intercollicular n.	-	++	+
interfascicular n.	+	+++	-
interpeduncular n.	+++	+++	++
med accessory oculomotor n.	-	+	-
n. posterior commissure	+	-	-
olivary pretectal n.	++	-	-
posterior commissure	++	-	-
posterior pretectal n.	++	+	-
rostral linear n. raphé	+	+	-
substantia nigra	+	-	-
substantia nigra, dorsal region	+ (cb)	-	-
supraoculomotor cap	-	++	-
ventral tegmental area	++	++	+/-
Periaqueductal gray			
anterior	++	++	+
anterior PAG, lateroventral region	+ (cb)	++	++
dorsomedial	+++	++	-
dorsolateral	+++	+++	++
lateral	++	++	+/-
ventrolateral	++	++	-
Superior colliculus			
commissure	+	+	-
brachium	++	+/-	-
deep gray layer	++	++	++
deep white layer	+	++	++
intermediate gray layer	+++	++	+/-
intermediate white layer	+++	+	+/-
optic nerve layer	++	++	+
Superficial gray	+	+	+++
zona layer	+/-	-	+++
Rhombencephalon			
Barrington's n.	+	++	+
central gray, pontine	+++ (cb)	+++	-
inferior olive, medial	+	++++	++++
lateral lemniscus	-	+	+
lateral parabrachial n.	+	+++	+
locus coeruleus	+/-	-	-
medial vestibular n., parvicellular	++ (cb)	+	+
median raphé n.	++++	++	-
n. incertus	++++ (cb)	++	++
paramedian raphé n.	++++	++	-
pontine raphé n.	+++ (cb)	+	-
prepositus hypoglossal n.	++	+	+
raphé cap	+++	+	-
raphé magnus	+/-	+	+
solitary tract n.	+	++	++
spinal trigeminal n., dorsomedial	-	++	+/-
spinal trigeminal n., interpolar	-	+/-	+
Tegmental system			
anterior tegmental n.	+++	+++	-
dorsal tegmental n.	++	++	+
dorsomedial tegmental area	+	+++	-
laterodorsal tegmental n., dorsal	+	++	++
laterodorsal tegmental n., ventral	-	+	++
pedunculo-pontine tegmental n.	++	++	-
posterodorsal tegmental n.	+	+	+
ventral tegmental n.	+	+	-

Table 11 : Evaluation qualitative (partie 3) de la distribution régionale des fibres rln-3 (rln3-like immunoreactivity), des ARNm de RXFP-3 et de la densité relative des sites de fixations de RXFP- dans le cerveau de souris C57Bl6/J (Smith et al., 2010)

Ces évaluations ont été faites en utilisant l'échelle suivante: (-) aucun signal détecté; (+/-) signal rare; (+) faible densité de signal dans la région; Signal de densité (++) modéré, (+++) élevé et (++++) très élevé. cb, corps cellulaires; n, noyau.