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Impact de la consommation aiguë chronique de médicaments à effets psychoactifs sur les fonctions cognitives : étude comportementale et pharmacocinétique chez le rat

Marie Heraudeau

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Spécialité **PHARMACIE**

Préparée au sein de l'**Université de Caen Normandie**

Impact de la consommation aigue et chronique de médicaments à effets psychoactifs sur les fonctions cognitives : étude comportementale et pharmacocinétique chez le rat

Présentée et soutenue par

HERAUDEAU MARIE

Thèse soutenue le 19/03/2026

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ASC / AUC : Aire Sous la courbe ou Area Under the Curve

ATS : ANOVA-Type Statistics

BHE : Barrière Hémato-Encéphalique

C_{max} : Concentration maximale

HED : Human Equivalent Dose

IP : Intraperitoneal

ISRS : Inhibiteur Sélectif de la Recapture de la Sérotonine

MWM : Morris Water Maze

NORT : Novel Object Recognition Test

ODM-tramadol : O-desméthyltramadol

OG : Oral Gavage

OMS : Organisation Mondiale de la Santé

SD : Standard Deviation

SEM : Standard Error of the Mean

$T_{½}$: Temps de demi-vie

T_{max} : Temps nécessaire pour atteindre la concentration maximale (C_{max})

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INTRODUCTION

Le **vieillissement de la population** constitue l'un des principaux défis démographiques et sanitaires du XXIe siècle. À l'échelle mondiale comme en France, la proportion de personnes âgées de 65 ans et plus ne cesse d'augmenter, entraînant des transformations des besoins du système de santé.

Les changements physiologiques liés au vieillissement entraînent un déclin progressif de nombreuses fonctions organiques, ce qui accroît la **vulnérabilité des personnes âgées aux maladies chroniques**. Cela se traduit par une augmentation du nombre de personnes consommant des **traitements de façon prolongée**, ainsi qu'un **allongement de la durée moyenne** de ces traitements chroniques.

La consommation chronique de médicaments expose les patients âgés à un **risque accru d'effets iatrogènes**. Cette population est particulièrement vulnérable aux effets indésirables en raison d'une **grande variabilité pharmacocinétique et pharmacodynamique**. Cette sensibilité accrue est notamment préoccupante pour les **substances à effet psychoactif**, qui du fait d'un passage de la barrière hémato-encéphalique, peuvent avoir un impact significatif sur les fonctions **cognitives**.

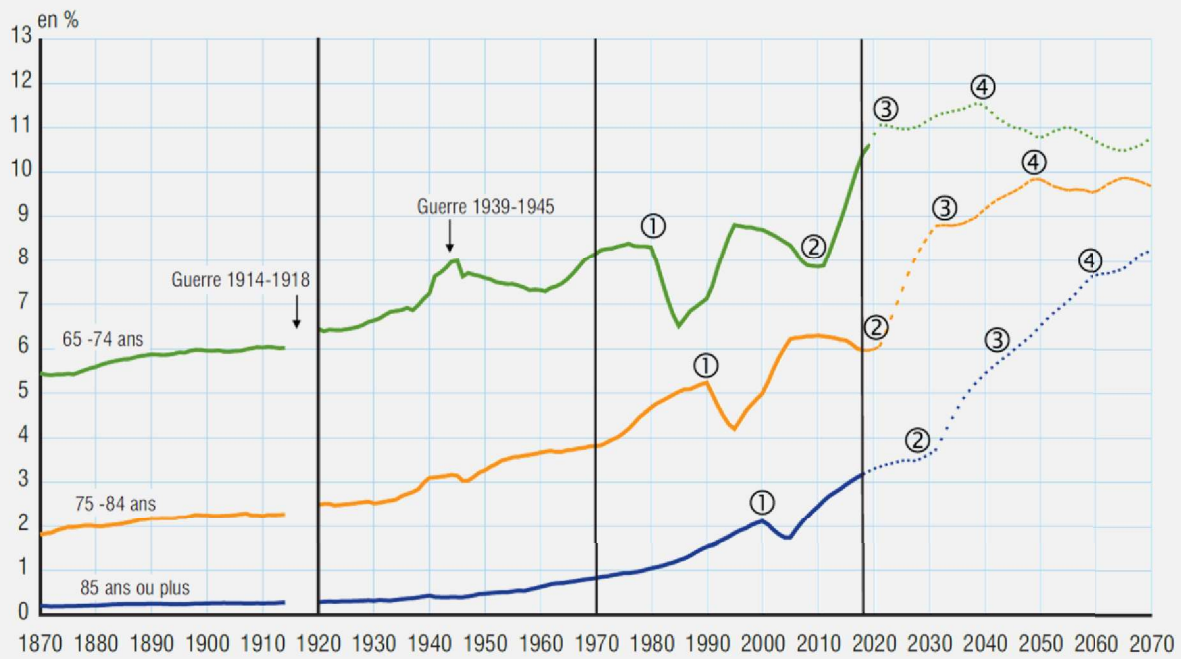
L'évaluation du lien entre la prise d'un médicament et des altérations des fonctions cognitives est cependant complexe. La forte variabilité interindividuelle, l'existence fréquente de comorbidités et la polymédication rendent difficile l'attribution causale d'un effet indésirable à un médicament donné. Dans ce contexte, les études précliniques représentent une approche intéressante, permettant d'isoler les effets d'une substance active et d'évaluer son impact sur les processus cognitifs dans des conditions expérimentales contrôlées.

Vieillissement démographique

Entre 1974 et 2024, la proportion mondiale des personnes âgées de 65 ans a presque doublé, passant de 5,5 % à 10,3 % et cette part devrait à nouveau doubler pour atteindre 20,7 % d'ici 2074 (UNFPA, 2024). L'Organisation Mondiale de la Santé (OMS) estime qu'en 2030, une personne sur six dans le monde aura 60 ans ou plus (OMS, 2024). En France, l'Insee estime que près de 22 millions de personnes seront âgées de 65 ans et plus en 2070 (Blanpain, 2018). La proportion de cette tranche d'âge dépassera un habitant sur quatre dès 2040 et atteindra près de 30 % en 2070 (Blanpain, 2018). Ce **vieillissement démographique** est encore plus marqué chez les plus âgés en France (**Figure 1**) et dans le monde où la part des 80 ans et plus devrait tripler entre 2020 et 2050 (OMS, 2024).

FIGURE 1

Proportion de personnes âgées de 65 à 74 ans, 75 à 84 ans et 85 ans ou plus de 1870 à 2070 (Blanpain, 2018)



Champ : France métropolitaine jusqu'en 1990, France hors Mayotte de 1991 à 2013, France à partir de 2014.
Lecture : en 2018, la France compte 10,4 % de personnes âgées de 65 à 74 ans.
Note : ① Début de l'arrivée des générations creuses 1914-1919 ; ② Début de l'arrivée des générations du *baby-boom* ; ③ Fin de l'arrivée des générations du *baby-boom* ; ④ Sortie des générations du *baby-boom*.
Sources : Human mortality database jusqu'en 1900 ; Insee, estimations de population et statistiques de l'état civil de 1901 à 2018 ; Insee, projections de population à partir de 2019, scénario central.

1. VIEILLISSEMENT DE LA POPULATION

Ce phénomène résulte principalement de deux tendances : la baisse des taux de fécondité et l'**allongement de l'espérance de vie** à la naissance qui a atteint 73,3 ans en 2024, soit une augmentation de 8,4 ans depuis 1995 (OMS, 2025a).

Vieillissement physiologique

Le vieillissement correspond à l'ensemble des changements physiologiques qui modifient progressivement les capacités physiques et intellectuelles de l'organisme avec l'âge (Boismal et al., 2020). Il résulte de l'interaction complexe entre des facteurs biologiques et environnementaux auxquels l'individu est exposé tout au long de sa vie.

Ce processus inévitable se traduit par un ralentissement progressif des systèmes physiologiques et une réduction des réserves fonctionnelles, **affectant l'ensemble des organes par des déclins graduels de leurs fonctions** (Flint & Tadi, 2025; Guilbaud et al., 2020). Sur le plan cardiovasculaire, il se manifeste par une diminution de la compliance myocardique et une rigidification des artères de gros calibre. Le système respiratoire est marqué par une réduction de l'élasticité pulmonaire et des volumes mobilisables. Sur le plan métabolique, la composition corporelle se modifie, associant une augmentation de la masse grasse et une diminution de la masse maigre. Le vieillissement de l'appareil digestif se traduit par une baisse de la motilité gastro-intestinale et des capacités d'absorption de l'intestin grêle, ainsi que par une diminution des fonctions hépatiques liée à la réduction de la masse et du débit sanguin hépatiques. La fonction rénale est également altérée, avec une diminution du nombre de glomérules fonctionnels et du débit de filtration glomérulaire (Flint & Tadi, 2025; Guilbaud et al., 2020). Enfin, le système nerveux central présente des modifications structurelles et fonctionnelles progressives, caractérisées par une réduction du volume de certaines structures cérébrales, un ralentissement de la conduction nerveuse et une diminution de la plasticité synaptique et des capacités de régénération neuronale. Ces modifications centrales peuvent s'accompagner d'un **déclin cognitif global** modéré avec l'âge (Murman, 2015).

Le vieillissement ne s'exprimant ni de manière uniforme selon les organes ni de façon identique entre les individus, il ne peut être appréhendé uniquement par l'âge

chronologique. L'OMS souligne ainsi l'absence de modèle-type définissant la personne âgée (OMS, 2024). Il correspond davantage à un déclin progressif et différencié des fonctions physiologiques, altérant le maintien de l'homéostasie et favorisant un état de **fragilité**, caractérisé par une **réduction des réserves physiologiques et une sensibilité accrue** aux facteurs de stress (Fried et al., 2001). Cette fragilité augmente le risque de développement pathologique, ce qui contribue à amplifier l'**hétérogénéité interindividuelle** marquée tant dans la vitesse d'évolution que dans l'expression clinique du vieillissement.

Personne âgée, pathologies chroniques et traitements chroniques

Le vieillissement de la population s'accompagne d'une augmentation de la vulnérabilité des personnes âgées aux **pathologies chroniques**. En effet, la fréquence des pathologies chronique augmente avec l'âge et tend à croître au fil du temps (Salomon et al., 2012). En France, 91 % des personnes âgées de 75 ans et plus présentaient au moins une maladie chronique en 2019 (Bagein et al., 2022), une proportion appelée à progresser avec le vieillissement démographique, faisant de cette évolution un enjeu majeur de santé publique.

Selon l'OMS, les maladies chroniques sont « des affections de longue durée qui évoluent lentement et nécessitent un traitement et des soins à long terme » (OMS, 2020). Elles figurent aujourd'hui parmi les principales causes de morbidité et de mortalité dans le monde. En France, les maladies cardio-neurovasculaires (32,2 %), le diabète (27,2 %), les tumeurs malignes (19,8 %) et les affections psychiatriques de longue durée (12,4 %) représentent les principales affections de longue durée (ALD) en 2022 (Lastier & Debeugny, 2024).

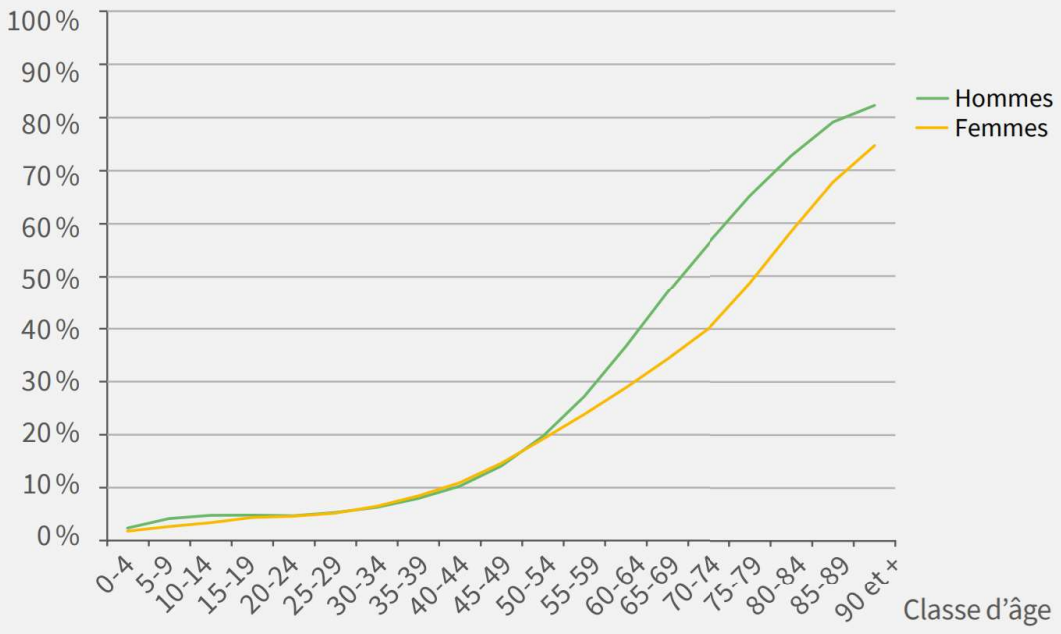
Parmi ces pathologies, **la dépression est particulièrement fréquente** chez les personnes âgées , elle concernerait plus d'une personne âgée sur trois dans le monde (Cai et al., 2023). Par ailleurs, **de nombreuses pathologies chroniques peuvent s'accompagner de douleurs chroniques**, lesquelles sont elles-mêmes fréquemment associées à des symptômes dépressifs. Il est estimé qu'environ 13 % des personnes

âgées présentent simultanément ces deux affections (Zis et al., 2017). En France, la prévalence de la douleur chronique augmente avec l'âge et dépasserait 50 % chez les personnes de plus de 75 ans (Bouhassira et al., 2008). À l'échelle internationale, elle se situe entre 38 % et 50 % selon les pays (LaRowe et al., 2024; Larsson et al., 2017; Pereira et al., 2014). L'intensité de la douleur chronique est par ailleurs associée à la persistance de symptômes dépressifs (Rouch et al., 2025).

Les maladies chroniques impliquent une **prise en charge thérapeutique de longue durée**. En 2022, 13,8 millions de français bénéficiaient du dispositif des affections de longue durée (ALD), soit 20,1% de la population, avec un âge moyen de 64 ans (Lastier & Debeugny, 2024). Cette proportion augmente fortement avec l'âge, atteignant 77 % chez les personnes de plus de 90 ans (**Figure 2**).

FIGURE 2

Pourcentage de la population bénéficiant d'une prise en charge en ALD, selon l'âge et le sexe, en France en 2022 (Lastier & Debeugny, 2024)



Source : Cnam (cartographie des pathologies et des dépenses de l'Assurance Maladie version de juillet 2024 – Tous régimes – France entière)

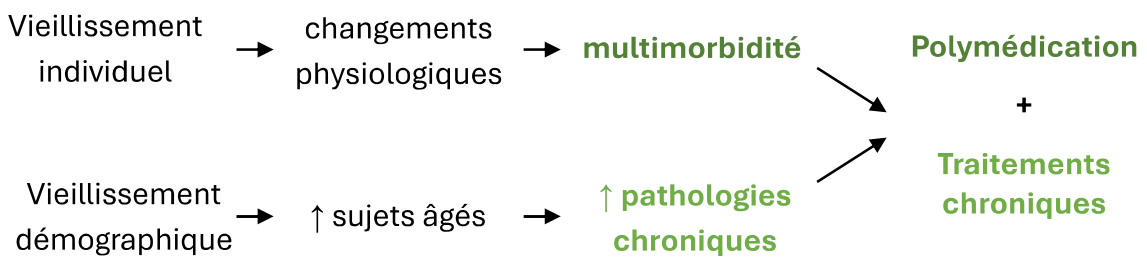
Les projections démographiques soulignent l'ampleur croissante de ces enjeux. Aux États-Unis, l'augmentation attendue de près de 140 % de la population âgée de plus de 80 ans d'ici 2050 devrait s'accompagner d'un triplement du nombre de personnes atteintes d'au moins une maladie chronique (Ansah & Chiu, 2023). Cette augmentation disproportionnée s'explique à la fois par l'apparition plus précoce de certaines pathologies mais aussi par les progrès médicaux qui permettent de vivre plus longtemps, entraînant un allongement de la durée des traitements (Kaczorowski et al., 2016).

Personnes âgées, polypathologie et polymédication

Au-delà de l'augmentation de la prévalence des pathologies chroniques avec l'âge, le vieillissement s'accompagne d'une hausse marquée de la **polypathologie**. En France, 21 % des personnes de plus de 75 ans présentent au moins trois pathologies chroniques (Bagein et al., 2022). Définie comme la coexistence d'au moins deux affections chroniques, la polypathologie concerne 55 à 98 % des personnes âgées et augmente avec l'âge, atteignant plus de 80 % après 85 ans (Marengoni et al., 2011; Salive, 2013). La polypathologie conduit fréquemment à la **polymédication** chez les personnes âgées, qui reçoivent en moyenne entre 5 et 7 médicaments (Halvorsen et al., 2017; Junius-Walker et al., 2007; Tsoi et al., 2014; Wauters et al., 2016). Généralement définie par la prise d'au moins cinq médicaments (Masnoon et al., 2017), la polymédication concerne environ 40 % des personnes âgées dans le monde, avec une prévalence croissante avec l'âge, atteignant près de 50 % après 80 ans (Wang et al., 2024).

POUR RESUMER

Le vieillissement démographique associé aux modifications physiologiques liées à l'âge entraine une augmentation de la prévalence de pathologies chroniques et avec elles, de la polymédication. Ainsi, les conséquences de la consommation de traitements chroniques liée au vieillissement de la population deviennent un réel enjeu de santé publique.



Médicaments et effets indésirables

La iatrogénie médicamenteuse est définie par l'OMS comme « toute réponse néfaste et non recherchée à un médicament survenant à des doses utilisées chez l'homme à des fins de prophylaxie, de diagnostic et de traitement ».

La prévalence des effets indésirables médicamenteux est complexe à établir, largement sous-estimée car insuffisamment rapportés (Hazell & Shakir, 2006) et varie selon le lieu d'étude et la population. **Les effets indésirables médicamenteux sont deux fois plus fréquents chez la personne âgée et leur proportion augmente avec l'âge** (Bégaud et al., 2002; Budnitz et al., 2006). En France, l'étude prospective multicentrique nationale IATROSTAT a estimé à plus de 200 000 le nombre d'hospitalisations annuelles liées à des événements iatrogènes, soit 8,5 %, proportion atteignant 10,6 % chez les patients âgés de 65 ans et plus (Laroche et al., 2023). Aux Etats-Unis, entre 2007 et 2009, près de la moitié des hospitalisations aux urgences pour événement iatrogène, chez les plus de 65 ans, concernaient des personnes âgées de 80 ans et plus (Budnitz et al., 2011).

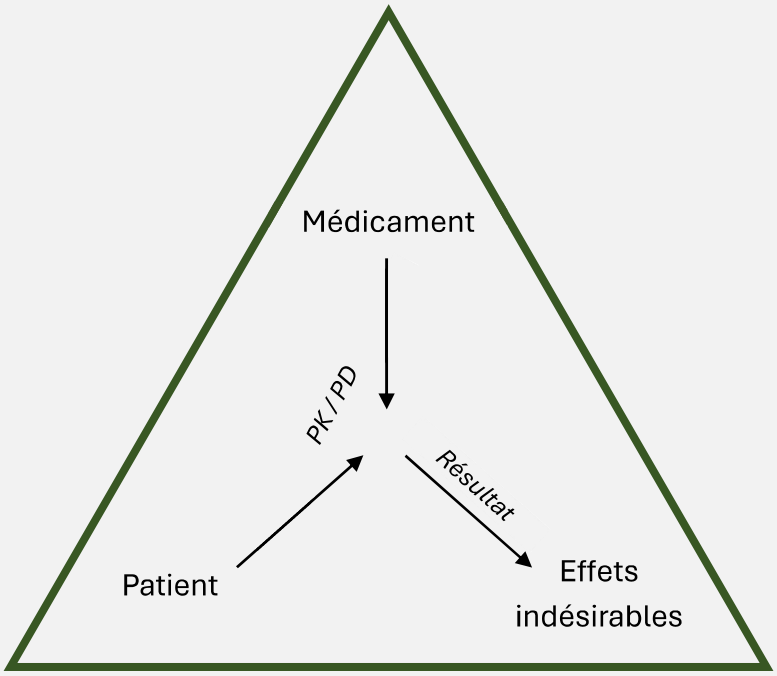
Les effets iatrogènes constituent par ailleurs une cause majeure de complications et de décès. Une étude prospective menée au Brésil a observé que 55 % des admissions hospitalières, chez des patients de 78 ans en moyenne, était due à la survenue d'événements indésirables, avec une mortalité de 41,5 % (contre 22,1 % lorsque l'hospitalisation n'était pas d'origine iatrogénique) (Szlejf et al., 2012).

Chez la personne âgée, **le risque d'événements iatrogènes augmente avec l'exposition prolongée et cumulative aux médicaments**, devenant significatif dès 3 à 4 traitements pris au long cours (Agostini et al., 2004). De plus, la gravité des effets indésirables survenant chez le sujet adulte non polymédiqué est moins importante que chez la personne âgée polymédiquée (Kim et al., 2024). La polymédication ne semble cependant pas suffire à expliquer à elle seule la fréquence plus élevée des événements indésirables chez les personnes âgées (Dubrall et al., 2020).

Ces données confirment que **la survenue d'un effet indésirable résulte de l'interaction entre les caractéristiques d'un médicament et la susceptibilité du patient** (**Figure 3**) et qu'il existe une vulnérabilité accrue des sujets âgés aux médicaments.

FIGURE 3

Relation entre les propriétés pharmacocinétiques (PK) et pharmacodynamiques (PD) d'un médicament et les facteurs individuels du patient dans la survenue d'un effet indésirable (adapté de Ray et al., 2015).



Pharmacologie du médicament et vulnérabilité de la personne âgée

Avec l'avancée en âge, plusieurs fonctions physiologiques subissent des modifications susceptibles d'influencer les paramètres pharmacocinétiques (**Figure 4**) et pharmacodynamiques des médicaments (Mangoni et al., 2025).

La **résorption** des médicaments est globalement peu altérée en termes de quantité absorbée, mais elle **peut être ralentie**. La diminution de la sécrétion d'acide gastrique, responsable d'une élévation du pH gastrique, peut altérer la dissolution des formes galéniques solides, l'état d'ionisation et la solubilité de certaines molécules. Le ralentissement de la vidange gastrique, associé à une diminution du débit sanguin splanchnique et parfois de la surface d'absorption intestinale, peut retarder l'atteinte de la concentration plasmatique maximale. Cependant, la biodisponibilité orale absolue reste généralement inchangée, à l'exception des médicaments soumis à un fort effet de premier passage hépatique (Mangoni et al., 2025; Péhourcq & Molimard, 2004).

La **distribution** des médicaments est affectée par les changements de composition corporelle : diminution de la masse hydrique et de la masse maigre associée à une augmentation de la masse grasse. Ces modifications réduisent le volume de distribution des médicaments hydrophiles (comme le tramadol ou la prégabaline) entrainant des concentrations plasmatiques plus élevées. Pour les médicaments lipophiles (comme la sertraline, la paroxétine ou l'aripiprazole), le volume de distribution est augmenté favorisant une accumulation tissulaire et prolongeant leur demi-vie. Avec l'âge, une diminution de l'albuminémie **accroît la fraction libre des médicaments** fortement liés aux protéines (comme la paroxétine ou la sertraline), favorisant ainsi une accumulation potentiellement toxique à dose inchangée (Mangoni et al., 2025; Péhourcq & Molimard, 2004).

Le **métabolisme hépatique diminue avec l'âge** en raison de la réduction de l'activité enzymatique des cytochromes P450, du volume et du débit sanguin hépatique. La clairance hépatique peut ainsi diminuer de 30 à 40%, prolongeant l'action de certains médicaments, en particulier pour les médicaments à métabolisme hépatique important (comme la paroxétine, la sertraline ou le tramadol), entraînant une augmentation de l'exposition systémique par prolongation de leur demi-vie (Ruscin et al., 2025). Par ailleurs, la réduction du métabolisme hépatique chez le sujet âgé peut diminuer l'effet de premier passage hépatique. Pour les médicaments fortement extraits par le foie, cela se traduit par une augmentation de la biodisponibilité et, par conséquent, du risque de toxicité.

L'**élimination rénale**, principale voie d'excrétion de nombreux médicaments et métabolites (comme la paroxétine, la sertraline et le tramadol), est significativement **réduite** du fait d'une altération de la filtration glomérulaire, d'un ralentissement du débit sanguin rénal et d'une diminution de la masse rénale. La clairance rénale est abaissée, entraînant une prolongation de la demi-vie des médicaments et favorisant leur accumulation (Mangoni et al., 2025; Péhourcq & Molimard, 2004).

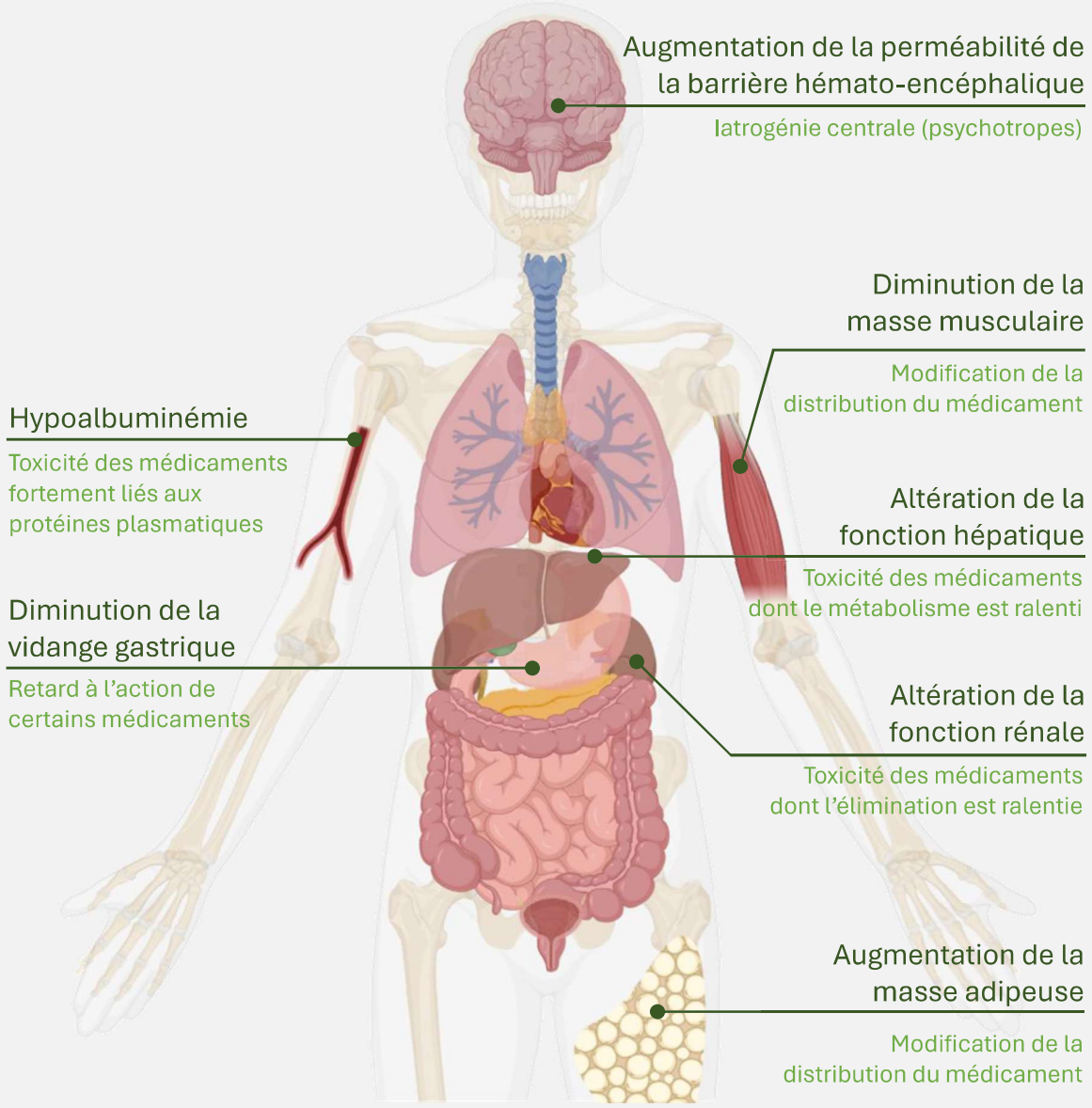
Indépendamment des modifications pharmacocinétiques, le vieillissement s'accompagne d'**altérations pharmacodynamiques** susceptibles, à concentration plasmatique équivalente, de modifier la réponse aux médicaments. Ces différences peuvent résulter à la fois d'une **diminution de la réserve cognitive**, altérant les performances basales du patient, et d'une **augmentation de la sensibilité aux médicaments**. Sur le plan mécanistique, ces altérations pharmacodynamiques résultent de changements de la densité, de l'affinité ou de la sensibilité des récepteurs, ainsi que d'altérations des voies de signalisation intracellulaire (Bowie & Slattum, 2007a).

Par ailleurs, le vieillissement peut favoriser une exposition cérébrale accrue aux médicaments. L'**altération de la perméabilité de la barrière hémato-encéphalique** associée à l'âge peut **majorer la pénétration des substances psychoactives** dans le système nerveux central, potentialisant et prolongeant les effets indésirables cognitifs et neurologiques des psychotropes (Farrall & Wardlaw, 2009).

Le vieillissement est un processus hétérogène entraînant des altérations fonctionnelles variables selon les organes et les individus. La combinaison des modifications pharmacocinétiques et pharmacodynamiques explique la **forte variabilité interindividuelle** de l'exposition médicamenteuse et de la réponse clinique chez le sujet âgé, qui se trouve donc, très certainement, souvent **surexposé au médicament**.

FIGURE 4

Représentation schématique des changements pharmacocinétiques associés à l'âge : effets du vieillissement sur l'absorption, la distribution, le métabolisme et l'excrétion des médicaments.



Conséquences de la consommation de médicaments sur la cognition

Parmi les effets indésirables les plus fréquemment rapportés chez la personne âgée, **ceux affectant le système nerveux central, et en particulier les processus cognitif, occupent une place importante** puisqu'ils représentent entre 13,9 et 18,21 % des effets indésirables (Budnitz et al., 2006; Doucet et al., 2002; Kim et al., 2024). Ces troubles peuvent se manifester de manière aiguë dès l'instauration d'un traitement ou apparaître de façon plus progressive après une exposition prolongée.

La **confusion** est un des syndromes gériatriques aigus les plus fréquents. La confusion peut être définie comme un **trouble neurocognitif aigu, transitoire et fluctuant**, se manifestant par une altération de la conscience, de l'attention et d'autres fonctions cognitives (Ramírez Echeverría et al., 2022). Le tableau clinique est variable et peut inclure des **manifestations psychomotrices**, celles-ci pouvant se présenter sous forme d'hyperactivité, d'hypoactivité ou d'un profil mixte (Mart et al., 2021). Le dépistage repose généralement sur la CAM (Confusion Assessment Method), un questionnaire réalisé en pratique courante évaluant les fluctuations de l'attention, de la pensée et de l'état de conscience (Inouye et al., 1990). Une étude prospective a montré que 26% des patients âgés en hospitalisation aiguë gériatrique présentent un épisode confusionnel (Wierenga et al., 2012). D'après une revue systématique couvrant la période 2004 à 2012, la prévalence de la confusion chez la population âgée varie entre 29 et 64% (Inouye et al., 2014). Les médicaments constituent une des causes principales de survenue de confusion chez les patients âgés (Jia et al., 2025)

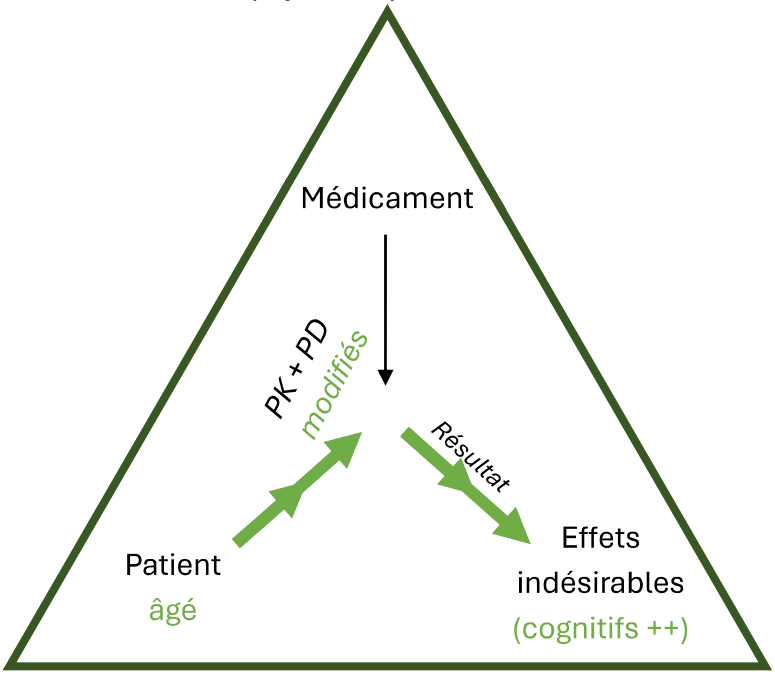
L'**usage chronique** de médicaments psychoactifs est parallèlement associé à un **risque accru de troubles neurocognitifs chroniques** (Barker et al., 2004; Reimers et al., 2025). Une déficience cognitive peut survenir à tout moment du traitement, le risque augmentant avec la durée d'exposition au médicament (Reallon et al., 2024). Concernant l'usage prolongé de médicaments, une étude de cohorte prospective menée en établissements de soins de longue durée aux États-Unis a révélé qu'environ 14 % des résidents présentaient au moins un effet indésirable lié aux médicaments sur 12 mois (Field et al., 2001). Environ un effet indésirable sur quatre est d'ordre neuropsychiatrique (Field et al., 2001; Gurwitz et al., 2005). Les effets les plus fréquemment rapportés

incluent des troubles de l'attention, de la concentration, de la **mémoire**, de la **flexibilité cognitive** ainsi qu'un ralentissement du traitement de l'information (Chippa & Roy, 2025; Reimers et al., 2025).

En résumé, les modifications pharmacocinétiques liées au vieillissement favorisent le risque d'accumulation du médicament et augmentent la vulnérabilité de la personne âgée aux effets indésirables, notamment cognitifs. Cette sensibilité est encore plus marquée pour les médicaments psychotropes, du fait d'une perméabilité accrue de la barrière hémato-encéphalique. Certains médicaments psychotropes sont fréquemment impliqués dans la survenue de troubles cognitifs chez la personne âgée (Chandramouleeshwaran et al., 2024a) et seraient responsables de 31,2 % des effets cognitifs rapportés (Doucet et al., 2002). Aux États-Unis, les médicaments du système nerveux central représentent la première cause (21,4 %) d'effets indésirables médicamenteux chez les patients admis dans un service d'urgences (Budnitz et al., 2006). Ils sont également identifiés comme la principale cause d'effet indésirable chez les personnes âgées présentant un déficit cognitif (Kanagaratnam et al., 2016).

POUR RESUMER

Les modifications pharmacocinétiques (PK) et pharmacodynamiques (PD) liées au vieillissement favorisent l'accumulation médicamenteuse et augmentent la sensibilité du système nerveux central, en particulier aux psychotropes. Ces médicaments sont fréquemment impliqués dans la survenue de troubles neurocognitifs aigus et chroniques, soulignant la vulnérabilité cognitive accrue du sujet âgé face aux médicaments psychotropes.



Médicaments psychotropes et iatrogénie chez la personne âgée

L'usage prolongé de substances psychoactives connaît aujourd'hui une progression notable, notamment chez la personne âgée. En France, **la prescription de psychotropes demeure significativement plus fréquente aux âges avancés** : 27 % de la population âgée de 75 ans ou plus ont eu un traitement psychotrope, contre 17 % chez les 65-74 ans et 9 % pour l'ensemble de la population (Bagein et al., 2022). Compte tenu du risque d'effets indésirables cognitifs associé à ces médicaments, cette tendance suscite des préoccupations croissantes en matière de santé publique. Elle peut avoir des conséquences importantes sur la qualité de vie des patients, en affectant leur mobilité et en favorisant la perte d'autonomie.

Aux Etats-Unis, les antalgiques opioïdes (5,9 % des cas), les antalgiques non opioïdes (3,0 %) et les antidépresseurs et stabilisateurs de l'humeur (2,8 %) représentent les 3 catégories de médicaments psychoactifs les plus fréquemment retrouvés chez les patients admis dans un service d'urgences (Budnitz et al., 2006). Chez les personnes âgées en établissement de soins de longue durée, les opioïdes constituent la première cause d'effets indésirables médicamenteux (6 %), devant les antidépresseurs et les benzodiazépines (5 % chacun) (Gurwitz et al., 2005). D'autres études ont identifié les opioïdes, les antipsychotiques, les anti-infectieux, les antiépileptiques et les antidépresseurs comme facteurs de risque indépendants d'événements indésirables médicamenteux chez les patients âgés vivant en maison de retraite (Field et al., 2001). Les antidépresseurs, anxiolytiques et antipsychotiques figurent, par ailleurs, parmi les trois classes de psychotropes les plus souvent impliquées dans les hospitalisations de patients de 70 ans en gériatrie (Doucet et al., 2002). Parmi les différentes classes pharmacologiques décrites dans ce paragraphe, plusieurs d'entre elles sont souvent suspectées de générer des risques accrus cognitifs chez le sujet âgé, sans qu'il soit réellement possible de distinguer quels médicaments sont plus à risque au sein d'une même classe (Reimers et al., 2025).

Pour ce travail, nous nous sommes intéressés à deux classes particulièrement prescrites chez le sujet âgé susceptibles d'entrainer des effets indésirables cognitifs : les **antalgiques opioïdes** et les **antidépresseurs**. Ces traitements sont fréquemment administrés de manière chronique pour la prise en charge de la douleur persistante et

des troubles dépressifs, deux affections dont la **prévalence augmente avec l'âge** (Thielke et al., 2010; Zimmer et al., 2020).

Tramadol

Le tramadol est un antalgique opioïde de palier II, possédant un mécanisme pharmacologique mixte. En effet, celui-ci combine une activité opioïde avec un effet inhibiteur de la recapture de la sérotonine et de la noradrénaline (Raffa et al., 1992).

Moins stigmatisé que la morphine, du fait de son classement dans le palier II de la classification de l'OMS, il est devenu l'**antalgique opioïde le plus prescrit** en France (Monzon & Nathalie, 2019), en Europe (Hider-Mlynarz et al., 2018) et dans le monde (Bigal et al., 2019). Chez la personne âgée, le tramadol est l'un des antalgiques les plus fréquemment administrés (Brocard et al., 2017; Chenaf et al., 2019), représentant plus de 40% des prescriptions d'opioïdes faibles chez les patients de 65 ans ou plus souffrant de douleur chronique (Bertin et al., 2013). De plus, la proportion de **prescriptions prolongées de tramadol augmente avec l'âge** (Hedenmalm et al., 2019), et la part des traitements d'une durée supérieure à deux ans a doublé au cours des dernières années (Monzon & Nathalie, 2019). Cette évolution illustre une tendance préoccupante à la **chronicisation de l'usage du tramadol chez le sujet âgé.** Pourtant, son double mécanisme d'action lui confère un profil de sécurité plus complexe et plus difficile à anticiper que les opioïdes classiques, surtout chez la population âgée particulièrement vulnérable aux effets indésirables des substances psychoactives.

Selon une étude coréenne évaluant l'association entre polypharmacie et effets indésirables graves chez les patients de 20 à plus de 80 ans, le tramadol était la molécule faisant le plus l'objet de signalements (Kim et al., 2024). Une étude de pharmacovigilance a suggéré un lien entre consommation de tramadol et la survenue de **troubles mnésiques** chez des patients de tout âge (Chavant et al., 2011). Par ailleurs, 14,6 % des notifications d'effets indésirables graves de pharmacovigilance liés au tramadol comportaient une déclaration de **confusion** chez des patients de 18 à 103 ans (Moulis et al., 2017). Cependant, l'imputabilité d'un médicament est souvent difficile à établir chez le sujet âgé du fait des comorbidités et de la polymédication fréquentes dans cette

population. Enfin, une revue récente de la littérature a rapporté que les opioïdes pourraient être responsables de ralentissement du temps de réaction, de diminution de l'attention et des capacités psychomotrices, de troubles de la conscience ou encore de confusion (Reimers et al., 2025). Dans cet article, les effets observés pour les opioïdes sont généralisés au tramadol, sans distinction entre les différentes molécules.

Les antidépresseurs Inhibiteurs Sélectifs de la Recapture de la Sérotonine (ISRS)

La dépression touche une proportion importante de personnes âgées à l'échelle mondiale : 6 % des patients dépressifs ont 60 ans et plus (OMS, 2025b). Les **ISRS** sont largement prescrits dans cette population et recommandés **en première intention** pour le traitement des épisodes dépressifs modérés à sévères (Haute Autorité de Santé, 2017). En 2023, ils représentaient 48 % des antidépresseurs prescrits chez les plus de 60 ans en France, la paroxétine et la sertraline étant **les plus utilisées** (15,5 % et 6,7 % respectivement) (Cognard et al., 2024). En inhibant le transporteur de la sérotonine, les ISRS renforcent la neurotransmission sérotoninergique et se montrent efficaces pour soulager les symptômes dépressifs chez la personne âgée (Tedeschini et al., 2011).

Néanmoins, d'après des recherches récentes, les avantages cliniques de ces traitements devraient être mis en regard des risques potentiels qu'ils pourraient engendrer sur le plan cognitif, notamment chez les patients à risque. Plusieurs études ont rapporté une association entre l'usage d'antidépresseurs et la **confusion** (Heser et al., 2018; Reimers et al., 2025; Wierenga et al., 2012). Parmi les ISRS, la paroxétine apparaît particulièrement impliquée dans ce risque confusiogène, notamment en raison de ses propriétés anticholinergiques (Doucet et al., 2002). Une étude pharmacoépidémiologique utilisant la base mondiale de pharmacovigilance VigiBase®, menée en collaboration avec le service de gériatrie du CHU de Caen, et à laquelle j'ai participé, a mis en évidence une association significative entre les déclarations de confusion et toutes les classes d'antidépresseurs (sauf les inhibiteurs mixtes de recapture de la sérotonine et de la noradrénaline) et au sein de la classe des ISRS, une association spécifique avec la paroxétine, mais pas avec la sertraline (Minoc et al., 2024) (**Annexe 1**). Ces résultats concordent avec ceux d'une étude de cohorte suggérant une

association entre l'usage de paroxétine et la survenue de confusion chez les personnes de 65 ans et plus (Heath et al., 2018).

POUR RESUMER

L'usage des psychotropes est particulièrement fréquent chez la personne âgée et s'accompagne de risques potentiels d'effets indésirables cognitifs aigus et chroniques. Les opioïdes, notamment le tramadol, et les antidépresseurs ISRS, notamment la sertraline et la paroxétine, figurent parmi les médicaments largement prescrits sur le long terme en gériatrie. La littérature suggère une possible association entre ces 3 médicaments et une augmentation du risque d'effets indésirables cognitifs aigus ou chroniques. Cependant, cette relation n'est pas pleinement établie. L'imputabilité d'un médicament dans la survenue de tels effets indésirables est difficile à déterminer chez la personne âgée. Cela s'explique d'une part par la possible confusion avec un déclin cognitif lié au vieillissement, et d'autre part par la polypathologie et la polymédication fréquentes dans cette population.

Chez la personne âgée

Large prescription + Consommation chronique

↗ Risque de survenue d'effets indésirables cognitifs :

- A l'initiation du traitement
- Après exposition chronique

4. LIMITES DES ÉTUDES CLINIQUES ET INTÉRÊT D'UTILISER LE MODÈLE ANIMAL

Limites des études cliniques

L'imputabilité d'un médicament dans la survenue d'un trouble cognitif chez la personne âgée est, à l'heure actuelle, très difficile à évaluer.

Cette difficulté s'explique notamment par les limites des essais cliniques, qui représentent la première source d'information sur les effets indésirables médicamenteux chez l'Homme. En effet, ils s'avèrent davantage conçus pour évaluer le bénéfice thérapeutique que le risque médicamenteux. **La durée des essais cliniques est souvent trop brève** au regard de la durée réelle d'exposition aux médicaments dans la pratique courante (Llewellyn-Bennett et al., 2016). En conséquence, les effets indésirables cognitifs liés à une administration chronique sont rarement évalués, d'autant plus lorsque le traitement est destiné à être poursuivi sur le long terme, voire à vie. En outre, les sujets âgés voire très âgés, sont souvent peu représentés dans les essais cliniques.

Les troubles cognitifs induits par les médicaments peuvent, par ailleurs, **mimer tout trouble cognitif lié à une pathologie ou au vieillissement**, rendant leur identification particulièrement complexe. Ces manifestations peuvent être interprétées à tort comme les symptômes ou l'aggravation d'une pathologie préexistante ou encore comme l'apparition d'une nouvelle affection (Meador, 1998). Cette confusion diagnostique est encore plus vraie dans le cas des personnes âgées **polypathologiques** (Moore & O'Keeffe, 1999).

Enfin, la **polymédication** qui caractérise cette population complique davantage l'attribution causale d'un effet cognitif à un médicament spécifique. Les interactions médicamenteuses, fréquentes dans ce contexte, rendent difficile la distinction entre l'effet d'une molécule isolée et celui d'une combinaison thérapeutique.

Dans cette perspective, le recours à des modèles animaux peut s'avérer particulièrement utile pour isoler et mieux comprendre les effets cognitifs potentiellement délétères d'un médicament suspecté.

4. LIMITES DES ÉTUDES CLINIQUES ET INTÉRÊT D'UTILISER LE MODÈLE ANIMAL

Intérêt de l'utilisation du modèle animal

Face aux limites des essais cliniques chez l'Homme, le recours aux modèles animaux apparaît comme une approche complémentaire pour l'investigation des effets indésirables cognitifs liés aux traitements médicamenteux.

Les modèles rongeurs offrent un contrôle expérimental strict, la possibilité de limiter les facteurs confondants tels que la polymédication et les comorbidités, qui compliquent l'interprétation des résultats chez l'Homme. Cette approche permet ainsi d'**isoler les effets d'un médicament** suspecté d'entrainer des effets indésirables cognitifs.

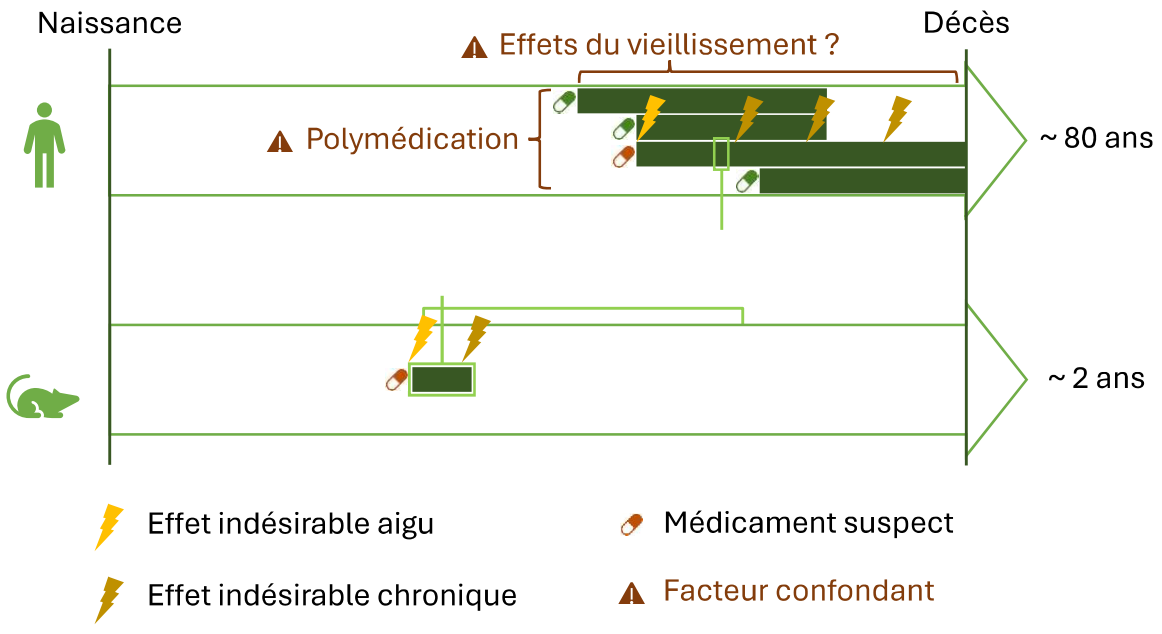
De plus, ces modèles permettent d'**observer les conséquences d'une administration chronique** sur des périodes correspondant à une fraction significative de la durée de vie de l'animal, qui est d'environ 2 ans pour les rats (Cameron et al., 1982). Cela reproduit plus fidèlement l'exposition prolongée des patients âgés aux traitements.

Les rongeurs présentent également des avantages pratiques et éthologiques notables : leur petite taille, leur facilité de manipulation et leur coût favorisent la réalisation d'expérimentations chroniques. Par ailleurs, ils présentent des similitudes importantes avec l'Homme au niveau des grandes fonctions biologiques, telles que la circulation sanguine, le métabolisme ou les fonctions centrales.

Le rat constitue l'une des espèces les plus couramment utilisées en recherche biomédicale en raison de son large répertoire comportemental, permettant d'explorer différents aspects de la cognition. Sa taille supérieure à celle de la souris offre un volume sanguin suffisant pour réaliser des prélèvements répétés et permet des analyses biologiques et pharmacocinétiques.

POUR RESUMER

L'imputabilité d'un médicament dans la survenue de troubles cognitifs chez la personne âgée est difficile à établir en raison de la polymédication, du risque de confusion avec les troubles liés au vieillissement ou à des pathologies associées mais aussi parce que la durée d'exposition au médicament dans les essais cliniques est largement inférieure à la durée d'exposition dans la vraie vie. Les modèles animaux permettent de palier à une partie de ces limites en isolant les effets d'un médicament pour une exposition chronique qui représente une part importante de la vie du rongeur.



Le tramadol et la paroxétine partagent plusieurs caractéristiques : une prescription fréquente et un usage souvent prolongé chez le sujet âgé, mais également des mécanismes d'action proche impliquant des composantes sérotoninergiques et anticholinergiques. Ils sont par ailleurs décrits dans la littérature comme plus à risque de générer des effets indésirables cognitifs aigus et chroniques chez les sujets âgés. Afin de préciser la genèse de la survenue d'effets indésirables cognitifs chez le sujet âgé et le type de processus cognitifs impactés, nous avons réalisé deux études chez le rat, en utilisant ces deux médicaments ainsi que la sertraline (à titre de comparaison car moins décrite que la paroxétine comme étant associé à des risques de troubles cognitifs).

L'objectif de cette thèse est d'**évaluer les effets cognitifs aigus et chroniques** associés à la consommation de trois substances psychoactives dans un modèle de rat surexposé : le **tramadol**, la **paroxétine** et la **sertraline**.

En s'appuyant sur les observations issues de la pratique clinique, ce travail vise, pour chacune de ces substances, à :

- **Evaluer les effets aigus**, de type confusion, survenant après instauration du traitement ;
- **Evaluer les effets chroniques**, à différents temps d'exposition, sur plusieurs processus cognitifs ;
- **Mettre en relation les effets comportementaux observés avec l'exposition** à ces en substances actives (par mesure des **concentrations plasmatiques**), après administration aiguë et chronique.

La mise en œuvre de protocoles d'administration chronique chez l'animal soulève des questions éthiques et méthodologiques, l'expérimentateur cherchant à rester au plus près de la réalité clinique tout en respectant le bien-être animal. Ainsi, au cours de cette thèse, un travail de développement méthodologique a été mené pour **concevoir et valider une méthode d'administration alternative**, plus adaptée à l'étude des administrations de substances en chroniques et moins stressante pour les animaux, tout en maintenant la validité scientifique attendue.

La partie *RESULTATS* de cette thèse sera présentée sous la forme de trois articles, correspondants à ces objectifs :

- ARTICLE 1 : Cognitive alterations after acute and chronic administration of tramadol in male Sprague-Dawley rats.

- ARTICLE 2 : Cognitive consequences of acute and chronic administration of SSRI antidepressants in male rats.

- ARTICLE 3 : Micropipette-guided Drug Administration (MDA) as a non-invasive chronic oral administration method in male rats.

MATÉRIELS ET MÉTHODES

1. PARAMÈTRES EXPÉRIMENTAUX UTILISÉS

Cette partie vient compléter les éléments de « Matériels et Méthodes » présentés dans les publications associées. Pour l'ensemble des expérimentations, **le même modèle animal a été utilisé, hébergé et traité dans des conditions identiques**.

Dans cette partie, nous avons souhaité justifier les choix du modèle animal, des doses administrées ainsi que de la méthode d'administration, en nous appuyant sur une comparaison avec leurs équivalents chez l'Homme, afin de **souligner la pertinence translationnelle** des résultats obtenus. De même, cette section vise à argumenter la pertinence des tests comportementaux employés, en précisant les fonctions cognitives évaluées et leur équivalence chez l'Homme.

Le protocole comportemental développé dans le cadre de cette thèse est original puisqu'à notre connaissance, il s'agit de la **première approche combinant une évaluation cognitive avec la mesure de l'exposition systémique** aux substances actives, à la fois **en condition aiguë et chronique**.

Des prélèvements sanguins ont donc été réalisé en aigu et en chronique, pour mettre en relation l'exposition systémique au médicament avec d'éventuelles altérations cognitives.

Par ailleurs, nous avons fait le choix d'évaluer les effets comportementaux à distance du pic plasmatique, de manière à s'affranchir des effets aigus et à étudier spécifiquement les effets résiduels liés à une exposition prolongée. Les administrations des substances actives étaient donc réalisées la veille des tests comportementaux (entre 16 h et 18 h), ces derniers ayant lieu le lendemain matin. À notre connaissance, ce mode d'administration, appliqué dans le cadre de protocoles chroniques, n'avait encore jamais été mis en œuvre pour les substances étudiées.

Enfin, les informations détaillées relatives au nombre d'animaux utilisés, aux protocoles expérimentaux, ainsi qu'aux différents tests comportementaux utilisés sont présentées dans chaque publication (voir partie *RESULTATS*).

1. PARAMÈTRES EXPÉRIMENTAUX UTILISÉS

Choix du modèle d'étude

Toutes les expérimentations ont été menées chez des rats mâles Sprague-Dawley adultes. La **souche Sprague-Dawley,** non consanguine et albinos, a été choisie en raison de son utilisation répandue (Gileta et al., 2022). Ce choix permet une comparaison directe de nos résultats avec les nombreuses données comportementales et pharmacocinétiques déjà publiées dans la littérature.

L'emploi exclusif d'animaux **mâles** visait à réduire la variabilité interindividuelle, garantissant à la fois une puissance statistique optimale et une meilleure comparabilité avec les études antérieures.

Durant la période de traitement, nos rats étaient âgés de 8 à 14 semaines. Même s'il est difficile de calculer une équivalence chez l'Homme, à cet âge, les rats sont considérés comme adultes (Andreollo et al., 2012). Le recours à des **rats adultes** répondait à la volonté d'éviter les biais associés au vieillissement tels que le déclin progressif des capacités cognitives (Robitsek et al., 2008), de l'activité locomotrice et du comportement exploratoire (Altun et al., 2007) ainsi que la diminution de la motivation ou la prise de poids excessive. Tous ces facteurs sont susceptibles d'altérer les performances comportementales et d'introduire une hétérogénéité non souhaitée dans les résultats. Par ailleurs, les rats âgés étant plus sensibles aux effets du stress (Sapolsky et al., 1987), l'utilisation d'animaux adultes permettait de limiter les effets confondants liés à ce paramètre.

Le choix d'utiliser des rats Sprague-Dawley mâles adultes a donc été justifié par la volonté de **simplifier** le modèle expérimental, d'assurer sa **comparabilité** avec les études existantes et de maîtriser au mieux l'**interprétation** des résultats obtenus.

Choix des doses étudiées

Comme indiqué dans l'introduction, les modifications pharmacocinétiques et pharmacodynamiques associées au vieillissement peuvent favoriser le risque d'accumulation systémique du médicament (Jansen & Brouwers, 2012). Cette observation permet de formuler l'hypothèse selon laquelle, **de nombreuses situations**

incluant la prise chronique médicamenteuse et la polymédication, conduiraient la personne âgée à être très souvent en situation de surexposition au médicament favorisant la survenue d'effets indésirables, notamment cognitifs. Afin de reproduire expérimentalement cette situation, le tramadol, la paroxétine et la sertraline ont été administrés à des doses suprathérapeutiques chez le rat adulte. Cela nous permet d'étudier spécifiquement les effets de la surexposition au médicament tout en évitant les biais liés à l'âge.

Les **doses maximales journalières recommandées** chez l'Homme ont été utilisées comme référence, soit respectivement 400mg pour le tramadol (*Base de Données Publique Des Médicaments*), 60 mg pour la paroxétine (*Base de Données Publique Des Médicaments*) et 200 mg pour la sertraline (*Base de Données Publique Des Médicaments*). A partir de ces valeurs, nous avons calculé les doses équivalentes chez l'animal selon la **formule d'équivalence inter-espèces** proposée par la Food and Drug Administration (FDA, 2005) :

$$Human\ Equivalent\ Dose\ (mg/kg) = \text{animal dose (mg/kg) x} \left(\frac{\text{animal weight (kg)}}{\text{human weight (kg)}}\right)^{0,33}$$

Cette équation tient compte du poids corporel moyen de l'Homme (70 kg) et de celui du rat adulte (0,5 kg), l'exposant 0,33 étant applicable pour des animaux d'un poids supérieur à 460 g, ce qui était le cas pour notre étude. Les doses équivalentes obtenues chez le rat étaient de 29,2 mg/kg pour le tramadol, 4,4 mg/kg pour la paroxétine et 14,6 mg/kg pour la sertraline. Cette approche ne tient cependant pas compte de certains paramètres individuels (âge, surface corporelle) ni des propriétés pharmacocinétiques spécifiques de chaque substance (volume de distribution). Elle constitue néanmoins une première estimation utile pour orienter le choix des doses expérimentales.

Sur la base de ces estimations, une recherche bibliographique a été menée afin d'identifier les posologies couramment utilisées dans les études précliniques. Pour le tramadol, les doses administrées chez le rat se situent autour de 20 mg/kg par voie intrapéritonéale (IP) (Hosseini-Sharifabad et al., 2016; Mehdizadeh et al., 2017; Mowaad et al., 2022) et 50 mg/kg par voie orale (Baghishani et al., 2018). Etant donné que l'administration IP induit des concentrations plasmatiques environ deux fois supérieures

à celles obtenues par voie orale (Evangelista Vaz et al., 2018), une dose orale équivalente doit être environ doublée pour atteindre des expositions comparables. Ainsi une dose orale de **50 mg/kg a été retenue comme dose suprathérapeutique**. La dose de **25 mg/kg**, correspondant à la moitié de cette valeur a été utilisée à titre de comparaison comme **condition thérapeutique**, soit entre 200 et 250 mg par jour chez l'Homme. Des données pharmacocinétiques rapportées dans la littérature pour une administration orale de tramadol de 20 mg/kg ont été utilisées pour établir une comparaison (Bao et al., 2023).

Concernant les antidépresseurs, les doses le plus souvent rapportées dans la littérature se situent entre 10 et 20 mg/kg pour la paroxétine (Ai et al., 2020; Han et al., 2015; Li et al., 2017; Myint et al., 2007; Tao et al., 2016) comme pour la sertraline (García-Gutiérrez et al., 2023; Melis et al., 2012; Sanchez et al., 2014; Tao et al., 2016). Conformément à ces données et en maintenant une posologie supérieure pour la sertraline par rapport à la paroxétine, conformément aux usages cliniques, des doses de **10mg/kg pour la paroxétine** et **16mg/kg pour la sertraline** ont été retenues. Ces dernières doses ont déjà été étudiées dans des études pharmacocinétiques chez le rat et permettaient donc d'envisager une comparaison (Li et al., 2017; Melis et al., 2012).

Choix du mode d'administration

Dans le but de reproduire au mieux les conditions de la prise médicamenteuse au long cours chez l'Homme, nous avons opté pour une méthode d'administration orale, celle-ci représentant la voie d'administration la plus couramment utilisée chez l'Homme (80% des formes pharmaceutiques distribuées) (*Les différentes formes de médicaments*, Vidal, 2021). Chez le rongeur, l'**administration orale est le plus souvent réalisée par gavage**. Cette technique présente plusieurs avantages, notamment un **contrôle précis de la dose et du moment de l'administration**. Toutefois, le gavage comporte également des inconvénients, surtout dans le cadre de protocoles chroniques où **la répétition des administrations accroît le risque de complications**. En plus des risques de lésions du tractus oro-gastrique (Brown et al., 2000), le gavage peut entraîner un stress chez les animaux puisque cette technique nécessite une contention de l'animal (Stuart &

Robinson, 2015). Ce stress a été observé dès les premières administrations dans nos propres expérimentations.

En parallèle de nos protocoles portant sur les psychotropes, nous avons donc cherché à mettre en place une méthode d'administration alternative, à la fois en adéquation avec la pratique clinique mais également plus respectueuse du bien-être animal. En réponse aux contraintes éthiques croissantes de la recherche animale, plusieurs travaux ont proposé des **approches alternatives** sans contention, à l'aide d'aliments appétents (Abelson et al., 2012; Küster et al., 2012; Teixeira-Santos et al., 2021). Ces méthodes nécessitent cependant plus de préparation et un isolement de l'animal pour s'assurer de la consommation de la dose dans son entièreté. En outre, le délai de consommation est souvent plus long.

Une autre approche fréquemment rapportée dans la littérature consiste à ajouter le principe actif directement dans l'**eau de boisson** mise à disposition des animaux. Au sein de notre équipe, j'ai participé à une étude de bioéquivalence utilisant cette méthode d'administration pour l'aripiprazole, un antipsychotique utilisé dans certains protocoles au sein de notre laboratoire (Chofflet et al., 2025) (**Annexe 2**). Le calcul de la posologie repose alors sur la consommation hydrique quotidienne moyenne, ajustée en fonction du poids corporel. Cette technique, ne nécessitant aucune contention, est non invasive, non stressante et peu chronophage. Cependant, certaines substances sont peu stables dans l'eau, d'autres peuvent modifier la consommation d'eau des animaux et ce mode d'administration continu **ne reflète pas la cinétique de l'administration orale humaine**, à doses uniques quotidiennes (Turner et al., 2011).

Afin de pallier à ces limites et de se rapprocher davantage des conditions d'une administration orale chez l'Homme, nous avons proposé une méthode alternative reposant sur le principe de l'**administration par micropipette**, récemment décrite chez la souris (Schalbetter et al., 2021). Cette méthode innovante et non invasive utilise un excipient appétent pour inciter l'animal à **consommer volontairement** la solution contenant le composé pharmacologique. Dans la perspective d'améliorer le bien-être animal et d'intégrer cette approche au sein du laboratoire COMETE, nous avons entrepris d'**adapter cette méthode au rat**. Bien que cette méthode n'ait pu être appliquée dans le cadre des protocoles relatifs au tramadol et aux antidépresseurs, elle s'inscrit dans les

interrogations actuelles sur le bien-être animal et sera intégrée dans de futurs travaux de l'équipe, concernant l'étude des effets de la prise chronique médicamenteuses.

Les résultats de cette méthode sont présentés dans l'article 3 intitulé : Micropipette-guided Drug Administration (MDA) as a non-invasive chronic oral administration method in male rats.

2. TESTS COMPORTEMENTAUX UTILISÉS

Evaluation du comportement de type confusion

L'évaluation des effets de type confusion induits par l'administration d'une substance pharmacologique chez l'animal demeure peu documentée dans la littérature. Une telle évaluation présente plusieurs difficultés méthodologiques. D'une part, le **moment d'apparition** des effets confusiogènes est imprévisible et d'autre part leurs **manifestations comportementales** ne sont pas clairement établies chez l'animal. Comme évoqué en introduction, la confusion chez l'Homme correspond à un état transitoire et fluctuant, pouvant se traduire par une altération psychomotrice se manifestant sous forme d'hypo- ou d'hyperactivité.

Afin d'explorer ces manifestations chez le rongeur, nous avons choisi d'utiliser un **test d'actimétrie** (**Annexe 3**) pour plusieurs raisons. Contrairement à la plupart des tests comportementaux effectués à un instant donné, l'actimétrie offre un **suivre continu** de l'activité locomotrice sur 24 heures, permettant d'observer d'éventuelles **fluctuations** au cours du temps. Réalisé dans des cages individuelles, ce test permet de mesurer **l'activité locomotrice**, paramètre qui peut être perturbée lors des manifestations de la confusion chez l'Homme. De plus, l'activité des rats est soumise à un rythme circadien, dont de légères perturbations au cours du temps peuvent refléter un comportement de type confusion. Ce test nous a donc sembler constituer un outil pertinent pour détecter d'éventuelles variations de l'activité locomotrice susceptibles de traduire un état de type confusionnel.

Le test d'actimétrie a été réalisé immédiatement après les premières administrations des substances psychotropes testées, donc en situation aiguë. Concernant les antidépresseurs, les données issues de la littérature chez l'Homme suggéraient, plus encore que pour le tramadol, la survenue de syndromes confusionnels lors de l'initiation du traitement. En conséquence, nous avons répété le test après plusieurs jours d'exposition aux antidépresseurs, afin d'évaluer la persistance ou l'évolution de ces effets au cours du temps.

2. TESTS COMPORTEMENTAUX UTILISÉS

Evaluation des performances d'apprentissage et de mémoire

Plusieurs fonctions cognitives sont susceptibles d'être altérées à la suite d'une exposition chronique à des substances psychoactives telles que le tramadol, la paroxétine ou la sertraline. Afin d'examiner ces effets, nous avons réalisé chez le rat, un **ensemble d'évaluations comportementales** permettant d'explorer **différents processus cognitifs**, notamment l'apprentissage, la flexibilité cognitive et la mémoire. Les tests choisis évaluent des fonctions cognitives homologues à celles observées chez l'Homme et sont **largement décrits dans la littérature**.

Dans le déclin cognitif lié à l'âge, la **mémoire épisodique** est particulièrement vulnérable (Tromp et al., 2015). Le protocole expérimental s'appuie donc sur l'hypothèse selon laquelle **la mémoire épisodique constitue la première fonction mnésique susceptible d'être altérée** tant par le vieillissement que par l'exposition chronique à des psychotropes. Cette forme de mémoire est définie comme la capacité à se remémorer des événements passés et leur contexte spatio-temporel (Tulving, 2002).

Dans cette perspective, nous avons combiné deux tests complémentaires ciblant des fonctions cognitives distinctes mais étroitement liées. Le test du **labyrinthe aquatique de Morris** (**Annexe 4**) est classiquement utilisé pour évaluer les **capacités d'apprentissage, de mémoire et d'orientation spatiale**, fonctions fréquemment altérées au cours du vieillissement (Costa et al., 2020). Ce test a été répété avec une modification de la règle d'apprentissage, afin d'évaluer la **flexibilité cognitive**, c'est-à-dire la capacité à inhiber une stratégie préalablement acquise pour en adopter une nouvelle. La **flexibilité cognitive** fait partie des fonctions cognitives les plus atteintes avec le vieillissement, elle est essentielle à l'adaptation aux situations nouvelles ou complexes (Collette, 2019).

Le **test de reconnaissance d'objet** mesure la **mémoire de reconnaissance** (**Annexe 5**). Selon les paramètres expérimentaux choisis, il peut renseigner sur la mémoire à court terme ou à long terme. Cette approche offre ainsi la possibilité d'examiner à la fois la rétention et la consolidation mnésique, en mobilisant de manière variable les mécanismes de stockage à long terme.

2. TESTS COMPORTEMENTAUX UTILISÉS

L'évaluation conjointe de ces deux tâches, réalisée à **différents temps d'exposition aux substances testées**, permet d'appréhender l'**évolution temporelle des altérations cognitives** induites par les traitements chroniques.

RÉSULTATS

Cognitive alterations after acute and chronic administration of tramadol in male rats

Cognitive effects of tramadol exposure in rats

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ARTICLE 1 – Cognitive alterations after acute and chronic administration of tramadol in male Sprague-Dawley rats.

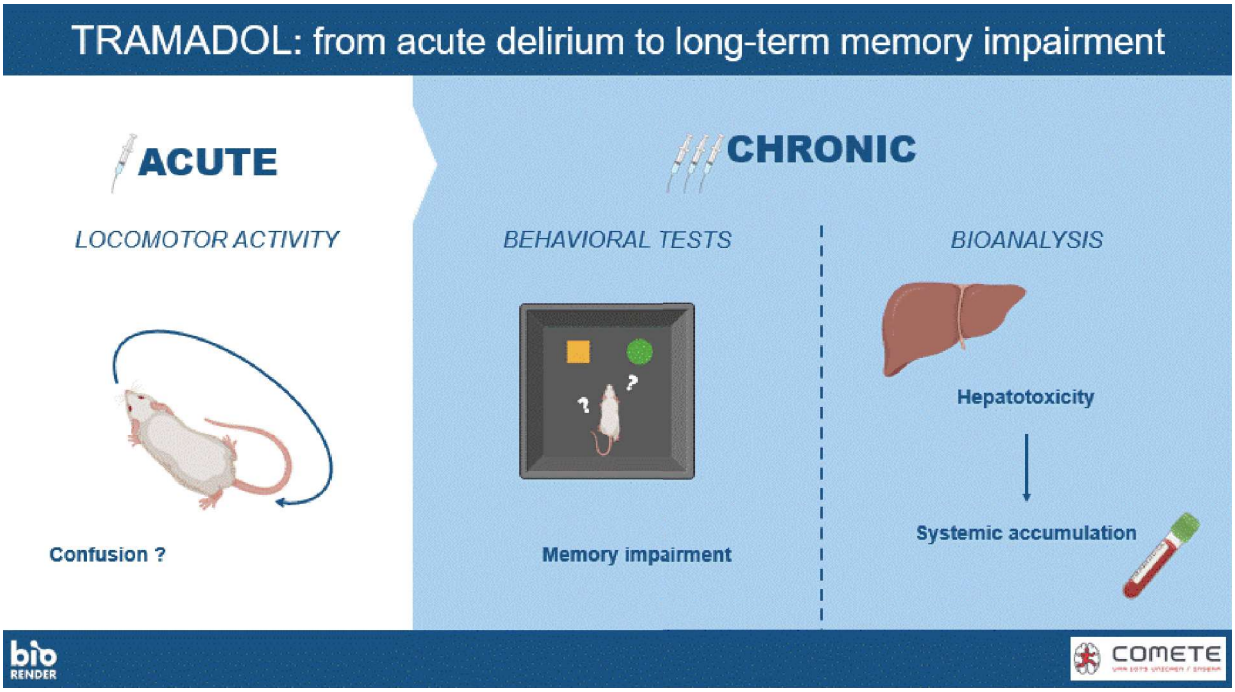
ABSTRACT:

Tramadol, a widely prescribed opioid analgesic, is often described as having cognitive adverse effects. However, the characterization of these short- and long-term effects and the relationship to tramadol exposure remains unclear. This study aimed to investigate the relationship between systemic tramadol exposure and cognitive outcomes of acute and chronic tramadol exposure in Sprague-Dawley rats. Animals received daily oral gavage of tramadol (25 or 50mg/kg) or vehicle for six weeks. Acute effects were evaluated via 24-hour locomotor activity following the first dose. Long-term cognitive performances were assessed using the Morris Water Maze (spatial memory) and the Novel Object Recognition Test (recognition memory), conducted 16h post-administration to minimize acute drug effects. Acute and chronic exposure to tramadol and liver function were evaluated respectively by measuring plasma concentrations of tramadol and its active metabolite O-desmethyltramadol, and hepatic transaminases (AST and ALT). Tramadol induced dose-dependent changes in locomotor activity, with hypoactivity at low dose and hyperactivity at high dose. While spatial memory remained unaffected after prolonged treatment, recognition memory was significantly impaired in both tramadol-treated groups, with deficits persisting up to six weeks after treatment cessation. Biochemical analysis revealed increases in hepatic transaminases associated with drug accumulation following chronic administration. These findings highlight that tramadol may induce acute behavioral perturbations that may reflect the delirium described in older adults. Chronic exposure may lead to cognitive deficits due to a drug accumulation and hepatic dysfunction. These results raise concerns regarding the long-term use of tramadol and the risks of overexposure, especially in vulnerable individuals.

KEY WORDS: behavior, animal, cognition, locomotor activity, memory, pharmacokinetics

ARTICLE 1 – Cognitive alterations after acute and chronic administration of tramadol in male Sprague-Dawley rats.

GRAPHICAL ABSTRACT:



1. INTRODUCTION

Tramadol is a centrally acting opioid analgesic primarily prescribed for moderate-to-severe pain in both acute and chronic conditions (Roulet et al., 2021). Its dual mechanism of action, combining mu-opioid binding activity with inhibition of norepinephrine and serotonin reuptake allows it to target mixed nociceptive and neuropathic pain, contributing to its widespread use (Raffa et al., 1992). In 2015, tramadol was one of the most commonly used mild opioids in Europe (Hider-Mlynarz et al., 2018) and the United States (Bigal et al., 2019), with prescription rates particularly high among older adults (Brocard et al., 2017; Chenaf et al., 2019; Hedenmalm et al., 2019). This trend is concerning, as tramadol is increasingly prescribed long-term in this population (Warner et al., 2022).

Older individuals are particularly vulnerable to iatrogenic adverse events due to comorbidities, polymedication and age-related pharmacokinetic and pharmacodynamic variability (ElDesoky, 2007). Compared with younger adults, changes in pharmacokinetic can result in systemic drug accumulation at equivalent administrated doses (Kaye et al., 1989). Consequently, chronic treatment in the elderly may results in drug overexposure, especially for psychoactive substances, partly attributable to age-related alteration of the blood-brain-barrier (Jansen and Brouwers, 2012). Clinical studies have suggested that both short and long-term tramadol use in older adults may be associated with cognitive disturbances, ranging from acute delirium to impairments in memory, language and attention, potentially related to its anticholinergic properties (Attoh-Mensah et al., 2020; Bassiony et al., 2017). However, interpretation of causality is complicated by confounding clinical factors such as underlying comorbidities or drug-drug interactions.

In this context, animal models may provide a valuable approach for characterizing the occurrence of adverse effects following drug administration, in controlled experimental conditions. Tramadol effects on cognitive capabilities have already been investigated in rodents following acute administration. In mice, a single dose (2.5 and 5mg/kg, IP) administered 30min before passive avoidance test led to a dose-dependent memory impairment (Jafari-Sabet et al., 2018). In Novel Object Recognition Test (NORT), Wistar rats receiving a single dose (20 mg/kg, IP) 90min before acquisition showed

impaired discrimination performances (Hosseini-Sharifabad et al., 2016a). Similarly, chronic tramadol treatment also demonstrated long-lasting deficits in recognition memory (Adekomi et al., 2019; Hosseini-Sharifabad et al., 2016a) and spatial memory and learning, with reduced time in the Morris Water Maze (MWM) target quadrant and increased escape latency (Adekomi et al., 2019; Baghishani et al., 2018; Mehdizadeh et al., 2017). However, most studies reporting memory impairment present two major methodological biases. First, reliance on single tests limits cognitive assessment. Second, behavioral testing is often conducted shortly after the last administration, making it difficult to distinguish acute effects related to peak plasma concentration from long-term effects occurring at steady state. This distinction is particularly relevant given that tramadol's half-life in rat is approximately 142min, but may be prolonged under conditions of chronic treatment (Sheikholeslami et al., 2016). Hosseini-Sharifabad and colleagues (Hosseini-Sharifabad et al., 2016a) and Mehdizadeh and colleagues (Mehdizadeh et al., 2017), conducted behavioral tests, respectively 90 and 30minutes after chronic administration protocol (20mg/kg/d, 21d, IP and 20 to 80mg/kg/d, 30d, IP). Interestingly, Adekomi and colleagues investigated the effects of tramadol 24 hours after the last dose of a 30-day treatment. In this study, authors used high doses (60mg/kg, SC, twice daily) to mimic tramadol misuse and highlighted cognitive alterations (Adekomi et al., 2019). Finally, cognitive impairments were also reported by Baghishani and colleagues and Mowaad and colleagues (respectively, 50mg/kg/d, OG for 28 days and 20mg/kg/d, IP for 2 months), but without specifying the administration-to-testing interval (Baghishani et al., 2018; Mowaad et al., 2022). Furthermore, none of these studies investigated the relationship between the observed cognitive effects and the pharmacokinetic data concerning tramadol after chronic administration. Consequently, to our knowledge, no data is available in rats regarding steady-state plasma concentrations of tramadol under chronic treatment conditions.

With an original design, this study aimed to model in adult rats the drug overexposure that occurs in elderly after chronic administration and to investigate alterations in short- and long- term cognitive processes associated with this overexposure. A further objective was to establish a relationship between pharmacokinetic parameters and behavioral outcomes following acute and chronic

tramadol administration at both therapeutic and supratherapeutic doses. We used a combination of widely used behavioral tests such as MWM and NORT to enable a comprehensive evaluation of cognitive functions while reducing biases inherent in single-test approaches.

2. METHODS

2.1. Animals

Sprague-Dawley rats (200-300g, 6 weeks old) were used for this study (Janvier Labs, France). To minimize confounding effects associated with age-related cognitive decline, the study was conducted in healthy adult rats subjected to an overexposure dose. This experimental choice also avoided the practical limitations inherent to behavioral testing in aged animals. Animals were allowed one-week acclimatization period and were randomly housed by four under a 12h light-dark cycle (lights on from 07:00 to 19:00). Water was provided *ad libitum* and food intake was restricted to 20g/rat/d to prevent excessive weight gain. Cage enrichment consisted of crinkle-cut shredded paper and a cardboard roll. Rats were handled daily (5min per day, 5 days) prior to experimentation. Experimental procedures were performed in accordance with the European Communities Council Directive (2010/63/UE) and approved by the regional ethical committee (Comité d'Ethique Normandie en Matière D'EXpérimentation Animale, CENOMEXA) (agreement number #43198). Animal studies are reported in compliance with the ARRIVE guidelines.

2.2. Pharmacological agent

Tramadol hydrochloride was obtained from injectable vials (100mg/2mL, CONTRAMAL®), extracted and lyophilized by the CERMN (Centre d'Études et de Recherche sur le Médicament de Caen Normandie).

2.3. Drug treatment schedule and experimental design of behavioral study

Before experimental (W-1), rats were randomly divided into 3 groups. From W1 to W6, they received daily oral gavage (OG) of tramadol 25mg/kg (TRAM25), tramadol 50mg/kg (TRAM50) or vehicle (control) (n=8/group) (**Fig. 1.**). Doses were determined based on clinical practice using Human Equivalent Dose (HED) calculation (Nair and Jacob, 2016). The 25mg/kg dose corresponds to a HED of 200-250mg (common daily human dose). The 50mg/kg dose, corresponding to a human equivalent dose (HED) exceeding 400 mg—the maximum recommended daily dose in humans—was selected to model overexposure. Tramadol was dissolved in saline. Flexible plastic probes (PHYMEP®) were used for gavage refinement (Õkva & Tamos, 2006), with a fixed volume of 1.5mL/kg. Groups were alternated during manipulations to avoid timing bias.

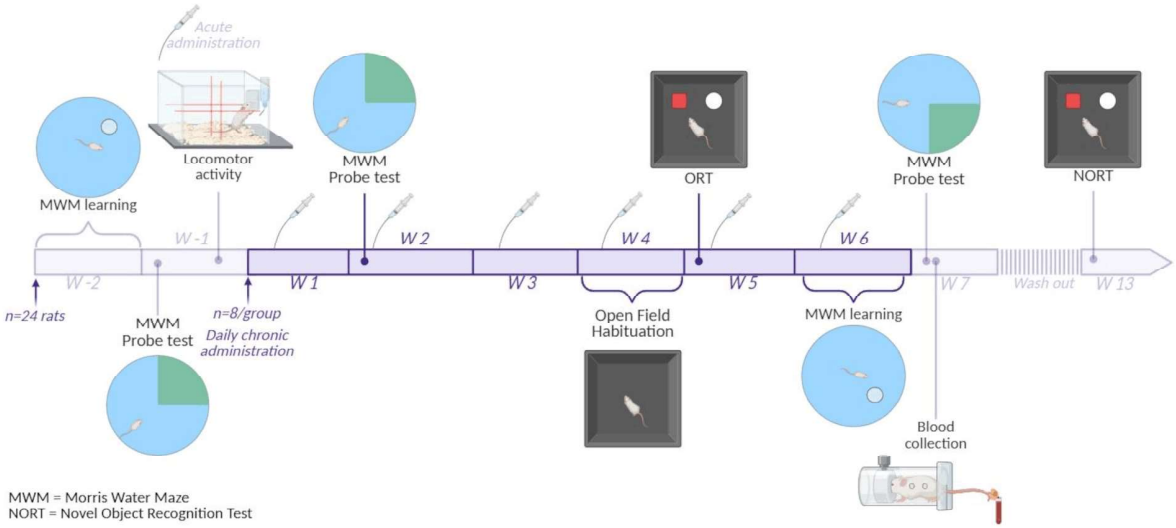


Fig. 1. General experimental design (created in Biorender.com)

OG was consistently administered in the late afternoon (16:00 to 18:00) to dissociate behavioral effects from acute peak drug levels. Behavioral testing occurred the following morning (09:00 to 12:00); ensuring a ≥16h interval between dosing and assessment. The final tramadol dose was administered in W6, one day before the final MWM probe test. A 6-week washout period preceded the NORT.

2.4. Behavioral tests

Behavioral testing was performed in twenty-four rats (n=8 per group) by an experimenter blinded to treatment group. Apparatuses were cleaned with 70% ethanol between animals, and rats were acclimated to the testing environment for 30 min prior to each test.

2.4.1. Infrared Actimeter system

Locomotor activity was assessed using Imetronic® (Pessac, France), as we previously described (Rizzolo et al., 2021). Actimeters consisted of plastic cages (33x21x19cm3) surrounded by two horizontal barrels with infrared beams (6.5cm and 16.5cm above the floor, respectively). The first OG administration of either saline or tramadol (25 and 50mg/kg) was performed around 6pm, rats were placed for 24h in the actimeter.

Recorded parameters included: (i) number of rearings, (ii) front and back activity, (iii) back-and-forth activity (see **Supplementary Fig. 1 and 2** for total horizontal and vertical activity). Data were sampled every 30min for 24h. Circadian rhythms parameters were determined using the cosinor.online application: cosinor, MESOR (Midline-Estimating Statistic Of Rhythm), amplitude (difference between the peak and the mean value of the wave) as well as acrophase (time at which the peak of rhythm occurs) (Molcan, 2023).

2.4.2. Morris Water Maze test (MWM)

Spatial learning was assessed using the MWM (Morris, 1984) and analyzed through video-tracking system (EthoVision XT, Noldus®). The initial session of MWM learning (W-2) and probe test (W-1) occurred prior to randomization (**Supplementary Fig. 3**).

Learning: rats underwent four daily trials for five consecutive days, with 60s inter-trial intervals. Rats failing to reach the platform within 120s were guided to it and remained for a 30s reinforcement period. Escape latency in seconds and total distance traveled were measured. Two MWM sessions (W-2 and W6) were performed, with the

platform located in the NE and SE quadrants respectively; distal visual cues were modified in the second session.

Probe test: with the platform removed, rats were tested for 60s, and time in the target quadrant was recorded. Probe tests were conducted 3 days after the last acquisition trial in W-1 and W7, and 17 days after the last acquisition trial in W2.

2.4.3. Novel Object Recognition Test (NORT)

Recognition memory was assessed using the NORT, as previously described (Heraudeau et al., 2023). Animal behavior was recorded and analyzed through video-tracking system (EthoVision XT, Noldus®).

Habituation: one-week prior testing, rats were acclimatized to the device (20min/day, 5 days). The percentage of time spent in the central zone were assessed as an index of anxiety-like behavior.

Object Recognition Test: the test comprised a training trial (T1), a 1-hour interval, and a test trial (T2). During T1, rats were exposed to two identical objects for 20min. During T2, one object was replaced by a novel one (N) while the other remained familiar (F). Exploratory behavior was defined as nose orientation within 2cm or nose contact but sitting on the object was excluded (Hosseini-Sharifabad et al., 2016b). The discrimination index was calculated: (eN - eF) / (eN + eF). A positive value indicates more time spent with the novel object whereas a negative one indicates more time spent with familiar object. Animals exploring <20s in either T1 or T2 were excluded. A second NORT was conducted eight weeks later using new objects.

2.5. Pharmacological analysis

2.5.1. Pharmacokinetic studies after acute administration

Pharmacokinetics were assessed following tramadol administration (25 and 50 mg/kg). 16 rats underwent two sample collections following each administration, with a two-week washout period between administrations (n=4 per time point). Blood was

collected from the tail vein at 0.25, 0.5, 1, 2, 4, 7, 18, and 24 hours after oral administration (**Table 1**), based on mouse pharmacokinetic profiles (Evangelista Vaz et al., 2018). Plasma was isolated (3000g, 20min, 4°C) and stored at -20°C. Tramadol and O-desmethyl (ODM)-tramadol plasma concentrations were quantified by LC-MS/MS using an ABSciex API 4500 QTRAP system (Framingham, MA, USA), as already described (Heraudeau et al., 2023). Acquisition was realized in multiple reaction monitoring (MRM) mode. Briefly, 40µL of internal standard (13C D3 tramadol, 1µg/mL), 200µL of 2N sodium hydroxide solution and 2.5mL of ethyl acetate were added to 200µL of plasma. The organic phase of the protein-precipitated mixture was extracted and dried then reconstituted in 1mL of A phase prior to LC-MS/MS quantification.

Rat number	Sampling times after acute administration (OG) of tramadol 25mg/kg or 50mg/kg							
	0.25 H	0.5 H	1 H	2 H	4 H	7 H	18 H	24 H
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								

Table 1. Blood collection after acute administration of tramadol in 16 male Sprague-Dawley rats. 2 collection sessions were performed 2 weeks apart. Rats 1 to 8 received tramadol 25mg/kg and rats 9 to 16 received tramadol 50mg/kg. A composite pharmacokinetic profile was generated from these data points.

PKanalix software (Lixoft®, Antony, France) was used to compute $t_{1/2}$ (time required for a 50% decrease in plasma concentration), t_{max} (time to achieve maximum concentration), C_{max} (maximum concentration), $AUC_{0\rightarrow 24h}$ (Area Under concentration-time Curve from Hour 0 to Hour 24) and $AUC_{0\rightarrow inf}$ (Area Under concentration-time Curve from Hour 0 to infinity).

2.5.2. Tramadol and ODM-tramadol concentrations after chronic administration

Twenty hours after the last dose, tail-vein blood was collected from tramadol-treated rats. Insufficient sampling in two rats per dose yielded n=6/group. Samples were processed as described in section 2.5.1.

2.6. Biochemical analysis: AST and ALT concentrations after acute and chronic administration

Twenty-eight rats were included in the biochemical analyses. Sixteen of these animals, previously used for the pharmacokinetic assessment, were allocated to the acute treatment group (n = 8 per dose), while twelve additional rats were included for the chronic treatment group (n = 6 per dose).Liver enzyme concentrations were measured approximately 20h after the last acute (n=16) or chronic (n=12) administration. Rats were administered pentobarbital (182.2mg/kg, IP, 5mL/kg) and were killed by decapitation 5min later. Blood was collected from the jugular vein, centrifuged (3,000g, 10min, +4°C) and plasma stored at -20°C. The plasma concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured through enzymatic method (Alinity c®, Abbott, USA). To assess the impact of chronic tramadol exposure on liver function, acute and chronic data for the 25 and 50mg/kg doses were pooled (detailed data in **Supplementary Fig. 4**).

2.7. Data and Statistical Analysis

Statistical analyses were performed using Prism (version 8.0; GraphPad® Software, USA) and R Studio (2023.06.0, Build 421, Posit Software, PBC). Normality of data was assessed by Kolmogorov-Smirnov and the homogeneity of variances by Bartlett test. Group comparisons were performed using one- or two-way analysis of variance (ANOVA) and were presented as mean +/- Standard Error Mean (SEM). Dunnett's test was used for post hoc comparisons. When the data deviated from normal distribution (e.g., amplitude of front and back activity, MESOR of horizontal activity, cumulative time in the center zone of OF and ALT plasma concentration), log-normalization was applied to improve comparability between groups, and limit the influence of extreme values. Welch's correction was used for data sets with inhomogenic variances were presented as median +/- interquartile range. Finally, when data sets did not meet one of the two criteria (normality and homogeneity of variances), non-parametric statistical tests were used (ATS: ANOVA-Type Statistics), followed by multiple pairwise comparison. When necessary (NORT and MWM probe test), a univariate t-test or Wilcoxon Signed Rank test was used to compare to a reference value (0 and 25%, respectively), parametric and non-parametric data respectively. Statistical significance was set at $p<0.05$. The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2025).

3. RESULTS

3.1. Locomotor activity

Cosinor analysis of back-and-forth activity did not reveal a significant group effect (ATS: F=1.74, p>0.05) nor a time x group interaction (ATS: F=0.39, p>0.05) but showed a significant time effect (ATS: F=100.10, p<0.0001) (**Fig. 2A**).

Cosinor analysis of front and back activity revealed a significant group effect (ATS: F=9.82, p=0.0001), a significant time effect (ATS: F=154.63, p<0.0001) and a significant time x group interaction (ATS: F=3.40, p=0.0098). Post-hoc analysis revealed that TRAM50 group differed from control group (multiple pairwise comparison: F=5.70, p=0.017) but not TRAM25 (multiple pairwise comparison: F=3.03, p>0.05) (**Fig. 2B**). Analysis of MESOR and amplitude revealed a significant difference between groups (**Fig. 2C and D;** one-way ANOVA: $F_{2, 21}$=12.22 and 15.98, p=0.0003 and p<0.0001 respectively), whereas no group effect was observed when considered acrophase values (**Fig. 2E;** one-way ANOVA: $F_{2, 21}$=0.88, p>0.05). A significant difference of MESOR values was observed between TRAM50 and control group, but not with TRAM25 (Dunnett's post-hoc test: p=0.015 and p>0.05, respectively). When considering amplitude values, TRAM25 showed a significant decrease, while TRAM50 showed a significant increase compared to the control group (Dunnett's post-hoc test; p=0.013 and p=0.027, respectively).

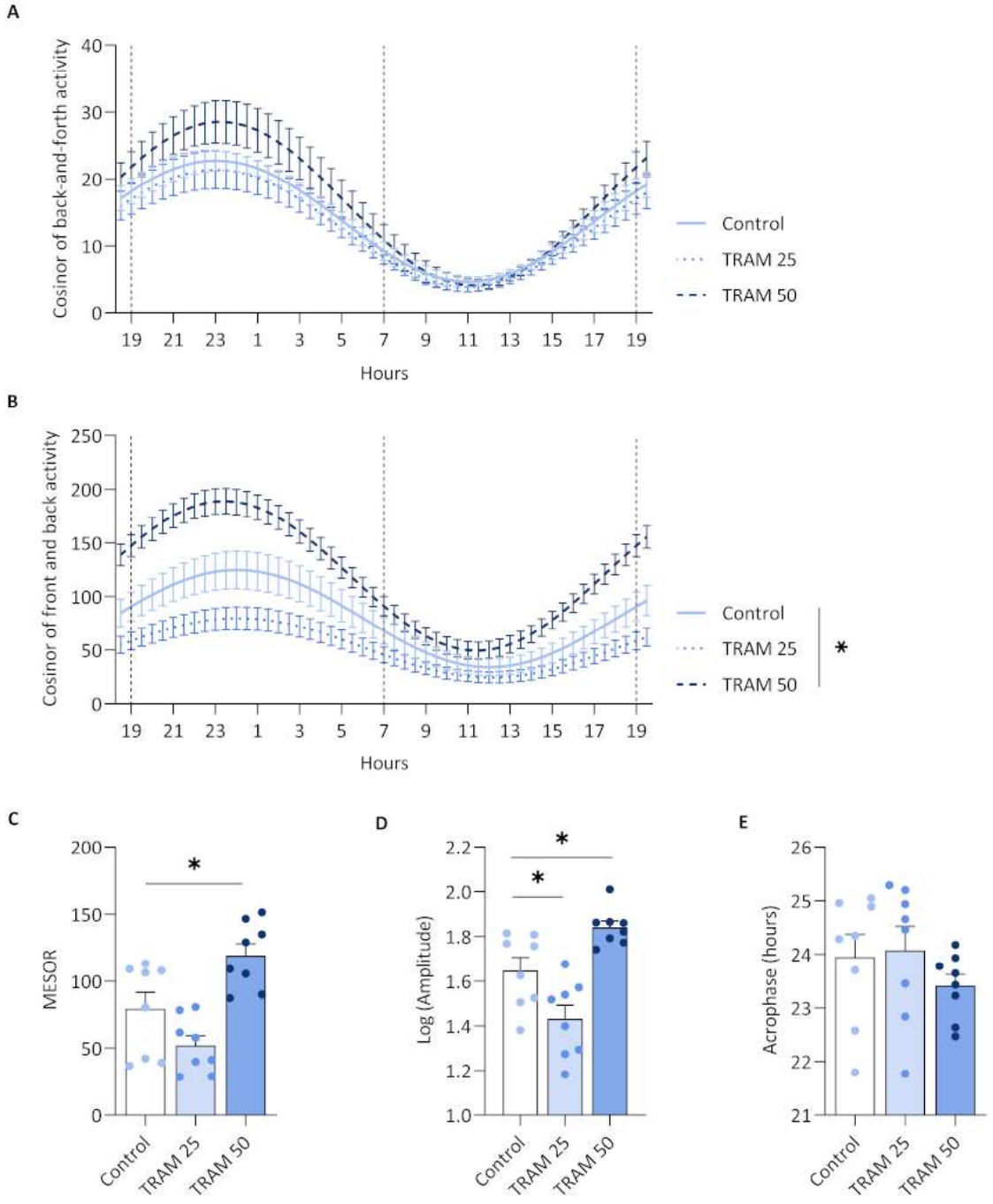


Fig. 2. Effects of acute administration of tramadol on (**A**) the cosinor of back-and-forth activity, (**B**) the cosinor of front and back activity and cosinor parameters of front and back activity: (**C**) MESOR, (**D**) amplitude and (**E**) acrophase (n=8/group). *p<0.05

3.2. MWM learning and probe test

Analysis of swimming distance at W6 revealed no significant group effect or time x group interaction, but a significant time effect (respectively two-way ANOVA: $F_{2, 21}$=1.12, $F_{8, 84}$=1.13 and $F_{4, 84}$=5.49 with p>0.05, p>0.05 and p=0.0006) (**Fig. 3A**).

Cumulative duration in target quadrant did not differ between groups for each time of exposure: on W2 (**Fig. 3B;** ANOVA: $F_{2, 21}$=0.12, p>0.05) and on W7 (**Fig. 3C;** Welch's ANOVA: $W_{2, 11.71}$=0.30, p>0.05).

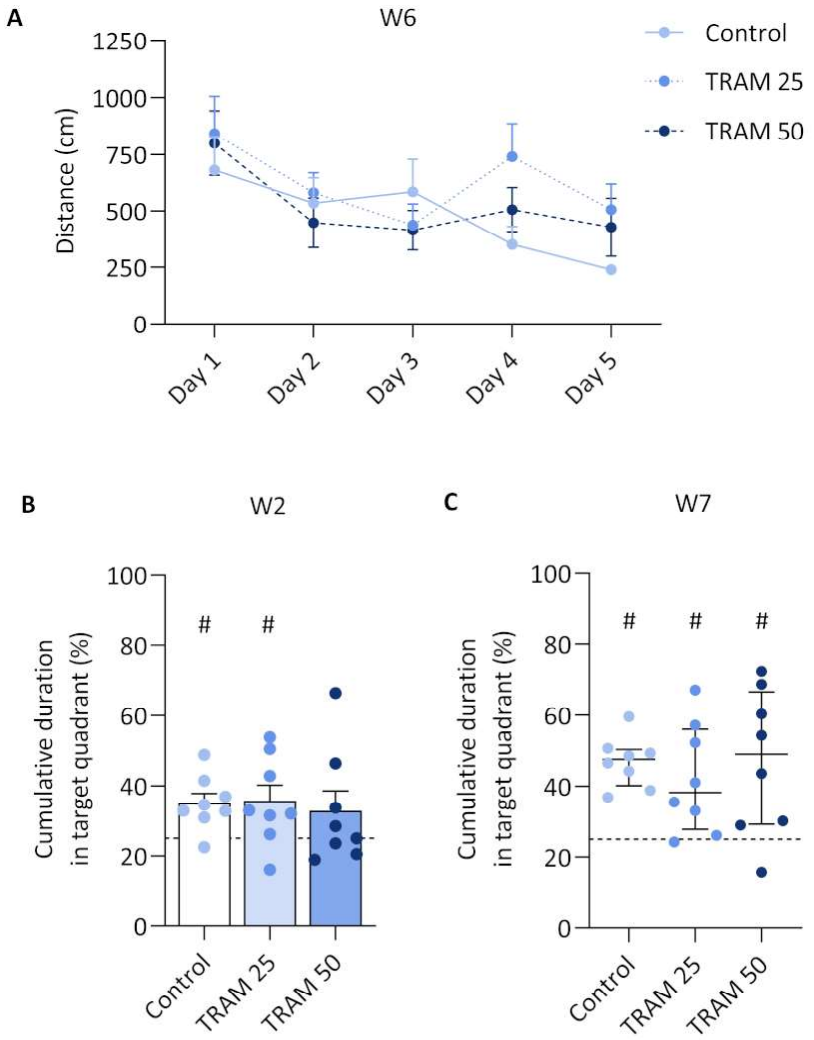


Fig. 3. Effects of 6 weeks of tramadol exposure on the distance moved to find the hidden platform across the 5 days of MWM learning (**A**). Effects of different tramadol exposure times on the cumulative duration in target quadrant during MWM probe test: after (**B**) 1 week (univariate t-test presented as mean +/- SEM) and (**C**) 6 weeks of administration (Wilcoxon Signed Rank presented as median +/- interquartile range due to unequal variances) (n=8/group). #p<0.05, compared with 25% threshold represented by the dashed lines.

Regardless of the probe test considered (W2 and W7), time spent in target quadrant was significantly different from the random exploration value of 25% for the control group (univariate t-test for W2: t_7=3.71, p=0.008 and Wilcoxon Signed Rank for W7: W=36.00, p=0.008). When considering TRAM25 animals, time spent in target quadrant was significantly higher that random value at W2 and W7 (univariate t-test: t_7=2.42, p=0.046 and Wilcoxon Signed Rank: W=34.00, p=0.016). Finally, performances of TRAM50 animals were below random value only at W2 (univariate t-test: t_7=1.380, p>0.05), but not at W7 (Wilcoxon Signed Rank: W=30.00, p=0.039).

Of note, swimming speed was similar whatever group of rats considered, at W2 (one-way ANOVA: $F_{2, 21}$=2.336, p>0.05) but not at W7, where velocity decreased in tramadol groups (**Supplementary Fig. 5**; Welch's ANOVA: $F_{2, 10.71}$=16.14, p=0.0006).

3.3. Novel Object Recognition Test

Prior to NORT, an OF test was carried out to assess anxiety-like behavior. On the first day of habituation, time spent in the central zone was not significantly different between groups (**Supplementary Fig. 6**; one-way ANOVA: $F_{2, 21}$=2.02, p>0.05).

Recognition memory was assessed in NORT and did not differ between groups: on W5 (**Fig. 4A;** one-way ANOVA: $F_{2, 20}$=0.88, p>0.05) and on W13 (**Fig. 4B;** one-way ANOVA: $F_{2, 21}$=1.20, p>0.05). Discrimination index was significantly different from threshold value of zero on W5 in control group (univariate t-test: t_6=2.59, p=0.041) but not in TRAM25 (univariate t-test: t_7=0.95, p>0.05) and TRAM50 groups (univariate t-test: t_7=0.84, p>0.05) (**Fig. 4A**). On W13, the ORT was repeated after a 6-weeks washout period following the treatment phase. The recognition memory was significantly different from threshold value on W13 in Control group (univariate t-test: t_7=3.53, p=0.010) but not in TRAM25 (univariate t-test: t_7=1.34, p>0.05) and TRAM50 groups (univariate t-test: t_7=0.89, p>0.05) (**Fig. 4B**).

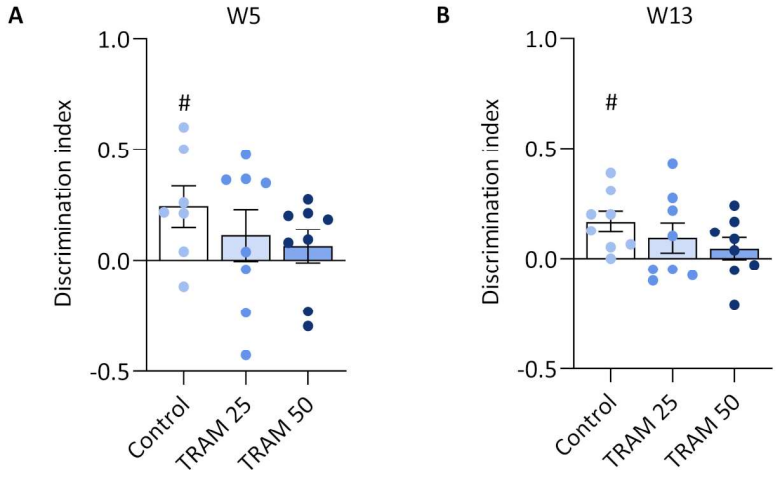


Fig. 4. Effects of tramadol exposure on the ORT discrimination index at W5 (n=8 for all groups except control in which one rat did not meet the 20s criterion of exploring in 20min of testing and was excluded from analysis) and W13 (n=8/group). #p<0.05 compared with 0.0 threshold represented by the dotted lines.

3.4. Tramadol and ODM-tramadol concentrations after acute and chronic administration

The mean plasma concentration-time curves of tramadol (**Fig. 5A**) and its metabolite ODM-tramadol (**Fig. 5C**) measured after OG administration of single doses of 25 and 50mg/kg of tramadol, are shown in **Fig. 5**.

Pharmacokinetic parameters for tramadol and ODM-tramadol were calculated and are shown in **Table 2**. For tramadol, the half-life (T½) increased from 3.61 ± 0.40 hours at 25mg/kg to 4.91 ± 3.00 hours at 50mg/kg. The maximum plasma concentration (Cmax) reached with TRAM50 (277.50 ± 71.83ng/mL) is higher than that reached with TRAM25 (224 ± 80.88ng/mL). Similarly, the AUC was more elevated with the TRAM50 dose, suggesting enhanced systemic exposure. For the primary metabolite ODM-tramadol, the half-life increased from 2.89 ± 0.22 hours to 5.34 ± 1.14 hours at 25 and 50mg/kg, respectively. Cmax value was also higher at TRAM50 (350.75 ± 85.92ng/mL) than at TRAM25 (300.75 ± 83.79ng/mL). Tmax for ODM-tramadol was delayed at the higher dose compared to TRAM25.

	$T_{½}$ (h)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0→24h}$ (ng.h/mL)	$AUC_{0→inf}$ (ng.h/mL)
TRAM25	3.61±0.40	1.00±0.71	224.00±80.88	748.33±240.26	753.73±241.36
TRAM50	4.91±3.00	0.69±0.88	277.50±71.83	1248.67±289.21	1342.20±487.68
ODM-TRAM25	2.89±0.22	1.00±0.71	300.75±83.79	839.40±93.49	843.51±93.95
ODM-TRAM50	5.34±1.14	2.00±0.75	350.75±85.92	1447.48±376.51	1491.81±376.18

Table 2. Plasma concentrations obtained after a single dose OG administration of tramadol hydrochloride (25 or 50mg/kg) to Sprague-Dawley rats. Values are mean ± SD (Standard Derivation), n=4 per time point. Pharmacokinetic parameters were calculated using PKanalix® software.

Quite surprisingly, 20h after the last OG of chronic daily administration protocol of 6 weeks, plasma tramadol and its metabolite were detected (**Fig. 5B** and **D**). Concentrations were significantly different between the two groups (TRAM25 and TRAM50) for both tramadol and its ODM-tramadol metabolite (unpaired t test: t_{10}=2.00, p=0.037 and Welch's t-test: $t_{5.370}$=5.00, p=0.0017).

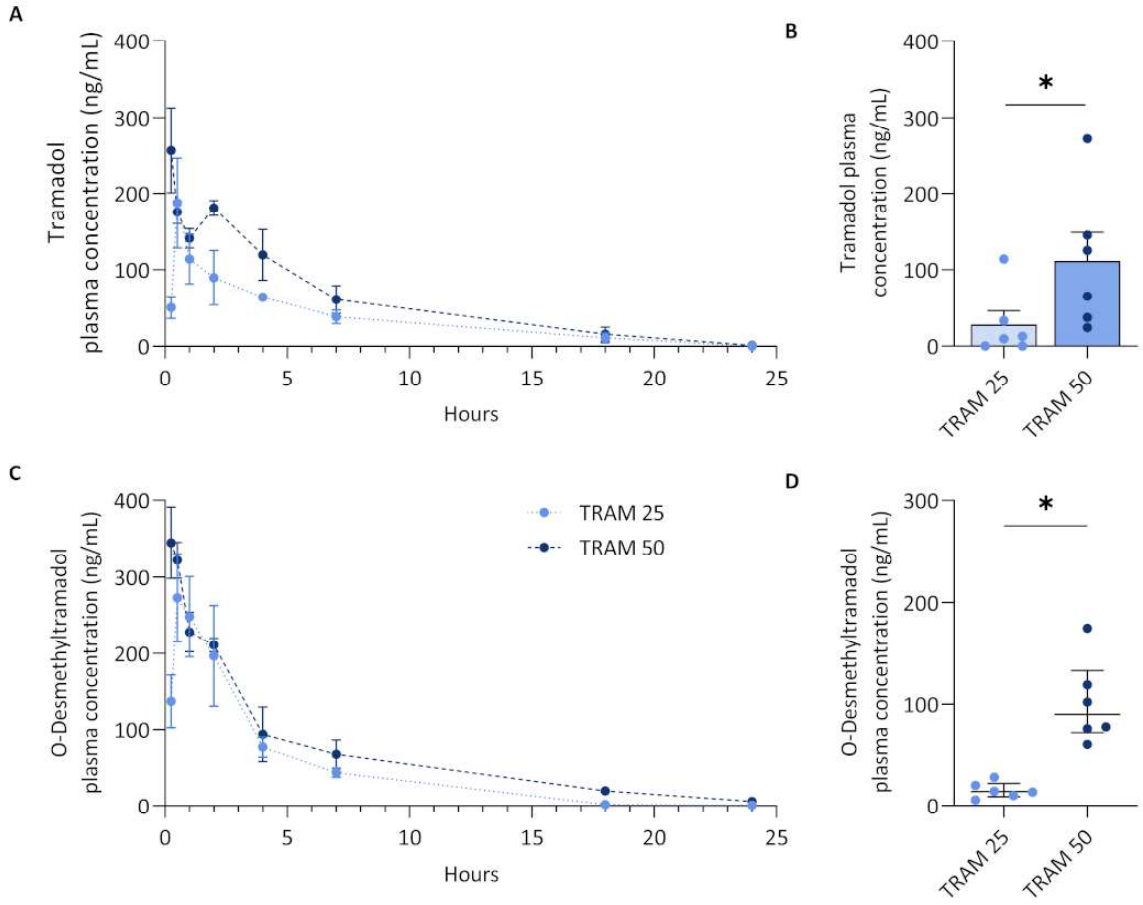


Fig 5. Tramadol and ODM-tramadol mean plasma concentration-time curves (respectively **A** and **C**) after a single administration *via* OG (n=4 per time point). Tramadol and ODM-tramadol plasma concentrations 20h after the last administration (W7) (respectively **B** and **D**) (n=6/group, obtaining adequate samples was not possible for four rats because of unsuccessful sampling or insufficient quantity of blood).

3.5. AST and ALT concentrations following acute and chronic administration

Following the pooling of data collected from rats treated with 25 and 50mg/kg under acute and chronic conditions, AST and ALT concentrations showed a significant group effect (**Fig. 6**; Welch's ANOVA test: $W_{2,\ 18.00}$=9.299, p=0.0017 and $W_{2,\ 15.46}$=37.00, p<0.0001 respectively). Post-hoc analysis revealed that AST and ALT concentrations were respectively 2.3 and 4.6 times higher in chronic condition compared to acute groups (**Fig. 6A** and **B**; Dunnett's t test: $t_{11.58}$=4.178, p=0.0037 and $t_{14.63}$=8.542, p<0.0001, respectively). Chronic groups significantly differed from control group for both AST and ALT parameters (Dunnett's t test: $t_{11.75}$=3.728, p=0.0084 and $t_{18.00}$=7.608, p<0.0001, respectively). Of note,

acute groups did not differ from control groups for both AST and ALT parameters (Dunnett's t test: $t_{17.13}$=1.786, p>0.05 and $t_{10.77}$=0.5671, p>0.05, respectively).

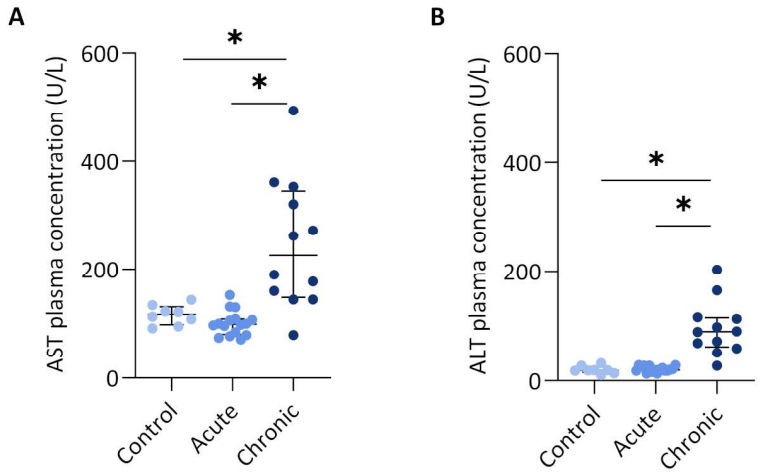


Fig. 6. Plasma concentrations of AST (**A**) and ALT (**B**) for control group (n=8) and combined tramadol treated animals, 20h after acute (n=16) and chronic (n=12) administration.

4. DISCUSSION

This study described for the first time the relationship between systemic tramadol exposure and alterations in cognitive performances in rats following both acute and chronic administration. We demonstrated not only behavioral changes following acute administration, but also long-term memory impairment after chronic tramadol exposure. These alterations were accompanied by systemic accumulation of tramadol and a time-dependent elevation of transaminase levels.

Locomotor activity was evaluated for 24h after a single oral dose. Whereas the low dose (TRAM25) produced a mild hypolocomotor effect, the high dose (TRAM50) unexpectedly induced hyperlocomotion. Tramadol's hypolocomotor effect has been previously documented in the literature after acute administration, potentially due to its agonist action at mu-opioid receptors (Elsukary et al., 2022; Szkutnik-Fiedler et al., 2012). In contrast, the hyperactivity observed at the higher dose may be explained by two pharmacological properties: (i) inhibition of serotonin and norepinephrine reuptake, which may produce antidepressant-like CNS stimulation; and/or (ii) anticholinergic effects mediated by muscarinic M1/M3 receptor inhibition (Minami et al., 2015; Nakamura et al., 2005). Indeed, the serotoninergic syndrome, marked by increased synaptic level of serotonin, is characterized in rats by a stereotyped behavior and hyperactivity (Jacobs, 1976). Otherwise, anticholinergic compounds (e.g. scopolamine) also elicit hyperactive behavior in rodents (Qiu et al., 2016). Regardless of the underlying mechanisms, hyperlocomotion resulting from stereotypical behavior can serve as a rodent model of human delirium. This phenomenon was observed in our study, where animals displayed increased random movements toward the front or rear of the cage, while back-and-forth activity remained unchanged. (Qiu et al., 2016; Soubrié et al., 1976). Delirium is characterized by abrupt impairments in cognitive functions and is commonly observed in aged patients during the initiation of drug treatments (American Psychiatric Association, 2013).

Spatial memory impairment appeared after one week of treatment in the TRAM50 group but were transient and absent after six weeks, contrasting with previous reports of

reporting learning and memory deficits following chronic tramadol administration (Baghishani et al., 2018; Mehdizadeh et al., 2017). This discrepancy may reflect methodological differences, particularly our decision to test animals outside the peak concentration window. In contrast, decreases in the discrimination index emerged after four weeks of treatment at both doses. Although no direct group differences were detected between treated and control animals, the deterioration in discrimination performance over time suggests that recognition memory may be sensitive to prolonged tramadol exposure. This result was in accordance with literature data showing that 21 days of tramadol administration (20 mg/kg, IP) induced a recognition memory deficit in the NORT (Hosseini-Sharifabad et al., 2016b). Interestingly, our results also showed for the first time that this deficit could last even six weeks after the last tramadol dose administration. Thus, tramadol has a long-lasting deleterious effect on recognition memory. Beyond the hippocampus, recognition and spatial memory are supported by distinct cortical networks, involving the medial prefrontal cortex for recognition memory (Chao et al., 2022) and the perirhinal and postrhinal cortices for spatial memory (Ramos, 2013). Although the exact mechanisms remain to be determined, one possible hypothesis is that these networks differ in their sensitivity to tramadol-induced neuronal damage, oxidative stress and neuroinflammation, rendering recognition-related circuits particularly vulnerable (Ezi et al., 2024).

Pharmacokinetic analyses showed that the 50mg/kg dose produced higher C_{max} and AUC values and a longer half-life than the 25mg/kg dose for both tramadol and its metabolite ODM-tramadol, consistent with a linear dose-dependent profile (Bao et al., 2023; Sheikholeslami et al., 2012; Zhao et al., 2010). Following oral administration, the authors obtained a $T_{½}$ of 1.58 to 7.554h, a T_{max} of 1.00 to 1.792h, a C_{max} of 162.063 to 190.23ng/mL and an AUC ranging from 456.28 to 724.84ng/mL.h depending on the dose administered (5.2 and 20mg/kg) (Bao et al., 2023; Zhao et al., 2010). Even within the same study, inter-animal variability, together with differences in dose, administration route, sampling time, and circadian rhythms, can lead to substantial discrepancies in half-life estimates across published data (Liu and Song, 2001).

After six weeks of daily administration, ALT and AST levels increased 4.6-fold and 2.3-fold, respectively, suggesting the onset of liver damage and supporting tramadol-induced hepatotoxicity. These findings align with earlier reports in Wistar rats showing that 14-day chronic administration of tramadol elevate serum liver enzymes, reduce liver antioxidant capacity, and induce histopathological alterations (Barbosa et al., 2020). Such hepatic impairment may modify pharmacokinetics by reducing hepatic clearance or saturating metabolic pathways, which is consistent with the systemic accumulation of tramadol and its active metabolite observed after chronic exposure. Although hepatotoxicity provides a plausible explanation, other mechanisms—such as the progressive saturation of elimination pathways, declining clearance over time, or altered enzymatic activity—cannot be excluded (El-Gaafarawi, 2006). Given that both tramadol and ODM-tramadol can cross the blood-brain barrier, elevated plasma concentrations are likely to result in increased cerebral levels (Kitamura et al., 2014). This systemic accumulation may thus extend to the central nervous system, contributing to the recognition memory impairments observed in our study.

One limitation of this study concerns the selected model. We deliberately used adult rats to investigate the effect of tramadol overexposure, a condition that most commonly occurs in elderly subjects following chronic treatment. However, the cognitive effects observed in our study are likely constrained by the use of adult animals, in which the mechanisms underlying the cognitive processes examined are not physiologically amplified by age. Another limitation is that our study was conducted in male rats and does not account for potential sex-dependent differences.

In conclusion, overexposure to tramadol induces both acute and long-term cognitive alterations. Our data suggest that, during the treatment initiation phase, the use of high, or even supratherapeutic, doses could contribute to delirium-like disorders. After chronic administration, tramadol induces liver damage and systemic accumulation, which may drive persistent deficits in recognition memory. These results parallel clinical observations in tramadol-treated patients, especially older adults, and underscore the need for cautious prescribing in individuals vulnerable to cognitive decline.

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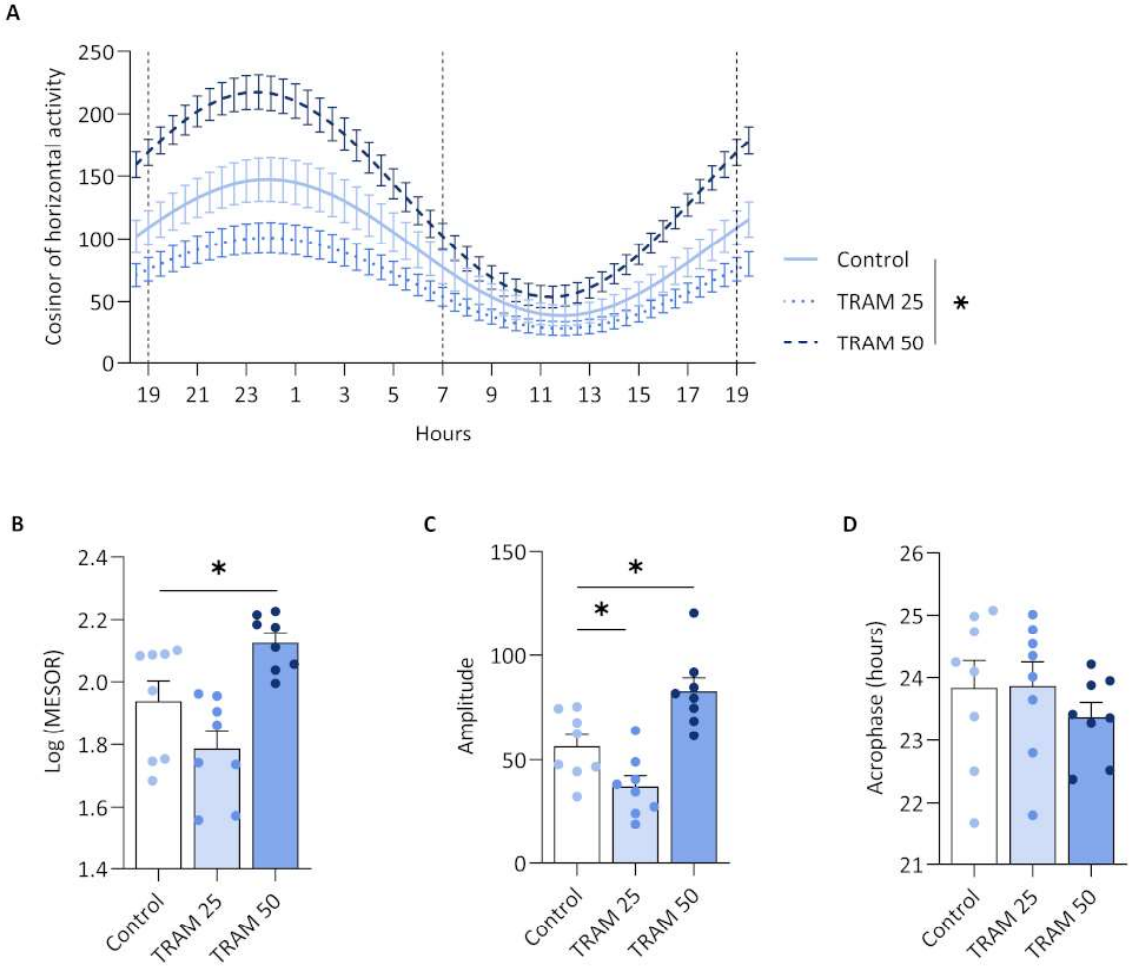
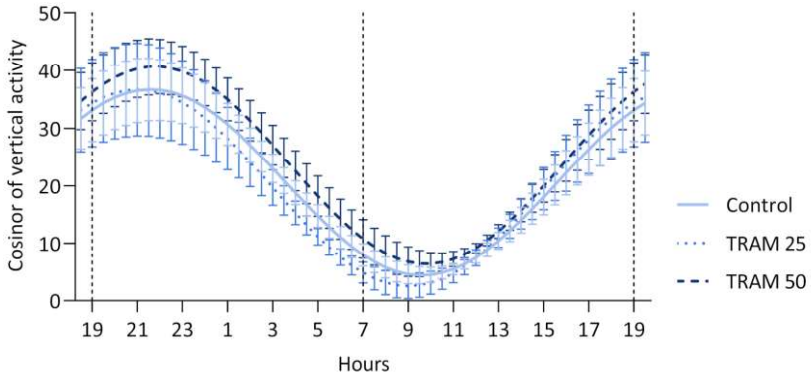
SUPPLEMENTARY MATERIAL

Cosinor analysis of horizontal activity revealed a group effect (ATS: F=10.38, p<0.0001), a time effect (ATS: F=167.30, p<0.0001) and a group x time interaction (ATS: F=2.93, p=0.020) (**Supplementary Fig. 1)**. Post-hoc analysis revealed that TRAM50 group differed from control group, but not TRAM25 (multiple pairwise comparison: F=6.09 and 3.34, p=0.016 and p>0.05, respectively) (**Supplementary Fig. 1A**). Analysis of MESOR and amplitude revealed a significant difference between groups (**Supplementary Fig. 1B and C;** one-way ANOVA: $F_{2, 21}$=10.41 and 16.26, p=0.0007 and p<0.0001, respectively), whereas no group effect was observed when considered acrophase values (**Supplementary Fig. 1D;** $F_{2, 21}$=0.59, p>0.05). A significant difference of MESOR values was observed between TRAM50 and control group, but not with TRAM25 (Dunnett's post-hoc test: p=0.038 and p>0.05, respectively). When considering amplitude values, TRAM25 showed a significant decrease, while TRAM50 showed a significant increase compared to the control group (Dunnett's post-hoc test: p = 0.007 and p = 0.049, respectively).



Supplementary Fig. 1. Effects of acute administration of tramadol on (**A**) the cosinor of horizontal activity and cosinor parameters: (**B**) MESOR, (**C**) amplitude and (**D**) acrophase (n=8/group). * $p<0.05$

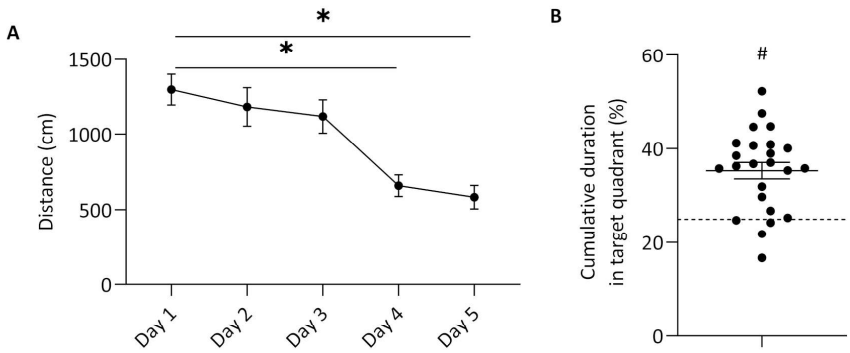
Cosinor analysis of vertical activity did not revealed a significant group effect (ATS: $F=1.23$, $p>0.05$), nor a time x group interaction (ATS: $F=0.31$, $p>0.05$) but showed a significant time effect (ATS: $F=76.12$, $p<0.0001$) (**Supplementary Fig. 2**).



Supplementary Fig. 2. Effects of acute administration on the cosinor of vertical activity (n=8/group).

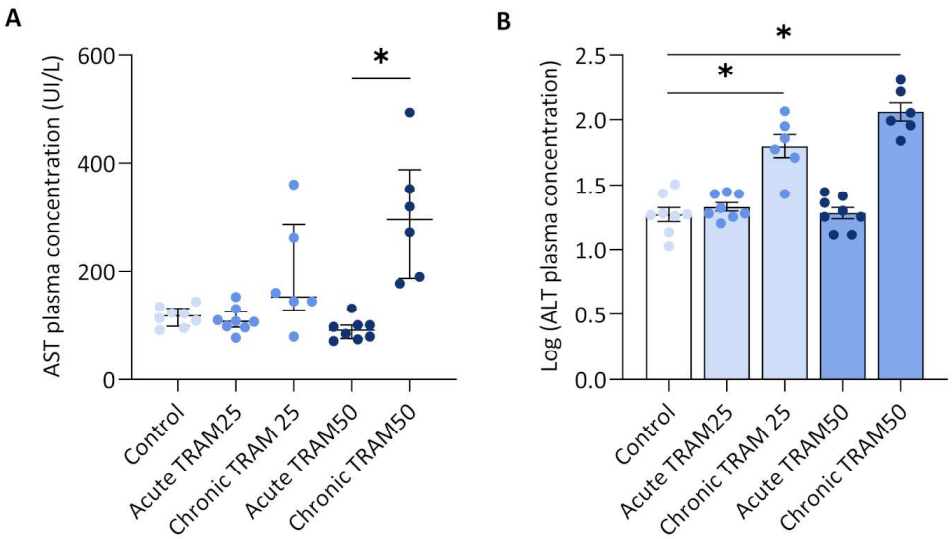
At W-2, during the five days of training and before group randomization, the distance moved by all the rats to find the hidden platform significantly decreased from D1 as of D4 (**Supplementary Fig. 3A**; one-way ANOVA: $F_{4, 115}$=10.33, p<0.0001), testifying that spatial learning occurred.

Spatial recognition memory was assessed by MWM probe tests after various periods of exposure to tramadol. The initial probe test was realized before groups randomization at W-1 and revealed a significant difference from threshold value of 25%, indicating that the spatial learning abilities of all rats remained comparable before treatment initiation (**Supplementary Fig. 3B**; univariate t-test: t_{23}=5.830, p<0.0001).

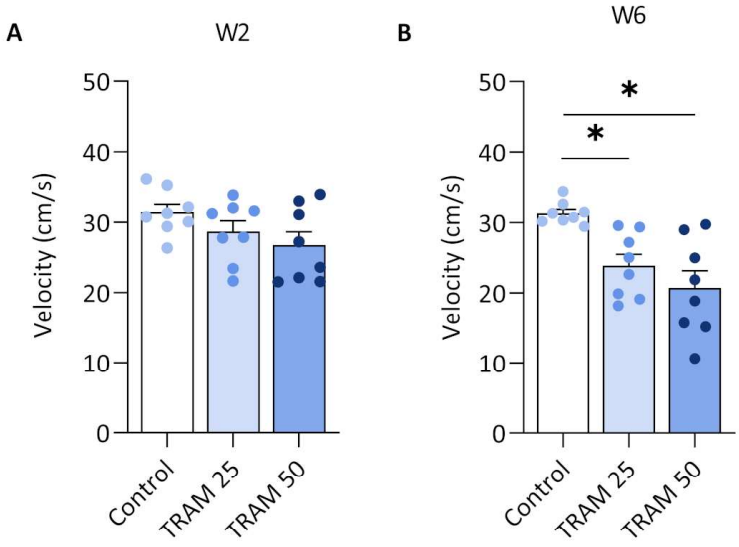


Supplementary Fig. 3. Evaluation of (**A**) the distance traveled to find the hidden platform over the 5-day MWM learning phase and (**B**) the cumulative time spent in the target quadrant during the MWM probe test, both prior to treatment (n=24). *p<0.05, compared with 25% threshold represented by the dashed line.

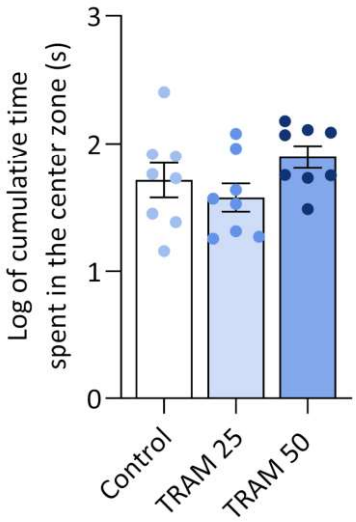
Analysis of AST and ALT plasma concentrations for the five groups showed a significant group effect (**Supplementary Fig. 4A**; Welch's ANOVA test: $W_{4, 13.59}$=5.937, p=0.0055 and **Supplementary Fig. 4B**; one-way ANOVA: $F_{4, 31}$=36.72, p<0.0001 after log normalization). Post-hoc comparisons revealed that AST concentrations were significantly different after chronic administration of tramadol at 50mg/kg (Dunnett's test, $t_{5.210}$=4.326, p=0.0493) whereas no such difference was observed in the TRAM25 group (Dunnett's test, $t_{5.375}$=1.943, p>0.05). Of note, no significant differences were found between the control and acute administration groups for either dose (Dunnett's test; TRAM25: $t_{13.32}$=0.6307, p>0.05; TRAM50: $t_{13.92}$=2.534, p>0.05). Dunnett's post-hoc tests of ALT concentrations revealed that chronic administration led to a significantly elevated ALT level compared to acute administration in the TRAM25 and the TRAM50 groups (p<0.0001). Of note, no significant difference was found between control and acute administration groups for either dose (p>0.05).



Supplementary Fig. 4. AST (**A**) and ALT (**B**) plasma concentrations for control group (n=8), TRAM25 (n=8) and TRAM50 (n=6) groups, 20h after acute and chronic administration of tramadol.



Supplementary Fig. 5. Effect of different tramadol exposure times on the velocity of rats during the MWM test: after (**A**) 1 week and (**B**) 6 weeks of administration (n=8/group). *p<0.05, compared with 25% threshold represented by the dashed line.



Supplementary Fig. 6. Evaluation of the cumulative time spent in the central zone during the OF test after 3 weeks of exposure to tramadol, a habituation conducted prior to ORT (n=8/group).

Cognitive consequences of acute and chronic administration of SSRI antidepressants in male rats

Cognitive effects of acute and chronic SSRIs in rats

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ARTICLE 2 - Cognitive consequences of acute and chronic administration of SSRI antidepressants in male rats

Abstract

Selective serotonin reuptake inhibitors (SSRIs) are first-line treatments for depression in older adults, who are particularly vulnerable to neurocognitive adverse effects due to age-related pharmacokinetic and pharmacodynamic changes. In 2023, SSRIs accounted for 48% of antidepressant prescriptions among older adults in France, with paroxetine and sertraline being the most frequently used. Among SSRIs, paroxetine has been associated with an increased risk of confusion, while evidence on the cognitive effects of chronic SSRI exposure remains limited.

This study compared the effects of paroxetine (10 mg/kg) and sertraline (16 mg/kg) in male Sprague-Dawley rats at doses modeling overexposure. Locomotor activity was assessed after treatment initiation, while cognitive performances were evaluated using the Morris Water Maze and Novel Object Recognition Test over six weeks of daily treatment. Pharmacokinetic analyses were conducted following acute and chronic exposure.

Acute sertraline administration increased rearing behavior, whereas paroxetine led to stereotypical activity. Following chronic exposure, only paroxetine impaired learning performance during MWM reversal learning. Pharmacokinetic analyses revealed accumulation of paroxetine but not sertraline.

These findings suggest that paroxetine and sertraline may induce transient cognitive disturbances during early treatment, consistent with confusion reported in clinical settings. Chronic overexposure to paroxetine may have impaired cognitive flexibility. These findings highlight that SSRIs differ in their neurocognitive impact, emphasizing the need for careful monitoring and personalized prescribing strategies in older adults at risk of drug accumulation and cognitive disturbances.

Keywords: cognition; locomotor activity; delirium; cognitive flexibility; pharmacokinetics

1. INTRODUCTION

Depression affects approximately 332 million individuals worldwide, including 6% of those aged 60 and over (WHO, 2025). Selective Serotonin Reuptake Inhibitors (SSRIs), which enhance serotonergic transmission by inhibiting the serotonin transporter, are recommended as first-line therapy (Pruckner & Holthoff-Detto, 2017). In 2023, SSRIs accounted for 48% of antidepressants prescribed to people aged 60 and over in France, with paroxetine and sertraline being the most commonly used (15.5% and 6.7% respectively) (Cognard et al., 2024) . In older adults, these drugs have been shown to alleviate depressive symptoms and demonstrate clinical efficacy (Tedeschini et al., 2011). However, emerging evidence suggest that the benefits of SSRIs in the elderly may warrant reevaluation, as older adults appears to be more sensitive to potential adverse effects, especially those concerning neurocognitive disruptions.

Indeed, pharmacoepidemiological studies indicate that the introduction of SSRIs may induce short-term neurocognitive adverse effects, such as delirium (Friedrich et al., 2022; Minoc et al., 2024). Delirium syndrome is an acute cognitive dysfunction characterized by a sudden and fluctuating impairment in alertness or attentional abilities, potentially affecting all cognitive domains. Delirium is particularly prevalent in older adults and it is associated with high morbidity and mortality (Siddiqi et al., 2006). Among the many risk factors, adverse drug reactions, particularly those related to antidepressants, play a significant role in the development of delirium, alongside advanced age, pre-existing neurocognitive disorders, and depression itself (Inouye & Charpentier, 1996). A population-based cohort study suggest that long-term use of SSRIs can lead to neurocognitive impairments affecting memory, language or learning (Carrière et al., 2009). Indeed, for optimal therapeutic effect, international guidelines recommend a minimum treatment duration of six months after symptom remission (Vos et al., 2004). While beneficial effects on the symptoms of depression are most often achieved, the consequences of these prolonged treatments on the overall functioning of the central nervous system and, in particular, on cognitive functions, remain poorly understood.

Several hypotheses have been proposed to explain the **associative relationship between antidepressant use and cognitive impairment in older adults**. One such

hypothesis focuses on the variability in anticholinergic burden among different antidepressants. Indeed, while neurocognitive disorders can occur with various medications, drugs with a high anticholinergic load appear to induce a higher risk, especially in older adults (Attoh-Mensah et al., 2020; Campbell et al., 2009; L. Han et al., 2001). To explore this hypothesis, we recently analyzed the association between antidepressant prescriptions and pharmacovigilance reports of delirium in older adults using the WHO VigiBase® (Minoc et al., 2024). A significant association between delirium and SSRIs class consumption was demonstrated (r-OR 1.14 [1.11–1.18]), and among this class, paroxetine appeared to be more associated with delirium (r-OR 1.29 [1.22–1.37]) than sertraline (r-OR 0.92 [0.87–0.97]). Notably, paroxetine exhibits high anticholinergic activity, whereas sertraline is considered to have a low anticholinergic profile (Sanchez et al., 2014; Al Rihani et al., 2021). Additionally to delirium reports, high anticholinergic burden antidepressants are thought to favor the occurrence of short and/or long-term cognitive impairments in old adults (Attoh-Mensah et al., 2020). Yet, no study has truly evaluated cognitive consequences of short- and/or long-term use of different SSRIs according to their anticholinergic activity. Indeed, due to polymorbidity and polypharmacy, examining the cognitive effects specific to one drug in the elderly appears difficult to achieve through clinical studies.

Due to the difficulties in studying the consequences of exposure to SSRIs in humans, many studies have examined cognitive outcomes in rats following exposure to SSRIs. Unfortunately, it remains contradictory. Several studies have reported an attenuation or normalization of cognitive deficits induced by pathological conditions. For example, paroxetine has been shown to improve learning and memory performance in the Morris Water Maze in a rat model of depression after acute administration (J. Han et al., 2015) and to reduce memory impairments in a mouse model of Alzheimer's disease following chronic treatment (Ai et al., 2020). Similarly, sertraline has been reported to alleviate learning and memory deficits in social stress–based models of depression after acute administration in rats (Ramanathan et al., 2003), and to improve learning performance in stressed rats or in pharmacologically induced models of cognitive impairment after chronic administration (Rad et al., 2022; Tao et al., 2016). In contrast, studies conducted in healthy rats have yielded divergent findings. Chronic administration

of sertraline or paroxetine had no effect on learning and memory performances (García-Gutiérrez et al., 2023; Myint et al., 2007; Naudon et al., 2007; Tao et al., 2016). Deleterious cognitive effects have been documented for paroxetine only, as acute administration was associated with memory impairments (Naudon et al., 2007), and chronic exposure induced both short- and long-term memory deficits (Menezes et al., 2018). Consequently, the acute and chronic cognitive effects of paroxetine and sertraline exposure in healthy animals remain contradictory and poorly understood. In particular, no study has yet addressed short-term cognitive alterations relevant to delirium-like states, the impact of prolonged SSRI administration with different anticholinergic activity, or the relationship between cognitive outcomes and blood drug concentrations as a parameter of drug exposure.

The present study aimed to compare the cognitive effects of two SSRIs with contrasting anticholinergic burdens (paroxetine and sertraline) on both short-term (delirium-like) and long-term (cognitive) processes in rats. In order to clarify the data available in the literature, and to relate the observed behavioral effects to the SSRIs exposure of rats, a combined pharmacokinetic study was conducted. This study was carried out in an overexposed adult rat model, in which high doses equivalent to supratherapeutic doses in human were deliberately used to to achieve supratherapeutic concentrations, such as those measured in the elderly treated by therapeutic doses.

2. METHODS

2.1. Animals

Sprague-Dawley rats (n=40) were purchased from Janvier Labs (France) at 6 weeks of age and were randomly housed by 4 in standard polycarbonate cages (59.8x38x20cm^3) upon arrival. Animal facility was maintained at a constant temperature (22 +/- 1°C) and humidity (50 +/- 10%) under a normal 12h light-dark cycle (lights on from 07:00 to 19:00). They had unrestricted access to tap water and a diet of 20g/rat/day of food to regulate weight gain during the time of experiment. After a week of acclimation to the facilities, rats were daily handled (5min/rat/d, 5 days) to accustom the rats to the experimenter. All the experiments were performed in accordance with the European Communities Council Directive (2010/63/UE) and approved by the regional ethical committee (Comité d'Ethique Normandie en Matière D'EXpérimentation Animale, CENOMEXA) (agreement number #43198).

2.2. Pharmacological agents

Paroxetine and sertraline, two widely prescribed antidepressants were selected for this study (Cognard et al., 2024). Their choice was guided by muscarinic cholinergic receptor affinity and pharmaco-epidemiological evidence linking some antidepressants to delirium (Friedrich et al., 2022; Minoc et al., 2024). Paroxetine displays higher muscarinic affinity than sertraline (respectively, Ki=72 and 430nmol/L) (Sanchez et al., 2014). Both compounds were obtained in powder form (Thermo Fisher®) and dissolved in a 1% Tween20 aqueous solution to account for their hydrophobic properties. Doses were derived from the maximum recommended daily human doses, *i.e.* 60 and 200mg for respectively paroxetine and sertraline (Shrestha et al., 2025). Using the Human Dose calculation (Nair & Jacob, 2016), these correspond to 5 and 16mg/kg in rats, respectively. To model potential drug overexposure in older patients, higher doses previously evaluated in pharmacokinetic studies in rats were used (10 and 16mg/kg respectively) (Li et al., 2017; Melis et al., 2012). All chemicals and reagents employed for pharmacokinetic analyses were of analytical grade. Different rats were used either for behavioral (n=24) or for pharmacokinetic (n=16) study.

2.3. Pharmacokinetic experiments

Rats were randomly assigned to two groups (n=8/group), receiving acute administration of either paroxetine (10mg/kg) or sertraline (16mg/kg) by oral gavage (OG). Each rat underwent two sample collections following each administration, with a two-week washout period between administrations (n=4 per time point). According to the hypothetic time of plasma pic which should be around 4,25h for paroxetine and 1h for sertraline (Li et al., 2017; Melis et al., 2012), blood samples were collected via puncture of the caudal vein at 0.5, 1, 2, 3, 4, 6, 12, 24 and 0.25, 0.5, 1, 2, 6, 8, 12 and 24 hours after oral administration for paroxetine and sertraline, respectively. Whole blood samples were then stored at -20°C just after collection until analysis.

2.4. Behavioral experiments

This protocol has previously been described and has been adapted for antidepressants (Heraudeau et al., submitted). All behavioral tests were conducted during the morning (from 9 to 12am). After a 30min acclimatization period to the testing room, behavioral experiments started with an experimenter blinded to the treatment. All devices cleaned with 70% ethanol at the beginning and between each animal to avoid odor cues. Animal behavior was recorded and analyzed through video-tracking system (EthoVision XT, Noldus®).

Rats were randomly divided into 3 groups (n=8 per group), received either vehicle (control group), paroxetine (PAROX, 10mg/kg) or sertraline (SERT, 16mg/kg). Oral gavage (OG, 1.5mL/kg) was performed once daily for six weeks (W1 to W6) in the late afternoon (between 4 to 6pm) to minimize potential confounding effects of acute drug action and peak plasma concentration (**Fig. 1**). Indeed, behavioral tests were conducted the following morning, ensuring a minimum delay 16h interval between administration and testing. To refine the procedure, administration was performed using flexible plastic probes (PHYMEP®) (Õkva & Tamos, 2006). It should be noted that, due to accident related to force-feeding by plastic tube, one rat from each the control and the sertraline group respectively died in W1 and W3.

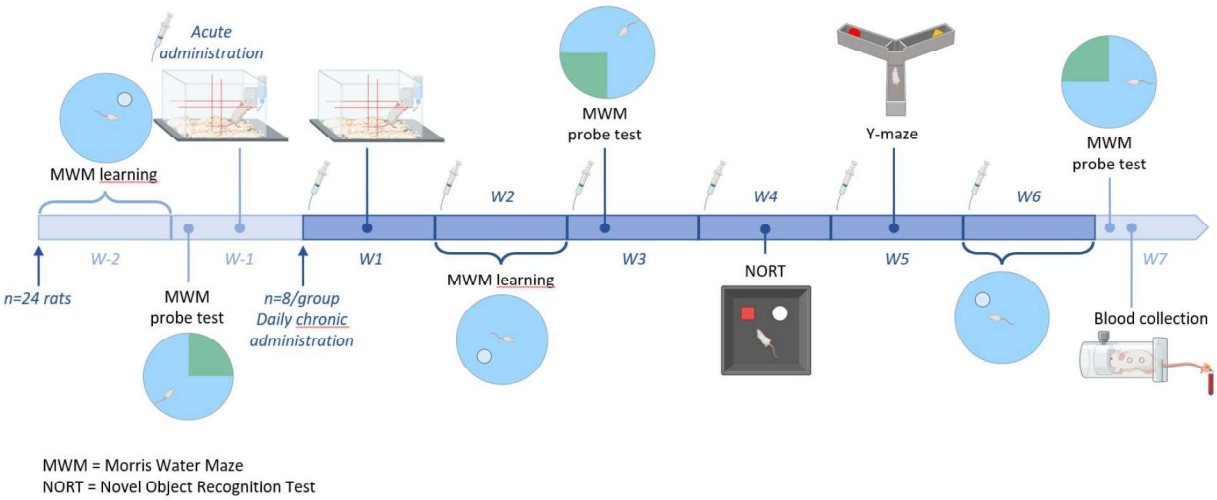

Fig. 1. Experimental design

2.4.1. Circadian locomotor activity

Imetronic chambers (33x21x19cm^3) and software (Imetronic®, Pessac, France) were used as previously described (Heraudeau et al., submitted). The following behavioral parameters were recorded: (i) number of rearings, (ii) front/back activity and (iii) back-and-forth activity (see **Supplementary Fig. 1** and **2** for front/back activity). Data were collected every 30min. Circadian rhythmicity was analyzed using cosinor analysis (cosinor.online; Molcan, 2023). When a difference was observed between one of the treated groups and the control group, cosinor parameters were calculated: (i) MESOR (midline-estimating statistic of rhythm): mean activity level over 24 hours, (ii) amplitude (difference between the peak and the mean value of the wave): difference between active and inactive phase activity and (iii) acrophase (time at which the peak of rhythm occurs): timing of peak activity within the 24-hour period.

To assess delirium-like effects of SSRIs, locomotor activity was monitored continuously for 24 hours at the start of treatment. Agitation or delirium-like behavior was indicated by a disproportionate increase in activity at either the front or the rear of the cage, associated by a reduction in overall horizontal activity (Heraudeau et al., submitted). Subsequently, circadian locomotor activity was again explored during W1 (after 3 days of administration) (see **Fig 1**).

2.4.2. Novel Object Recognition Test (NORT)

Recognition memory was evaluated in the open field (100x100x45cm^3) at W4 with a 2h inter-trial interval to probe short-term memory, whereas long-term memory was assessed in the Y-maze at W5 with a 24h interval to evaluate memory consolidation and retrieval (Wietrzych et al., 2005).

At W4, animals underwent a 20min habituation session 24h before testing to reduce novelty-induced stress. The percentage of time spent in the central zone and locomotor velocity were measured to ensure that the groups did not differ in anxiety-related behavior or baseline locomotor activity. The NORT protocol in open field was already described (Heraudeau et al., submitted). In short, the task included a familiarization phase (20min with two identical objects), a 2h inter-trial interval and a recognition phase (20min) with one familiar (F) and one novel (N) object. Exploration was defined as active investigation (nose within 2cm or direct contact), excluding climbing or sitting. A discrimination index [(eN − eF)/(eN + eF)] was calculated and animals with <20s of exploration in either phase were excluded.

At W5, the same procedure was applied with modified durations as described in the literature: a 15min familiarization phase, a 24h inter-trial interval and a 5min recognition phase (Walling et al., 2016). Rats were placed in the arm without an object and objects differed from those used in the open field.

2.4.3. Morris Water Maze test (MWM)

The spatial memory and cognitive flexibility performance was assessed using MWM (Morris, 1984). The initial session of MWM learning (W-2) and probe test (W-1) were conducted prior to the randomization of the rats. The MWM learning and probe tests were conducted as already described (Heraudeau et al., submitted). In short, MWM learning involved 4 daily training trials over 5 consecutive days, with 60-s inter-trial intervals. Rats failing to reach the platform within 120s were guided to it for a 30s reinforcement period. Three sessions were conducted: W-2, W2 (reversal, platform relocated), and W6 (with modified distal cues), with the platform respectively placed in NE, SO and NO quadrants.

The total distance travelled to reach the platform was assessed. Reversal learning at W2 was specifically included to evaluate cognitive flexibility, as it requires the animal to inhibit a previously learned spatial strategy and adapt to the new platform location. Learning performance on Day 1 (D1) of each training session was compared between groups to assess cognitive flexibility, specifically the ability to adapt to a reversal.

In the MWM probe test, the platform was removed, and time spent in the target quadrant was measured during 60s trials at three time points: 3 days after W-2 and W6 training, and 17 days after W2 training. Swimming speed was measured as an indirect marker of emotional state, ensuring that performance deficits reflect genuine memory processes rather than affective alterations.

After the last MWM probe test, blood samples were collected from the tail vein of the rats treated with paroxetine or sertraline, 20h after the final dose.

2.4.4. Blood sample analysis

Paroxetine and sertraline were extracted from rat blood by liquid–liquid extraction with D6-venlafaxine as an internal standard, as previously described (Heraudeau et al., 2023). LC–MS/MS analysis was performed on an ABSciex API 4500 QTRAP with ESI+ (Electrospray Ionization Source in positive mode) in MRM (Multiple Reaction Monitoring) mode. Separation was achieved on a reversed-phase Biphenyl column (2.1 × 100mm, Phenomenex, USA) at 0.5mL/min, 40°C. The lower limit of quantification was 5ng/mL. The following pharmacokinetic parameters were calculated using PKanalix software (Lixoft®, Antony, France): $t_{1/2}$ (time required for a 50% decrease in plasma concentration), t_{max} (time to achieve maximum concentration), C_{max} (maximum concentration) and $AUC_{0\rightarrow 24h}$ (Area Under concentration-time Curve from Hour 0 to Hour 24). For paroxetine, the terminal elimination half-life ($t_{1/2}$) was calculated without considering the 6-hour point. It should be noted that the values obtained are therefore approximate and based on a single-compartment model.

2.5. Data and Statistical Analysis

Statistical analyses were performed using Prism (version 8.0; GraphPad® Software, USA) and R Studio (2023.06.0, Build 421, Posit Software, PBC). Normality of data was assessed by Kolmogorov-Smirnov and the homogeneity of variances by Bartlett test. For all behavioral tests, group comparisons were performed using one- or two-way analysis of variance (ANOVA) and were presented as mean +/- Standard Error Mean (SEM). A mixed-effects model was used to analyze D1 learning performances over time, accounting for both within-subject dependency and inter-individual variability. Dunnett's test was used for post hoc comparisons. Welch's correction was used for data sets with inhomogenic variances were presented as median +/- interquartile range. Finally, when data sets did not meet one of the two criteria (normality and homogeneity of variances), non-parametric statistical tests were used (ATS: ANOVA-Type Statistics or Mann-Whitney), followed by multiple pairwise comparison.

When necessary (NORT and MWM probe test), an univariate t-test was used to compare to a reference value (0 and 25%, respectively). Statistical significance was set at $p<0.05$. The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2025).

3. RESULTS

3.1. Locomotor activity

3.1.1. Vertical activity

After a single administration, cosinor analysis of vertical activity revealed a significant group and time effect (respectively, ATS: F=4.26 and 72.87, p=0.015 and p<0.0001), but no group x time interaction (ATS: F=2.46, p=0.085) (**Fig. 2**). Vertical activity of sertraline group, but not paroxetine, significantly differed from control group (multiple pairwise comparison: F=4.34 and 0.86, p=0.037 and >0.05, respectively) (**Fig. 2A**). Analysis of MESOR and amplitude revealed a significant difference between groups (**Fig. 2B and C;** one-way ANOVA: $F_{2, 21}$=6.47 and 6.73, p=0.0065 and p=0.0055 respectively), whereas no group effect was observed when considered acrophase values (**Fig. 3D;** one-way ANOVA: $F_{2, 21}$=0.87, p>0.05). A significant difference of MESOR values was observed between sertraline and control groups, but not with paroxetine (Dunnett's post-hoc test: p=0.0304 and p>0.05, respectively). When considering amplitude values, no difference was shown for sertraline or paroxetine compared to the control groups (Dunnett's post-hoc test; p>0.05).

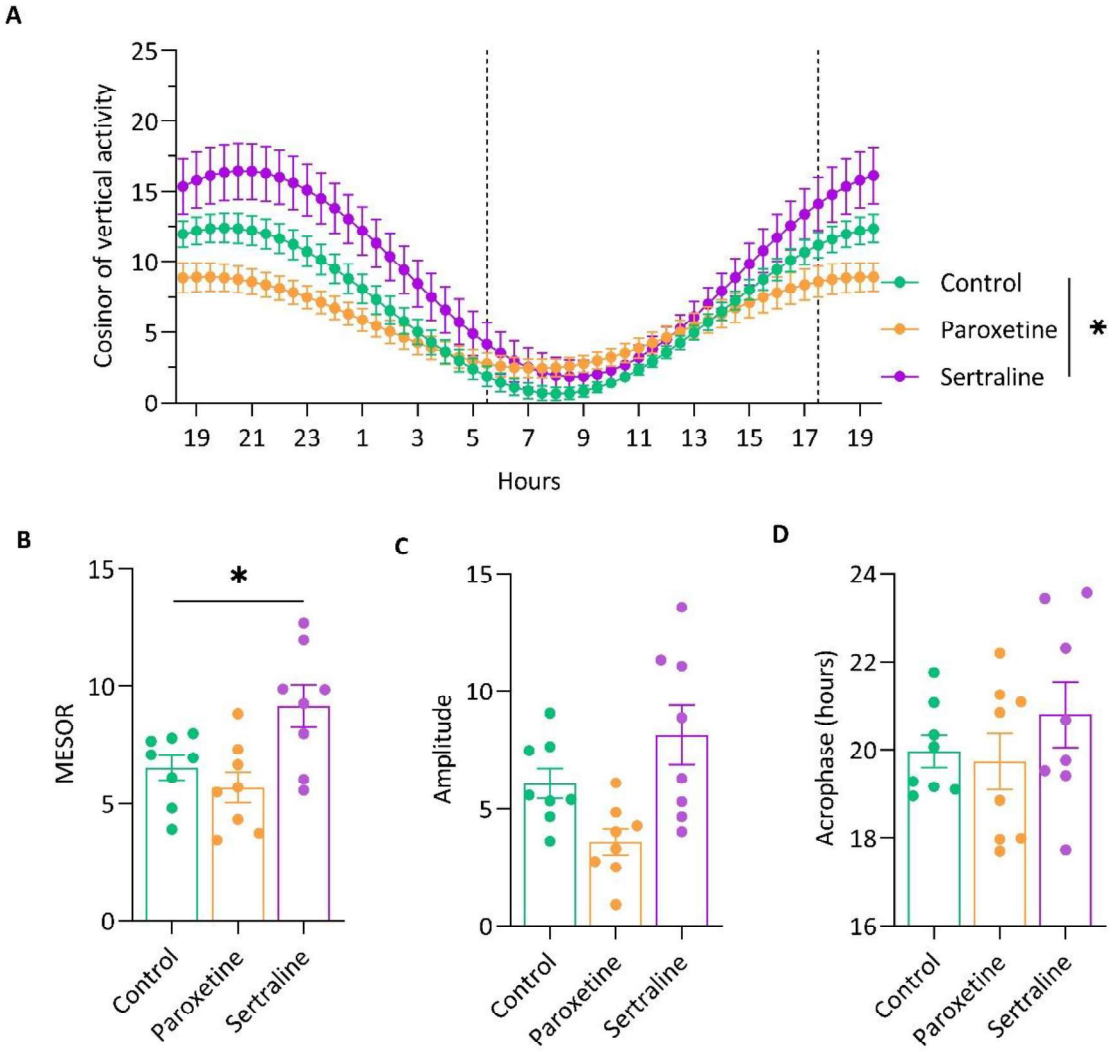

Fig. 2. Effects of acute administration of paroxetine or sertraline on (**A**) the cosinor of vertical activity and cosinor parameters: (**B**) MESOR, (**C**) amplitude and (**D**) acrophase (n=8/group). *p<0.05

After 3 days of administration, cosinor analysis of vertical activity did not reveal a significant group effect (ATS: F=1.11, p>0.05) but a significant group x time interaction (ATS: F=3.11, p=0.017) and a significant time effect (ATS: F=81.41, p<0.0001) (**Supplementary Fig. 3A**). Group x time interaction revealed that paroxetine, but not sertraline group, significantly differed from control group (multiple pairwise comparison: F=5.06, p=0.0074 and F=2.12, p>0.05, respectively). Post-hoc analysis of MESOR, amplitude and acrophase did not show any significative difference between groups (respectively Welch's ANOVA: $F_{2, 12.60}$=1.711, p>0.05; $F_{2, 12.22}$=2.030, p>0.05 and one-way ANOVA: $F_{2, 20}$=3.018, p>0.05) (**Supplementary Fig. 3**).

3.1.2. Horizontal activity

Cosinor analysis of total horizontal activity only reveal a significant time effect, after either acute or 3day administration (respectively, ATS F=50.73 and F=37.99, p<0.0001) (**Fig. 3A and B**).

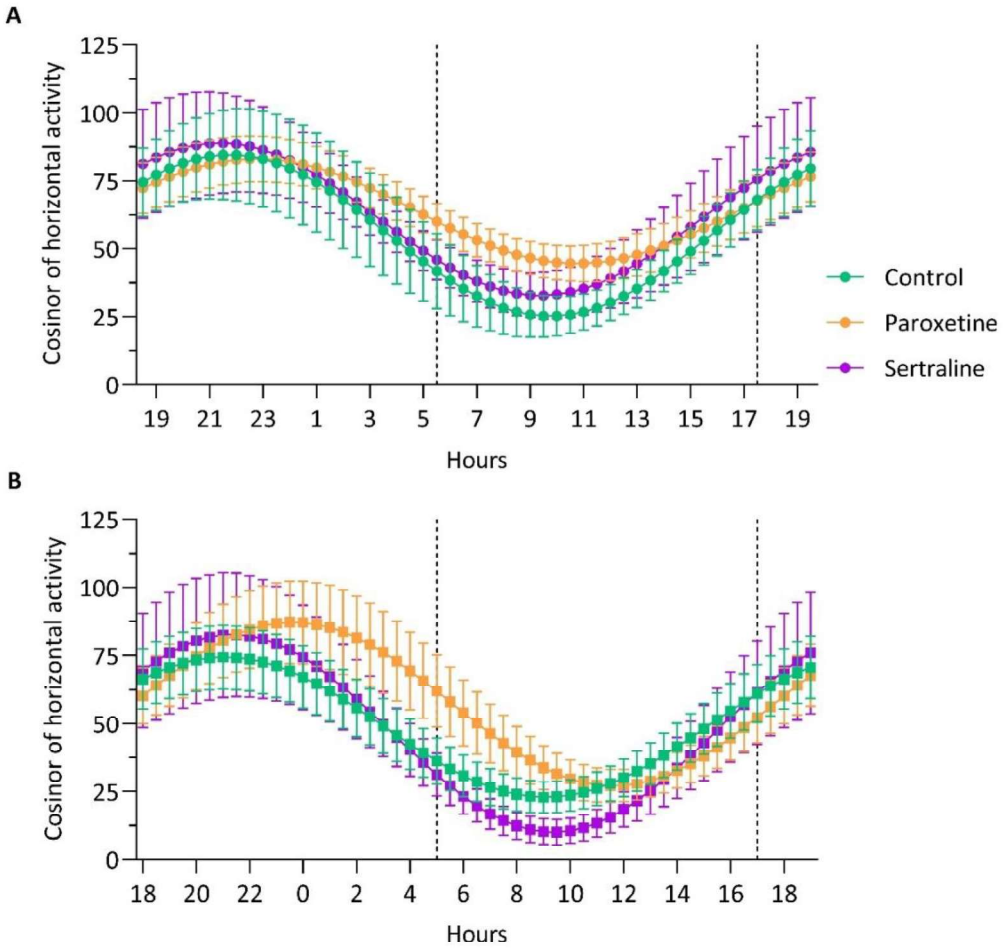

Fig. 3. Effects of (**A**) acute and (**B**) after 3 days of administration of paroxetine or sertraline on the cosinor of horizontal activity (acute: n=8/group; after 3 days: n=8/group, n=7 for control group).

After a single administration, cosinor analysis of back-and-forth activity did not reveal a significant group effect (ATS: F=0.41, p>0.05) nor a group x time interaction (ATS: F=0.88, p>0.05) but showed a significant time effect (ATS: F=53.26, p<0.0001) (**Supplementary Fig. 4**).

After 3 days of administration, cosinor analysis of back-and-forth activity revealed a significant group effect (ATS: F=4.72, p=0.011), a significant group x time interaction (ATS: F=3.01, p=0.019) and a significant time effect (ATS: F=94.70, p<0.0001) (**Fig. 4**). Post-hoc analysis revealed that paroxetine and sertraline groups significantly differed from control group (multiple pairwise comparison: F=10.15, p=0.0014 and F=6.38, p=0.015 respectively). A significant group x time interaction was found for paroxetine and sertraline groups compared to control group (multiple pairwise comparison: F=4.51, p=0.013 and F=3.08, p=0.042 respectively). Analysis of MESOR and amplitude showed a significative group effect (respectively Welch's ANOVA test: $F_{2, 11.03}$=6.590, p=0.0131 and $F_{2, 12.03}$=4.801, p=0.0293) (**Fig. 4B and C**) but not acrophase (one-way ANOVA: $F_{2, 21}$=3.387, p>0.05) (**Fig. 4D**). Analysis of MESOR values revealed a significant difference between paroxetine, but not sertraline, and control group (Dunnett's post-hoc test: $t_{9.385}$=3.109, p=0.024 and $t_{7.673}$=2.330, p>0.05, respectively) (**Fig. 4B**). Dunnett's post-hoc analysis of amplitude values did not show significant differences between paroxetine or sertraline and control groups (respectively $t_{11.42}$=2.327 and $t_{8.213}$=2.496, p>0.05, respectively) (**Fig. 4C**).

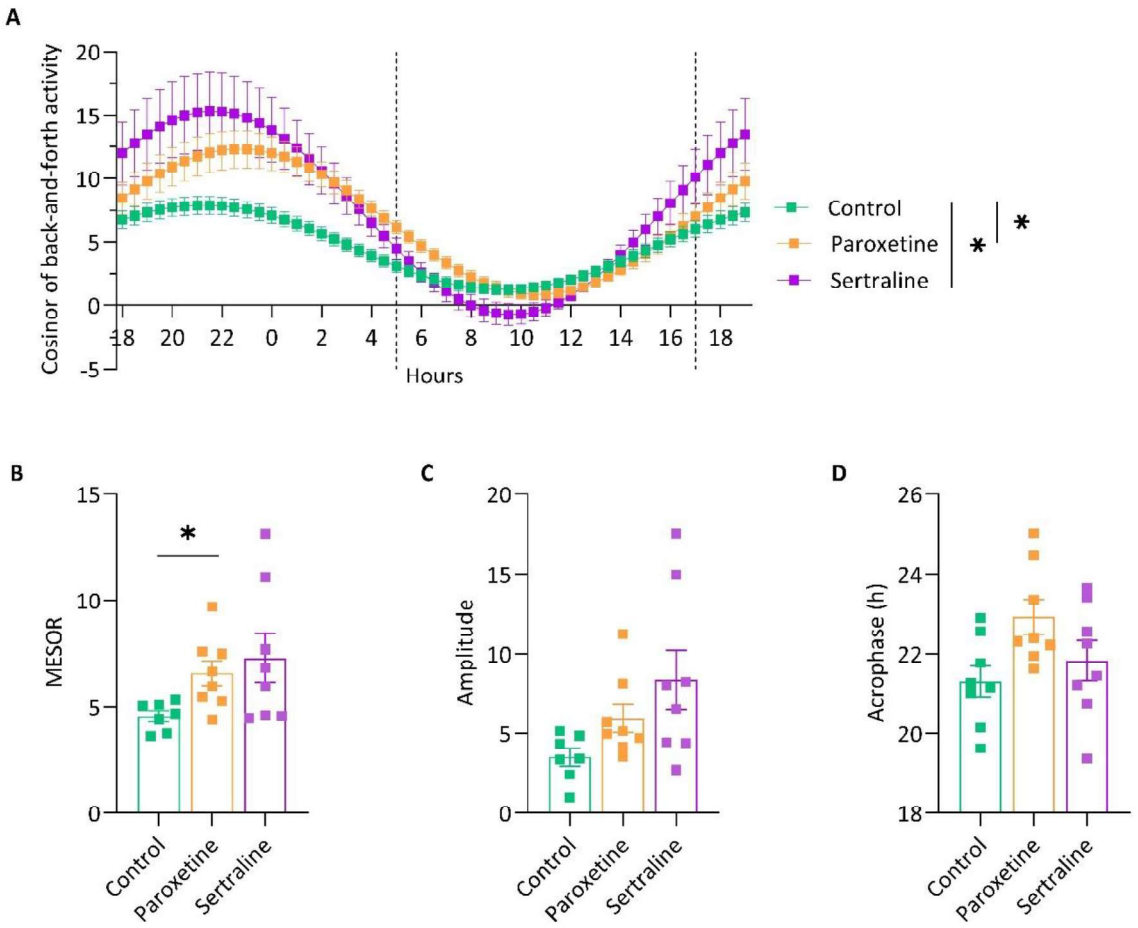

Fig. 4. Effects of 3 days of administration of paroxetine or sertraline on (**A**) the cosinor of back-and-forth activity and cosinor parameters: (**B**) MESOR, (**C**) amplitude and (**D**) acrophase (n=8/group, n=7 for control group). *p<0.05

3.2. MWM learning

Analysis of distance traveled to reach the hidden platform revealed a significant group effect at W6 but not at W2 (respectively two-way ANOVA: $F_{2, 19}$=3.533, p=0.049 and $F_{2, 20}$=0.9877, p>0.05), a significant time effect (respectively two-way ANOVA: $F_{4, 76}$=12.06, p<0.0001 and $F_{4, 80}$=22.92, p<0.0001) and no group x time interaction (respectively two-way ANOVA: $F_{8, 76}$=1.524, p>0.05 and $F_{8, 80}$=1.542, p>0.05) (**Fig. 5**). Post-hoc analysis of group effect at W6 revealed a significant difference between paroxetine and control group but not between sertraline and control group (Dunnett test: p=0.037 and p>0.05 respectively) (**Fig. 5B**).

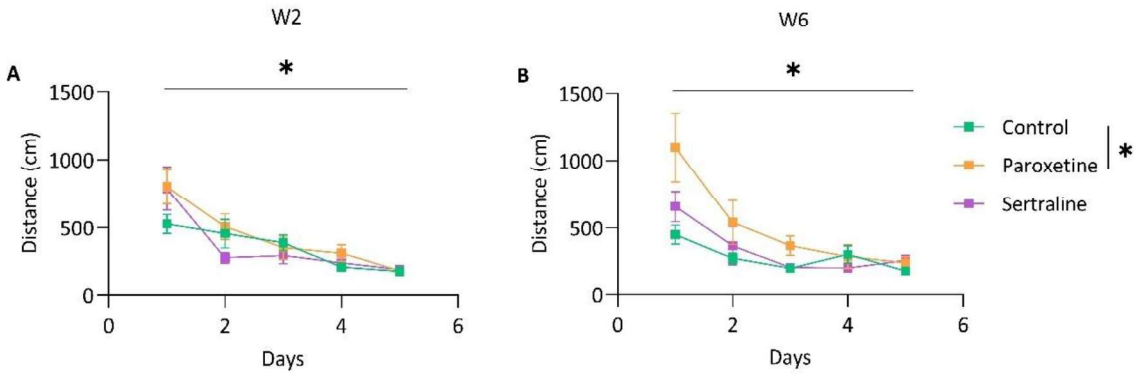

Fig. 5. Effects of 1 and 5 weeks of paroxetine or sertraline exposure on the distance moved to find the hidden platform across the 5 days of MWM learning (W+2: n=8/group, n=7 for control group; W+6: n=7 for control and sertraline groups; n=8 for paroxetine group). *p<0.05

Further analysis of distance traveled to reach the hidden platform during D1 of MWM learnings revealed a significant group and time effect (respectively, Mixed model: $F_{2, 61}$=3.350 and 7.659, p=0.042 and 0.0011), but no group x time interaction (Mixed model: $F_{4, 61}$=1.108, p=0.3608) (**Fig. 6**). Post-hoc analysis revealed a significant difference between paroxetine and control groups but not between sertraline and control groups (Dunnett test: p=0.0338 and p>0.05 respectively). Compared to W-2 performances, only control group had a significant decrease of the distance move on D1 of the W6 learning (Dunnett test: p=0.0034). Dunnett's multiple comparisons showed a significant comparison between paroxetine and control group on W6 (p=0.0074).

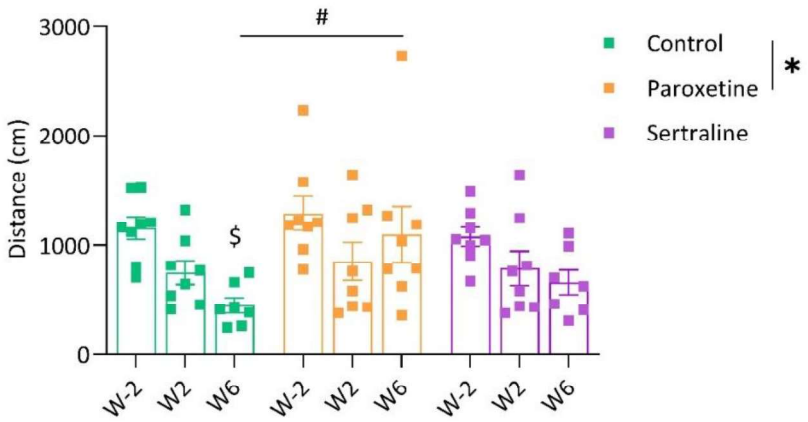

Fig. 6. Effects of 1 and 5 weeks of paroxetine or sertraline exposure on the distance moved to find the hidden platform across the day 1 (D1) of MWM learning (W-2: n=8/group; W+2: n=8/group, n=7 for control group;

W+6: n=7 for control and sertraline groups; n=8 for paroxetine group) compared to the first learning session on W-2. *p<0.05 compared to control group. $p<0.05 compared to W-2. #p<0.05 compared to the control group for the same week.

3.3. MWM probe test

Cumulative duration in target quadrant did not differ between groups for each time of exposure: on W3 and on W7 (respectively one-way ANOVA: $F_{2, 20}$=0.8528, p>0.05 and $F_{2, 19}$=0.7791, p>0.05) (**Supplementary Fig. 6A and B**).

Swimming speed was significantly different between groups at W3 and W7 (respectively, one-way ANOVA: $F_{2, 20}$=6.829 and 14.38, p=0.0055 and 0.0002) (**Fig. 7**). At W3, velocity showed a significant difference between paroxetine and control groups (Dunnett's post-hoc test: p=0.0030, **Fig. 7A**) whereas at W7 a significant difference was showed for paroxetine and sertraline with control group (Dunnett's post-hoc test: p<0.0001 and p=0.0321 respectively, **Fig. 7B**).

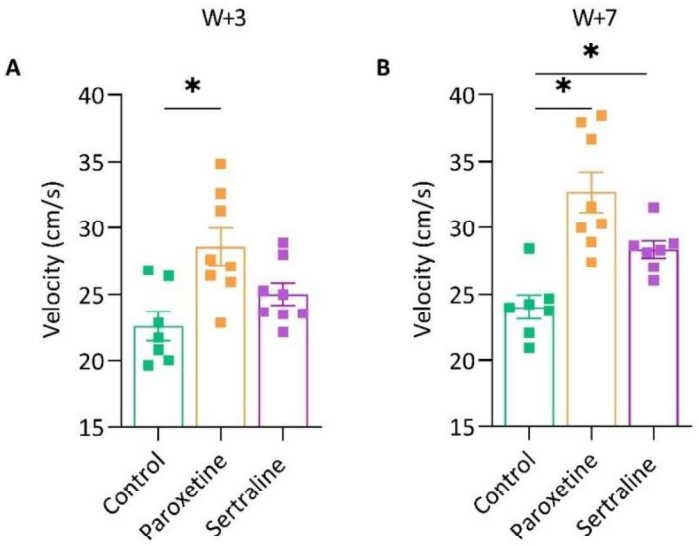

Fig. 7. Effects of different paroxetine or sertraline exposure times on the rats' velocity during MWM probe test: after (**A**) 2 weeks and (**B**) 6 weeks of administration (W+3: n=8/group, n=7 for control group; W+7: n=7 for control and sertraline groups; n=8 for paroxetine group). *p<0.05.

3.4. Novel Object Recognition Test

An OF test was carried out 24h prior to the NORT to evaluate anxiety-like behavior. Time spent in the central zone was not significantly different between groups (**Supplementary Fig. 7A**; one-way ANOVA: $F_{2, 19}$=1.504, p>0.05). Of note, velocity was similar whatever group of rats considered (**Supplementary Fig. 7B**; one-way ANOVA: $F_{2, 19}$=1.19, p=0.3509).

Recognition memory was assessed in NORT and did not differ between groups: on W4 and W5 (**Fig. 8A and B;** one-way ANOVA: $F_{2, 19}$=0.2249, p>0.05 and $F_{2, 19}$=0.1865, p>0.05, respectively). The recognition memory was significantly different from threshold value in control, paroxetine and sertraline groups on W4 (respectively univariate t-test: t_6=10.13, p<0.0001; t_7=5.404, p=0.001 and t_6=3.545, p=0.012) and W5 (respectively univariate t-test: t_6=6.841, p=0.0005; t_7=3.950, p=0.0055 and t_6=8.231, p=0.0002).

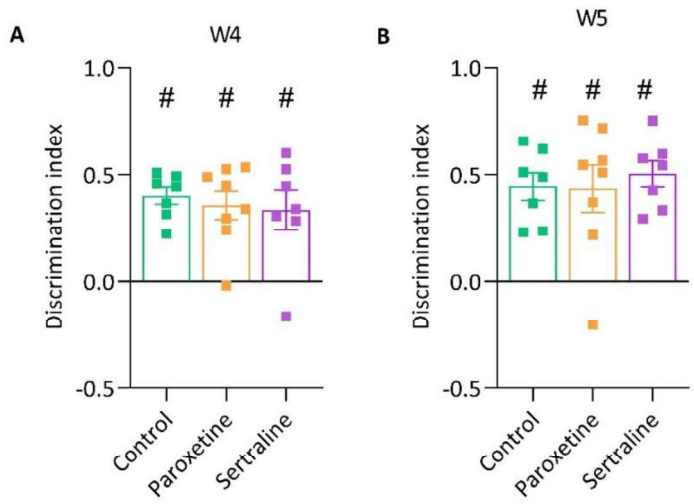

Fig. 8. Effects of chronic administration of paroxetine or sertraline on the recognition memory (n=7 for control and sertraline groups; n=8 for paroxetine group) during (**A**) W4 and (**B**) W5. #p<0.05 compared to the threshold value of 0.0.

3.5. Paroxetine and sertraline concentrations

3.5.1. Pharmacokinetic analysis after acute administration

The mean plasma concentration-time curves of paroxetine (**Fig. 9A**) and sertraline (**Fig. 9B**) after OG administration of single doses of 10 and 16mg/kg respectively are shown in **Fig. 9**. Pharmacokinetic parameters for paroxetine and sertraline were calculated and are shown in **Table 1**.

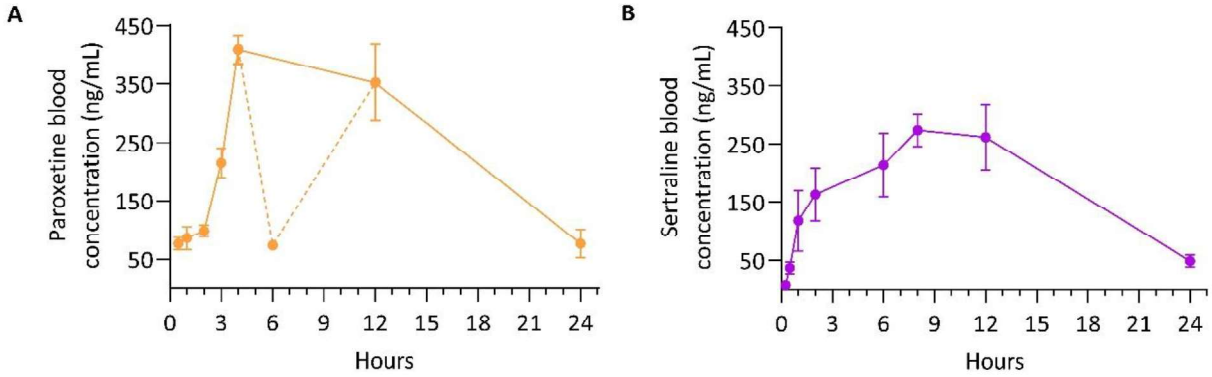

Fig. 9. Mean blood concentration-time curves following a single oral administration of paroxetine (**A**) and sertraline (**B**) (n=4 per time point).

	$T_{½}$ (h)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0→24h}$ (ng.h/mL)
PAROX	8.31±3.61	6.00±2.00	419.00±29.98	4,946.82±548.64
SERT	6.09±0.68	9.13±1.13	312.00±37.91	4,421.40±682.70

Table 1. Plasma concentrations obtained after a single OG administration of PAROX or SERT (10 or 16mg/kg respectively) to Sprague-Dawley rats. Values are mean ± SEM, n=4 per time point ($t_{1/2}$ was calculated without considering the 6-hour point for paroxetine group).

3.5.2. Concentrations after chronic administration compared to acute administration

Residual paroxetine concentrations significantly increased after chronic administration compared to acute administration (Mann-Whitney test: U=0, p=0.0040)

(**Fig. 10A**). Residual sertraline concentrations did not differ after acute or chronic administration (Mann-Whitney test: U=9, p>0.05) (**Fig. 10B**).

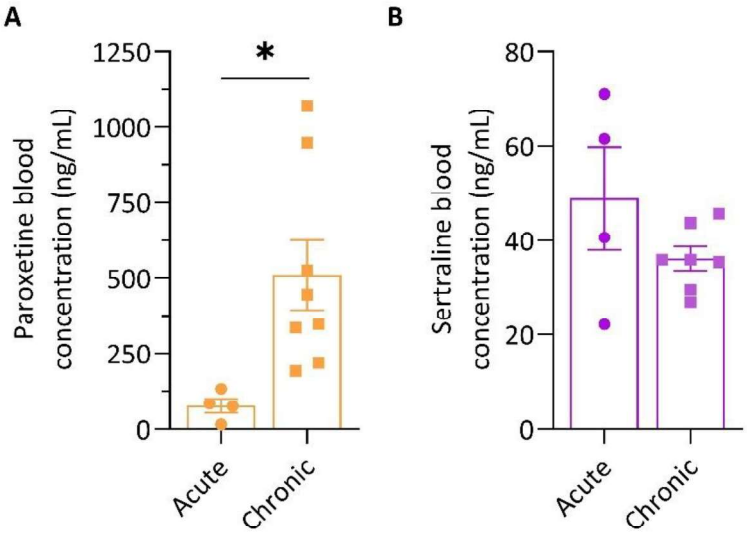

Fig. 10. Residual paroxetine and sertraline blood concentration after acute (n=4/group, t=24h after administration) and after 6 weeks of daily administration (n=8 for paroxetine and n=7 for sertraline, t=20h after the last administration). *p<0.05.

4. DISCUSSION

Our study evaluates for the first time neurocognitive consequences of short-term (after treatment introduction) and long-term (prolonged use) administrations of two widely prescribed SSRIs in elderly patients, paroxetine and sertraline. The main results of this study concern the behavioral disturbances observed during the treatment initiation period, resulting in different types of locomotor hyperactivity that could be interpreted as delirium-like effect. Furthermore, chronic administration of paroxetine, but not sertraline, led to a systemic accumulation associated with learning and flexibility cognitive disturbances.

Subtle changes in locomotor activity after acute administration of sertraline and paroxetine were monitored over a 24-hour period. Sertraline, but not paroxetine, induced a significant vertical hyperactivity, as indicated by an elevated MESOR. This acute stimulatory effect aligns with previous findings in mice, which showed that sertraline (8.9 mg/kg, SC), in contrast to paroxetine (7.9mg/kg, SC), increased spontaneous locomotor activity measured in actimeters between 60 ans 120min post-administration, without specifying whether the measured activity was vertical or horizontal (Sanchez et al., 2014). The differential effects of this compounds may be attributable to their distinct pharmacological profiles. Beyond serotonin transporter (SERT) inhibition, sertraline partially inhibits the dopamine transporter (DAT), with 20-fold higher affinity than paroxetine (Tatsumi et al., 1997), consistent with dopaminergic modulation of motor activity (Kitaichi et al., 2010). Thus, the partial inhibition of DAT by sertraline could be involved in the modulation of motor exploratory behaviors, such as rearing, compared with paroxetine.

Given that delirium-like effects in humans may appear after the first dose or within a few days of treatment, locomotor activity was re-assessed in rats after three days of SSRI administration. Both SSRIs increased back-and-forth activity without affecting total horizontal locomotion, suggesting stereotypical behaviors. Notably, only paroxetine significantly elevated the mean back-and-forth activity (MESOR) over 24 hours. To the best of our knowledge, this type of assessment had never been carried out before, our

results are thus original. This hyperactive profile, emerging after 3 days, consisted of repetitive, non-exploratory movements reminiscent of a delirium-like state, similar to patterns observed after tramadol administration in rats (Heraudeau et al., submitted). Moreover, a phase shift in circadian activity was observed, with paroxetine delaying the acrophase of back-and-forth activity from 21:45 to 23:48 compared with controls, indicating a potential treatment-induced circadian disturbance.

Acute hyperactivity in rodents has been already interpreted as a model of human delirium (Qiu et al., 2016), correlating with psychomotor agitation symptoms (Shenvi et al., 2020). SSRIs can provoke reversible agitation at treatment initiation in elderly patients (Sinclair et al., 2009), with delirium episodes typically appearing 3–4 days after onset (Kogoj, 2014; Masson, 2016). Our results are consistent with pharmacoepidemiological data collected by our team using WHO databases, which have shown that paroxetine, but not sertraline, is significantly associated with delirium reports (Minoc et al., 2024). Nevertheless, isolated cases of delirium under sertraline have also been described in humans (Friedrich et al., 2022).

Mechanistically, SSRIs generally stimulate the central nervous system. Paroxetine has been demonstrated to exhibit a higher SERT affinity than sertraline, which has the potential to increase synaptic serotonin levels (Tatsumi et al., 1997). As it was already suggested in the literature data, this may result in the manifestation of stereotyped behaviours or hyperactivity, which are characteristics of serotonin syndrome (Haberzettl et al., 2013). Paroxetine greater norepinephrine transporter (NET) affinity, combined with dopaminergic modulation, may further elevate locomotor activity (Mitchell et al., 2006). Additionally, paroxetine's anticholinergic properties, particularly M1 muscarinic receptor affinity, may contribute to stereotypical behaviors, as shown by hyperactivity following muscarinic antagonism with scopolamine (Qiu et al., 2016; Sanchez et al., 2014).

Following 6 weeks of daily administration, pharmacokinetic results showed that the residual paroxetine blood concentrations are much higher than after a single administration, suggesting a systemic accumulation, whereas sertraline concentrations remained unchanged. Interestingly, this increased exposure to paroxetine after chronic

treatment, is associated with cognitive impairments. Indeed, animals treated with paroxetine, but not sertraline, exhibited an increased travel distance during MWM learning compared to controls, suggesting an alteration in spatial learning performance. More specifically, the comparison of performance on D1 during the different learning sessions carried out at W-2, W2 and W6, highlights a gradual increase in performance for the control group, despite a reversal of the platform location. This progression is not observed in rats treated with ISSRs. Moreover, the distance covered at D1 of W6 is higher for the paroxetine group than for the control group and the overall performance at D1 for the paroxetine group is significantly worse than those of the control group. This lack of improvement in performance for the paroxetine group during learning tasks with reversed instructions probably reflects an impairment in cognitive flexibility and the adoption of ineffective search strategies, as it was suggested in prior studies (Nolan & Lugo, 2018; Saab et al., 2011). However, memory retention capacities did not appear to be impaired by chronic administration of sertraline or paroxetine. Indeed, neither paroxetine nor sertraline affected cumulative duration in the target quadrant during the MWM probe test or the discrimination index in the NORT. Overall, chronic administration of paroxetine or sertraline did not impair spatial memory retention or recognition memory, but paroxetine, for which we have demonstrated a systemic accumulation, could generate learning ability alterations and cognitive flexibility failure.

Previous studies have reported heterogeneous effects of chronic paroxetine or sertraline administration on learning and memory, largely depending on the experimental model and the presence or absence of pre-existing cognitive deficits (Ai et al., 2020; García-Gutiérrez et al., 2023; Menezes et al., 2018; Myint et al., 2007; Naudon et al., 2007; Rad et al., 2022; Tao et al., 2016). In contrast to these variable findings on memory processes, the present study specifically demonstrates a deleterious effect of chronic paroxetine on cognitive flexibility in healthy animals.

Although additional data are needed to explain this type of alteration, several mechanistic hypotheses can be formulated to explain these impairments. First, chronic exposure to paroxetine, may lead, probably *via* a systemic accumulation, to hyperactivity of the serotonergic system. This hyperactivity could lead, as has already been

demonstrated in the hippocampus, to alterations of long-term potentiation and synaptic plasticity (Villani & Johnston, 1993). Second, paroxetine's strong affinity for the M1 cholinergic receptor can generate anticholinergic effect that may disrupt hippocampus-dependent spatial learning (Sanchez et al., 2014). These effects may be exacerbated by systemic accumulation of paroxetine. In our study, conversely, sertraline, which has not shown systemic accumulation, did not induce spatial learning deficits. The data from the literature rather suggest memory-enhancing effects particularly by facilitating spatial memory consolidation and preventing cognitive impairment (Mele et al., 2004). This protective, or even facilitating effect could be linked to the pharmacological profile of sertraline, which have a weaker anticholinergic activity, combined with a partial inhibitory effect on the dopamine transporter (DAT), thereby increasing extracellular dopamine in striatal and accumbal regions (Kitaichi et al., 2010). This dichotomy aligns with literature showing that cholinergic activation in the hippocampus is necessary for memory encoding, while a reduction in acetylcholine actually facilitates information retrieval (Hasselmo, 2006). Overall, these findings support the hypothesis that SSRIs with substantial anticholinergic properties, such as paroxetine, may contribute to long-term cognitive decline (Attoh-Mensah et al., 2021).

Interestingly, during probe test, increased swimming velocity was observed for the paroxetine group at W2 and W6, and for the sertraline group at W6. In lack of improved performance in the probe test, this hyperactivity could reflect an increase in stress state, particularly for the paroxetine treated group. Besides, the animals in this group displayed the most handling difficulties during OG administration. This type of administration method is known to generate an additional stressor (Turner et al., 2012). These observations are consistent with literature reporting that paroxetine may enhance stress-related behaviors and sensitivity to environmental stressors after acute or repeated exposure (Amodeo et al., 2015; Drapier et al., 2007).

The pharmacokinetic data from our study revealed both a secondary plasma concentration peak around 12h after acute administration and a progressive systemic accumulation of paroxetine after repeated administration. The secondary peak may reflect enterohepatic recirculation, which prolongs drug exposure by temporarily

reintroducing the compound into circulation, as it was already suggested (Pollack & Brouwer, 1991). Although not documented for paroxetine, similar phenomena have been reported for other SSRIs in rats (Kreilgaard et al., 2008). In rats, paroxetine undergoes extensive hepatic metabolism and is predominantly excreted via the bile rather than urine, unlike in humans, which may favor potential enterohepatic recycling (Matsunaga et al., 2013)

The combination of significant biliary excretion and saturable metabolism can explain the long-term accumulation observed after repeated administration of paroxetine (Matsunaga et al., 2013). Importantly, these pharmacokinetic data were obtained in adult rats, yet accumulation was still observed after repeated administration. Therefore, it can be hypothesized that in elderly subjects, who are already at risk of drug accumulation, this effect may be even more pronounced. Indeed, in humans, for equivalent doses, plasma concentrations of paroxetine were significantly higher in elderly than in adults (Kaye et al., 1989). Given that paroxetine crosses the blood-brain barrier (Biegon et al., 1993), we could assume that elevated plasma levels may lead to increase cerebral exposure, potentially contributing to cognitive deficits. Although the paroxetine brain/serum ratio in mice (1.1) does not indicate preferential accumulation in the brain (Kreilgaard et al., 2008), plasma accumulation of the drug is nevertheless likely to increase brain concentrations. This increased central exposure, combined with the pharmacological properties of paroxetine, may exacerbate behavioral effects and contribute to the progressive worsening of cognitive deficits over time.

When compared with literature values, our pharmacokinetic results showed route- and age-dependent differences. For paroxetine, oral administration produced a longer half-life (80.14 *versus* 11.80h), delayed (6.00 *versus* 4.25h) and higher (419.00 *versus* 143.36ng/mL) peak concentration, and higher overall exposure (4,946.82 *versus* 1,425.99ng.h/mL) than intraperitoneal administration (Li et al., 2017). Sertraline peak concentration (312 *versus* 156ng/mL) and time (9h *versus* 1h) also differed from prior reports, potentially due to differences in animal age (Melis et al., 2012).

5. CONCLUSION

Whereas acute sertraline only induced hyperactivity, acute paroxetine produced both hyperactivity and stereotyped behaviors, consistent with a delirium-like state. Chronic paroxetine exposure, likely due to pronounced plasma accumulation, can lead to deficits in cognitive flexibility. These findings highlight that subtle differences in the pharmacological profiles of SSRIs can translate into meaningful differences in the risk of cognitive adverse effects, particularly with long-term treatment. The results of this study complement existing literature on the risks of cognitive impairment associated with some SSRIs and also confirm that knowledge of the pharmacological profiles of each drug within the same class can allow for the personalization of treatments according to patient profiles, particularly for the risk of cognitive disorders.

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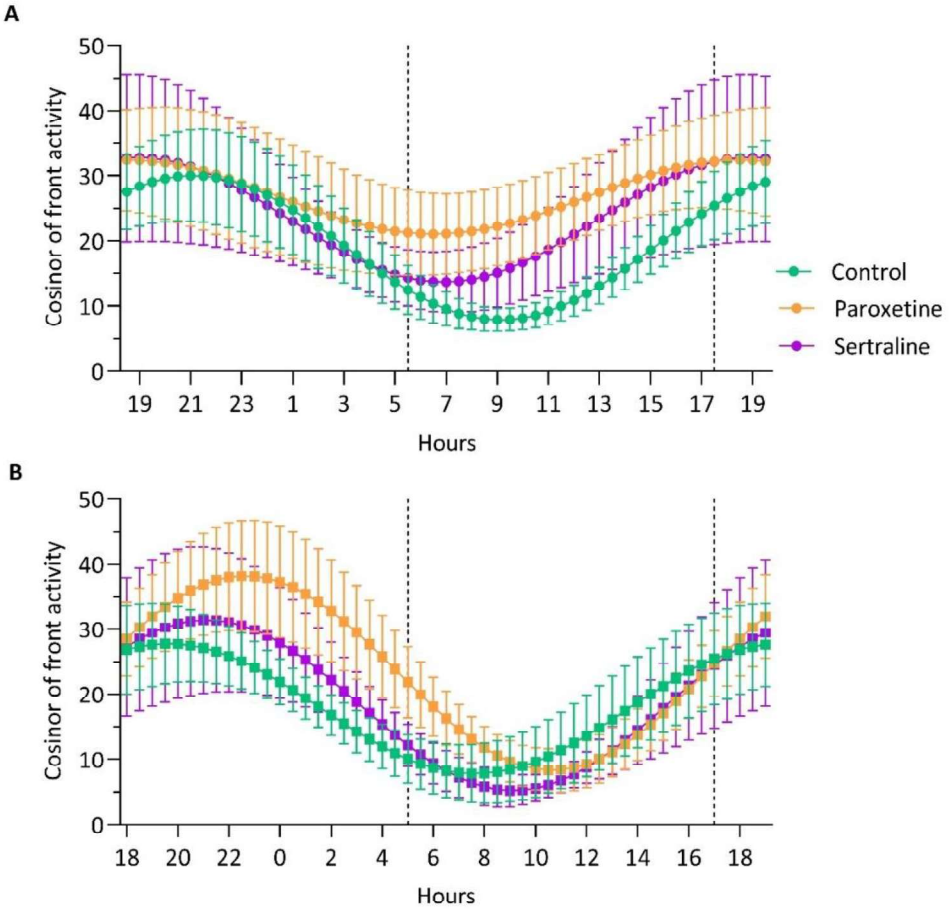
Supplementary Material

1. Locomotor activity

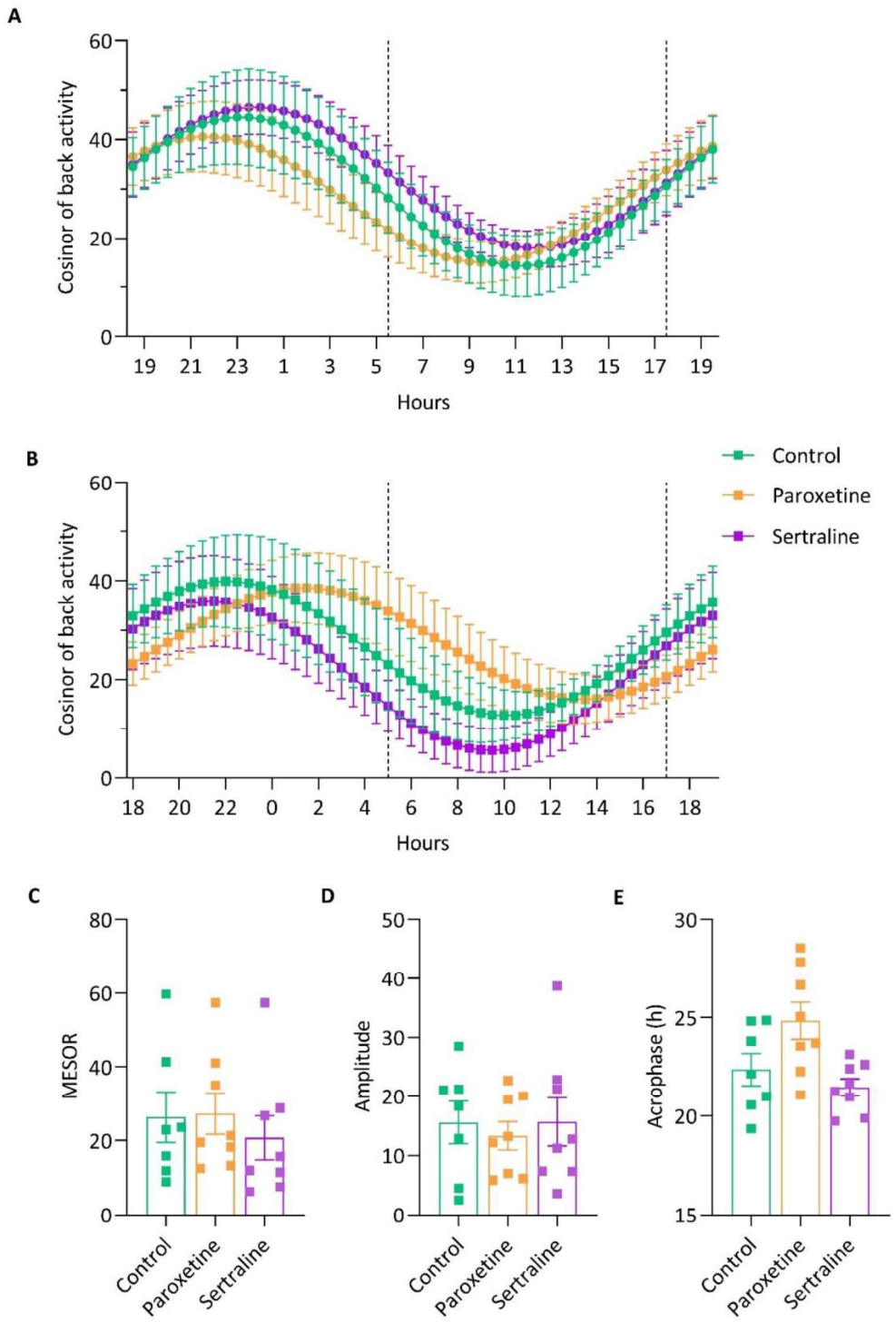
Whether after acute or after 3 days of administration, cosinor analysis of front activity did not reveal a significant group effect (ATS: respectively F=0.44 and F=0.61, p>0.05) nor a significant group x time interaction (ATS: respectively F=1.72 and F=0.86, p>0.05) but a significant time effect (ATS: respectively F=7.86, p<0.001 and F=21.67, p<0.0001) (**Supplementary Fig. 1A and B**).



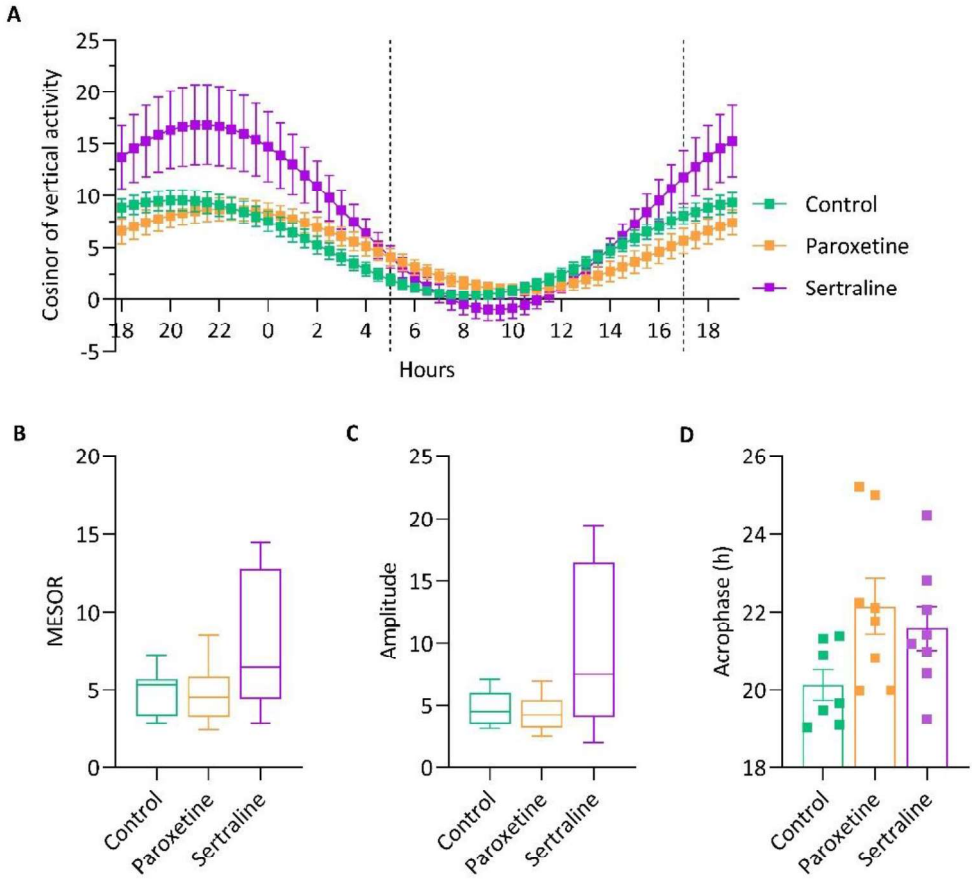
Supplementary Fig. 1. Effects of (**A**) acute and (**B**) after a 3-day administration of paroxetine or sertraline on the cosinor of front activity (acute: n=8/group; after 3 days: n=8/group, n=7 for control group).

After a single administration, cosinor analysis of back activity did not reveal a significant group effect (ATS: F=0.211, p>0.05) nor a group x time interaction (ATS: F=0.73, p>0.05) but showed a significant time effect (ATS: F=20.58, p<0.0001) (**Supplementary Fig. 2A**).

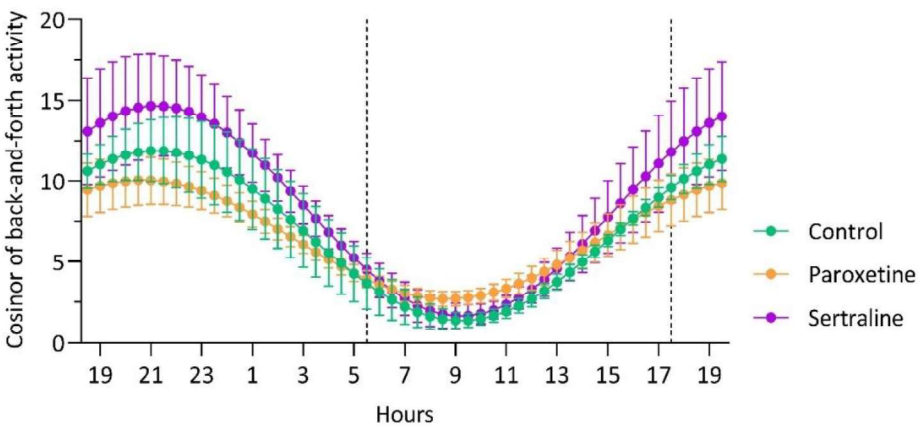
After 3 days of administration, cosinor analysis of back activity did not reveal a significant group effect (ATS: F=0.67, p>0.05) but a significant group x time interaction (ATS: F=3.23, p=0.021) and a significant time effect (ATS: F=29.81, p<0.0001) (**Supplementary Fig. 2B**). Post-hoc analysis revealed that group x time interaction significantly differed between paroxetine and control group (multiple pairwise comparison: F=3.96, p=0.026) but not for sertraline group (multiple pairwise comparison: F=0.25, p>0.05). Analysis of MESOR and amplitude did not show significative difference between groups (respectively one-way ANOVA: $F_{2, 20}$=0.3450, p>0.05 and $F_{2, 20}$=0.1547, p>0.05) (**Supplementary Fig. 2C and D**). Analysis of acrophase revealed a significant group effect (one-way ANOVA: $F_{2, 20}$=5.457, p=0.0128) but Dunnett's post-hoc analysis revealed no difference between paroxetine or sertraline compared to control group (p>0.05) (**Supplementary Fig. 2E**).



Supplementary Fig. 2. Effects of (**A**) acute administration of paroxetine or sertraline on the cosinor of back activity (n=8/group). Effects of a 3-day administration of paroxetine or sertraline on (**B**) the cosinor of back activity and cosinor parameters: (**C**) MESOR, (**D**) amplitude and (**E**) acrophase (n=8/group, n=7 for control group). *p<0.05



Supplementary Fig. 3. Effects of a 3-day administration of paroxetine or sertraline on the cosinor (**A**) and the parameters of vertical activity: (**B**) MESOR, (**C**) amplitude and (**D**) acrophase. (n=8/group, n=7 for control group).

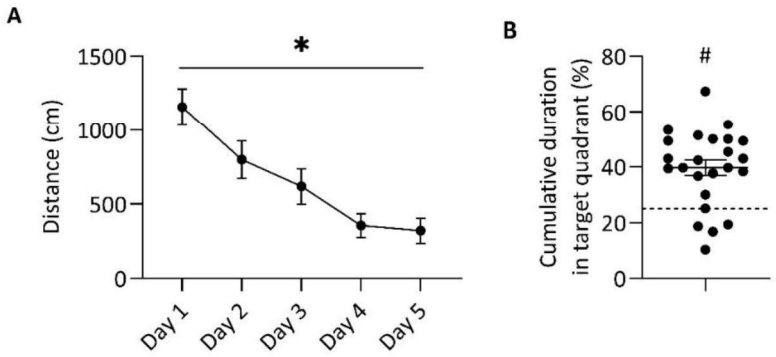


Supplementary Fig. 4. Effects of acute administration of paroxetine or sertraline on the cosinor of back-and-forth activity (n=8/group).

2. Morris Water Maze

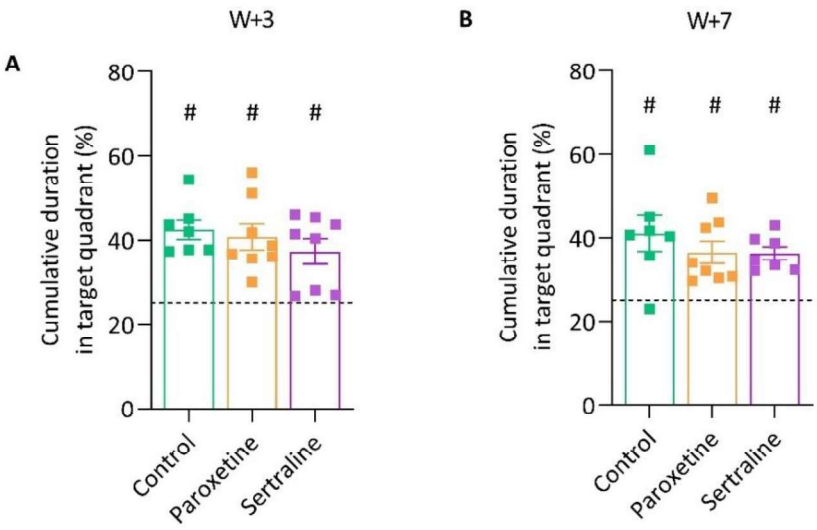
At W-2, during the five days of training and before group randomization, the distance moved by all the rats to find the hidden platform significantly decreased from D1 to D5 (**Supplementary Fig. 5A**; one-way ANOVA: $F_{4, 115}$=43.77, p<0.0001), testifying that spatial learning occurred.

Spatial recognition memory was assessed by MWM probe tests after various periods of exposure to paroxetine or sertraline. The initial probe test was realized before groups randomization at W-1 and revealed a significant difference from threshold value of 25%, indicating that the spatial learning abilities of all rats remained comparable before treatment initiation (**Supplementary Fig. 5B**; univariate t-test: t_{23}=5.202, p<0.0001).



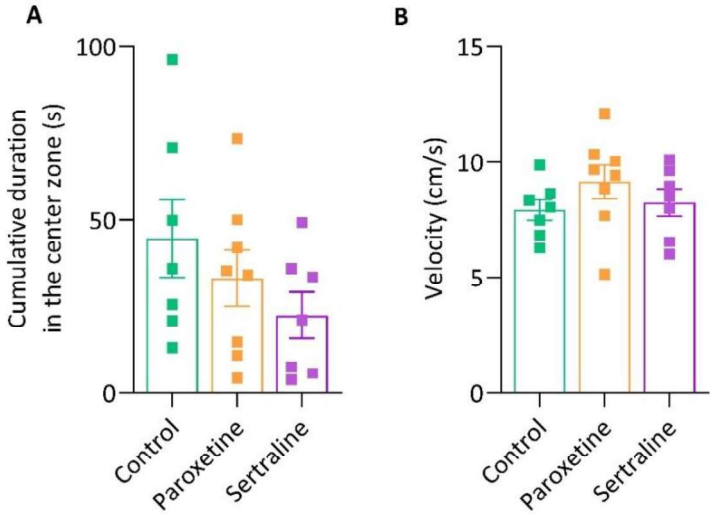
Supplementary Fig. 5. Evaluation of (**A**) the distance traveled to find the hidden platform over the 5-day MWM learning phase and (**B**) the cumulative time spent in the target quadrant during the MWM probe test, both prior to treatment (n=24). *p<0.05, compared with 25% threshold represented by the dashed line (n=8/group).

Regardless of the probe test considered (W3 and W7), time spent in target quadrant was significantly different from the random exploration value of 25% for the control group (univariate t-test for W3: t_6=7.634, p=0.0003 and for W7: t_6=3.749, p=0.0095), for paroxetine group (univariate t-test for W3: t_7=5.138, p=0.0013 and for W7: t_7=4.328, p=0.0034) and for sertraline group (univariate t-test for W3: t_7=4.066, p=0.0048 and for W7: t_6=7.025, p=0.0004) (**Supplementary Fig. 6**).



Supplementary Fig. 6. Effects of different paroxetine or sertraline exposure times on the cumulative duration in target quadrant during MWM probe test: after (**A**) 2 weeks and (**B**) 6 weeks of administration (W+3: n=8/group, n=7 for control group; W+7: n=7 for control and sertraline groups; n=8 for paroxetine group). #p<0.05, compared with 25% threshold represented by the dashed lines.

3. Novel Object Recognition Test



Supplementary Fig. 7. Effects of chronic administration of paroxetine or sertraline on (**A**) the time spend in the center zone and (**B**) the velocity of rats during Open Field test (n=7/group; n=8 for paroxetine group).

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Micropipette-guided Drug Administration (MDA) as a non-invasive chronic oral administration method in male rats

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ABSTRACT

Background: In preclinical studies resorting to rodents, the effects of prolonged oral intake of active substances are difficult to evaluate. Indeed, to get closer to clinical reality, oral gavage (OG) is frequently used but the repetition of administrations induces risks of lesions of the digestive tract, and stress for animals which can compromise the quality of the results.
New method: This study describes the development of a non-invasive oral administration method in male Sprague Dawley rats, as a safe alternative of OG, more faithful to clinical reality and limiting biases in pharmacokinetics and/or pharmacodynamics interpretation. Micropipette-guided Drug Administration (MDA) is based on the administration by micropipette of a sufficiently palatable vehicle for the animals to voluntarily take its contents.
Results: MDA was not demonstrated as less stressful than OG. A pharmacokinetics equivalence between MDA and OG was demonstrated for pregabalin administration but not for aripiprazole. Despite the use of a sweet vehicle, the MDA method does not result in weight gain or significant elevation of blood glucose and fructosamines level. Regarding the time needed to administrate the solution, the MDA method is significantly faster than OG.
Comparison with existing method(s): Contrastingly to procedures using food or water, this method allows for a rigorous control of the time and dose administered and is delivered in discrete administration windows which is therefore closer to the clinical reality. This method appears particularly suitable for pharmacological evaluation of hydrophilic compounds.
Conclusions: The MDA procedure represents a respectful and adapted pharmacological administration method to study the effects of chronic oral administration in rats.

1. Introduction

Drug consumption is associated with a risk of adverse effects, the probability of which varies according to patient (age, sex, weight, *etc.*) and the progression of the pathology. Prior to marketing, drug adverse effects are identified during clinical trials. As a result, a specific warning is given to patient during prescription to enable detection of adverse effects as early as possible. Unfortunately, some adverse effects may go undetected during clinical trials. The duration of exposure investigated is too short (relative to a lifelong treatment administered for chronic disease) to identify late-onset adverse effects. Post-marketing pharmacovigilance systems and real-life pharmaco-epidemiological studies may identify some associations between a given drug or a drug class and an adverse effect. However, this new signal needs to be confirmed and studied by further investigations. Animal models could help to better understand the reasons for late-onset adverse effects and thus improve our drug prescription practices and make them safer.

Oral route is the preferred method of drug administration (80% of pharmaceutical forms distributed), mainly because it is less invasive and less expensive. In the field of preclinical research on rodents, oral gavage (OG) is hence used to promote the transition to clinical use. However, while OG technique has the advantage of precise control over time and dose of administration, it also does have drawbacks. Indeed, a certain degree of technical skills is required from experimenters, since the

Abbreviations: MDA, Micropipette-guided Drug Administration.

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animal has to be restrained. In addition to discomfort, restraint can be a source of stress for animals (Stuart and Robinson, 2015). Beyond all this, repeated OG can also lead to aspiration pneumonia, as well as irritation, injury or even rupture of pharyngeal, esophageal and/or gastric membranes (Brown et al., 2000).

In line with evolving ethical concerns and in order to respect animal welfare as much as possible, new less invasive oral administration techniques have been developed. One such strategy consists in adding the drug either to the feed (Abelson et al., 2012; Diogo et al., 2015; Hovard et al., 2015) or to the drinking water (Zhan et al., 2019). Although these are stress-free methods for animals as no interaction is required, animals need to be housed individually to ensure correct dosing. Ideally, rats should be housed in groups to reduce the stress levels (Sharp et al., 2002). They might also be criticized from a pharmacological point of view. Indeed, constant access to a pharmacological substance (*via* food or water) could lead to a different plasmatic profile to a clinical situation with oral administration at specific time intervals (Turner et al., 2011a,2011b).

Recently, an innovative non-invasive method called Micropipette-guided Drug Administration* (MDA) has been described in the mouse (Scarborough et al., 2020). This method takes advantage of the sweet and appetizing properties of sweetened condensed milk, which is used as a vehicle for drug delivery. Its palatability induces voluntary ingestion of the micropipette solution by the animals. Taking into account ethical concerns, this method also appears to be more comparable to oral administration used in humans. Authors also demonstrated that MDA was less stressful for mice than OG and intraperitoneal (IP) administration (respectively Scarborough et al., 2020; Schalbetter et al., 2021). However, the effect of using a sweet vehicle (often criticized) on glucose metabolism has never been evaluated. Techniques using this type of delivery, such as those using syringes, are often criticized for being very time-consuming (Atcha et al., 2010). For the first time, we compared the time required for the administration procedure by OG *vs* MDA.

Herein, we aimed to adapt this method for use in rats. Rats have a wider behavioral repertoire than mice and have a higher blood volume, which is sometimes necessary when performing numerous biochemical assays. The practicality / compatibility of the method was evaluated and compared to the commonly used oral gavage (OG) method. This included assessment of animals motivation / willingness over-time (over repeated administrations), animal welfare (corticosterone, glucose and fructosamine levels). Furthermore, a comparative study of the pharmacokinetic properties of the two methods (MDA *versus* OG) was performed using two psychoactive substances. For this purpose, the antiepileptic drug pregabalin and the antipsychotic drug aripiprazole were chosen. These are two widely used drugs prescribed for long-term illness(Evoy et al., 2021; Rhee et al., 2020). They are of particular interest for such pharmacokinetic studies because of their different physicochemical properties.

2. Materials and methods

2.1. Ethical concerns

Experimental procedures were performed in accordance with the European Communities Council Directive (2010/63/UE) and approved by the regional ethical committee (Comité d'Ethique NOrmandie en Matière d'EXpérimentation Animale, CENOMEXA) (agreement number: 35428).

2.2. Animals

Male Sprague-Dawley rats (n = 45), 6 weeks of age at the start of the protocol were used (Janvier Labs, France). Animals were randomly housed in pairs in standard polyacrylic cages (42x26x18,5 cm^3) with free access to food and tap water. Cage enrichment consisted in crinkle cut shredded paper and a cardboard roll. The animal facility was maintained under a normal 12 h light-dark cycle (lights on from 7 am to 7 pm), with a constant temperature (22 ± 1 °C) and humidity (50 ± 10%). During the first week following arrival, rats were left undistributed (acclimation to animal facility). During the second week, daily handling sessions were performed to accustom the rats to the experimenter (5 min per day, 7 days).

2.3. Oral administration methods and general experimental design

Random tables were used to separate animals into 2 groups according to the oral administration method: OG (n = 22) or MDA (n = 23) (see Fig. 1). Treatment (either OG or MDA method) was performed daily (early in the morning) for 3 weeks (from D1 to D21) in the administration room separate from the animal facility. Of note, to avoid additional stress, animals were acclimated in the administration room for 30 min

OG group: the procedure began on D1. Briefly, rats were restrained by the trunk (see Fig. 2 A) and a probe was inserted into the mouth all way down to the stomach. Of note, a flexible plastic probe (PHYMEP®) was used for gavage refinement (Okva and Tamos, 2006). In addition, to limit mortality or adverse respiratory effects, the gavage volume was set at 1.5 mL/kg.

MDA group: a two-phase training procedure is required. As a one-week procedure, this training can be performed during the animal's handling phase to save time (as we did here) (see Fig. 1). During the first 3 days, animals were habituated to the smell and taste of a sweetened condensed milk solution (RÉGILAIT® full-fat milk: saccharose 45%, fat 8%, defatted lactic dry extract 20%), diluted at 3/10 with rodent bottle water. A 2 mL cup of this appetizing solution was placed in the cage each day. On the last four days of the week, a micropipette P1000 tip (Starlab®) containing the solution was presented to the rats through the cage grid. The pipette was left in place until the rat licked the tip and swallowed the solution (see Fig. 2B and Supplementary material – Video). The administration volume was set at 0.5 mL/kg. If an animal refuses to consume the micropipette content, a gentle restraint can be made for administration.

2.4. Effects of the administration method on the animal welfare

In each group (OG and MDA), 14 rats were used. They were treated daily for a chronic period (3weeks) (Fig. 3).

2.4.1. Body weight evolution

On each morning of the experimental protocol, just prior to administration, all animals in these groups (n = 28) were weighted from D-6 to D21.

2.4.2. Behavioral assessment

All devices were cleaned with 70% ethanol at the beginning and between each animal to avoid odor cues. Behavioral tests were performed 1 h after oral administration.

On D1, *i.e.* after the first oral administration, an elevated plus maze test was performed. The polyvinyl chloride apparatus consisted of 4 arms (50x10cm^2), with a central platform (10x10cm^2; Imetronic®). Two opposite arms were open (40Lux) and other two were closed by a 30 cm high wall (10Lux). The apparatus was elevated 75 cm above the floor. A rat was placed on the central platform facing an open arm and allowed to explore the apparatus for 5 min. The number of entries and the percentage of time spent in the open arms were calculated and used as an index of anxiety-like behavior (Lister, 1987).

On D20, *i.e.* after 3 weeks of daily administration, an open field test was performed. The apparatus consisted of a white polyvinyl chloride open box (100x100x45cm^3, 10Lux at the center, 7Lux at the corners). The rat was placed in the center of the apparatus and allowed to explore freely for 20 min (Soares-Silva et al., 2022). The percentage of time spent in the center was calculated and used as an index of anxiety-like

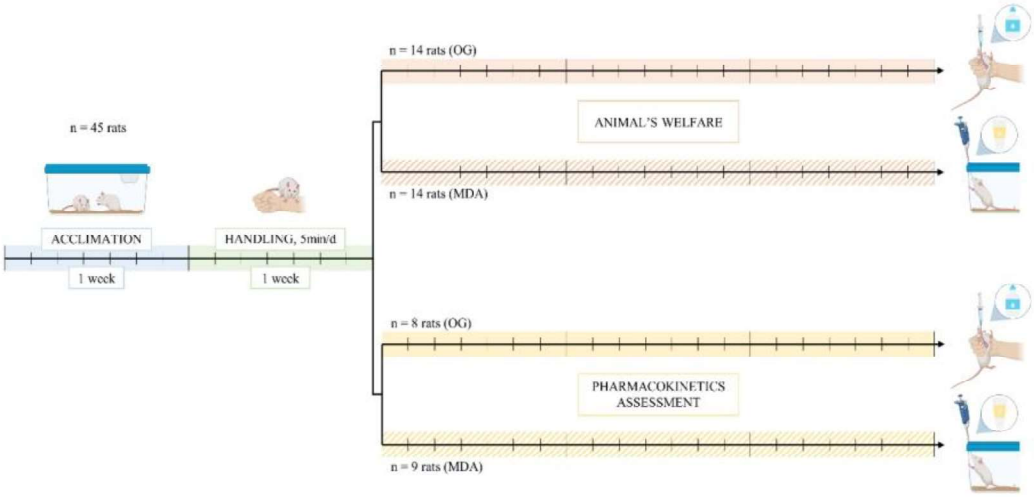

Fig. 1. General experiment design (created in BioRender.com).

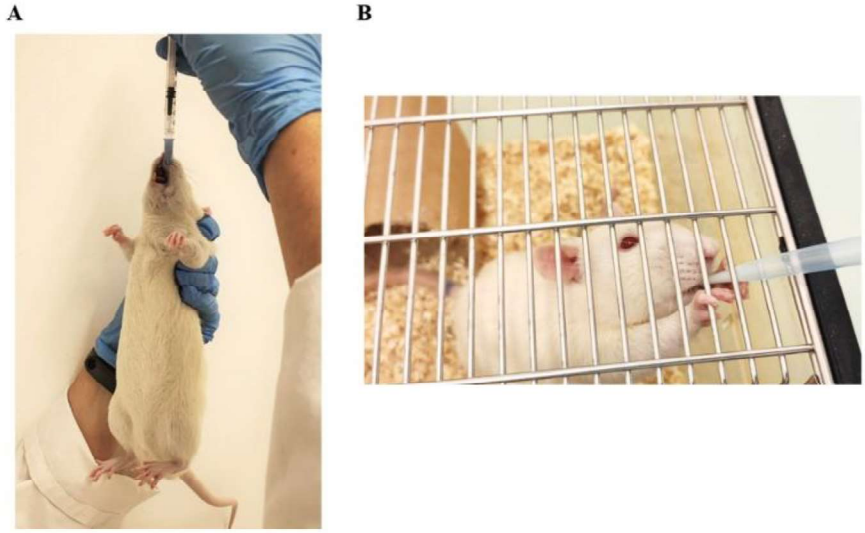

Fig. 2. (**A**) Restraint of the rat during OG method, (**B**) Micropipette displays through cage grid during MDA method.

behavior.

2.4.3. Biochemical analysis

To compare both acute and chronic effects of OG *versus* MDA, blood samples were collected at two time points of the treatment procedure, either 30 min after a single administration (n = 4) or after 3 weeks of daily treatment (n = 10). Rats received an intraperitoneal bolus of pentobarbital (120 mg/kg in a volume of 2,19 mL/kg) and were killed by cervical dislocation 5 min later. Blood was then collected from the jugular vein in either 2 mL sodium fluoride or 4 mL sodium heparin tubes. Blood collected in sodium fluoride tubes was centrifuged (3000 g, 10 min, Room Temperature) and the supernatant plasma was collected and stored at − 20 °C until analysis. Glycemia was measured through glucose hexokinase method (DxC 700 AU, Beckman-Coulter Instruments, USA). Blood collected on sodium heparin tubes was centrifuged (3000 g, 20 min, 4 °C) and the supernatant plasma was collected and stored at − 20 °C until analysis. Fructosamine levels were determined through colorimetric method (Konelab, Thermo Fisher Diagnostic). Corticosterone concentration was measured as a stress indicator (Marin et al., 2007) using liquid chromatography/tandem mass spectrometry (LC-MS/MS). Chromatography was performed on an ABSciex API 5500 QTRAP triple quadrupole mass spectrometer (Framingham, MA USA) equipped with an electrospray ionization source.

2.5. Effects of the administration methods on the pharmacokinetics

Two drugs, pregabalin and aripiprazole (see below; n = 4 rats per group, except for MDA-aripiprazole with n = 5 rats) were studied to compare the two methods of oral administration (OG *versus* MDA) from a pharmacological point of view (see Fig. 4).

2.5.1. Pharmacological agents

The two psychotropic drugs were chosen because of their different physicochemical properties: pregabalin (Merck®) and aripiprazole (Thermo Fisher®). The former, pregabalin, is a hydrophilic compound (logP=−1.78), while the latter, aripiprazole, is a lipophilic compound

Video S1. Administration process of Micropipette-guided Drug Administration.A video clip is available online. Supplementary material related to this article can be found online at doi:10.1016/j.jneumeth.2023.109951.

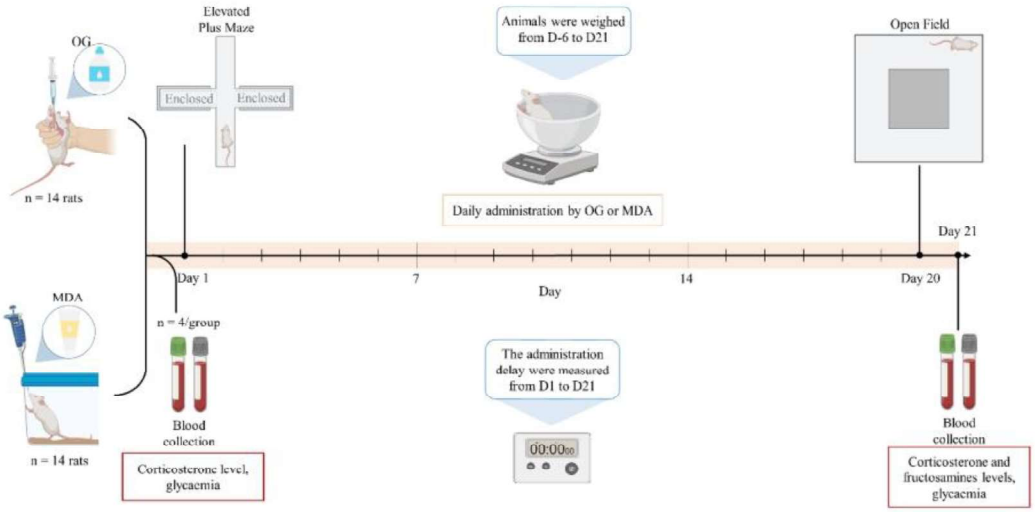

Fig. 3. Experimental protocol of effects of the administration method on the animal welfare *(created in BioRender.com)*.

(logP=5.30, pKa_1 =7.46, pKa_2 =13.51). Doses of pregabalin and aripiprazole (10 and 3 mg/kg, respectively) were chosen according to literature pharmacokinetics data (Lau et al., 2013; Raish et al., 2019). Considering its physicochemical properties, aripiprazole was first prepared in a mixed of Tween 20 (1% solution (Martins et al., 2008)) and acetic acid (0.17‰ solution), and finally in the vehicle solution (pH=6.18). Vehicle solution was either diluted in sweetened milk or in saline, according to oral methods used (MDA and OG, respectively).

2.5.2. Pharmacokinetic studies

At the end of the 3-weeks treatment period, rats were sacrificed by cranial percussion and decapitation one hour after the last administration. This killing method was used because it does not need any sedation and thus avoids any drug interaction with the anesthetic. Blood was collected directly from the jugular vein into sodium heparin tubes (4 mL capacity). Samples were centrifuged (3000 g, 20 min, RT) and the supernatant plasma was then collected and stored at − 20 °C until analysis.

Pregabalin and aripiprazole plasma concentrations were measured using liquid chromatography/tandem mass spectrometry (LC-MS/MS) according to an adaptation of the analytical conditions described by Zhang et al. (2022) and Kirschbaum et al. (2010) respectively. Chromatography was performed on an ABSciex API 4500 QTRAP triple quadrupole mass spectrometer (Framingham, MA, USA) equipped with an electrospray ionization source. Acquisition was realized in multiple reaction monitoring (MRM) mode. Separation was performed on a

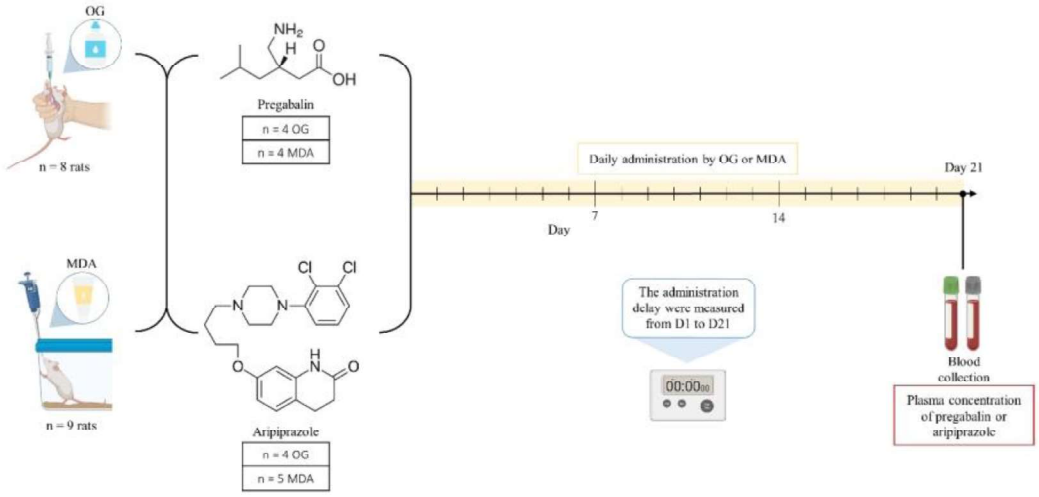

Fig. 4. Experimental protocol of assessment of pharmacokinetics equivalence *(created in BioRender.com)*.

reversed-phase Biphenyl column (2.1x100mm, Phenomenex, USA). Mobile phases consisted of 1 mM ammonium formate in water and 0.02% formic acid, (A phase) and ammonium formate 1 mM in methanol and 0.02% formic acid (B phase), with gradient elution at 0.5 mL/min. Column temperature was maintained at 40 °C. All analyses were performed using positive ion ESI (ESI+).

Pregabalin was extracted from rat plasma using protein precipitation. To 100 µL of plasma were added, 200 µL of internal standard (D6-Pregabalin, 0,5 mg/L) and 100 µL of water were added. The supernatant of the protein precipitated mixture was extracted and reconstituted in 200 mL of acetonitrile for a second centrifugation prior to LC-MS/MS quantification.

Aripiprazole was extracted from rat plasma using liquid-liquid extraction. To 100 µL of plasma were added 50 µL of internal standards (D6-Venlafaxine and D3-Olanzapine, 250 ng/mL), 500 µL of 0,25 M sodium hydroxide solution and a mixture of ethyl acetate/dichloromethane/hexane (40/22/38 v/v). The organic phase was extracted and dried, then the extract was reconstituted in 100 mL of A mobile phase prior to LC-MS/MS quantification.

2.6. Applicability of the administration method to large groups

The delay to perform oral administrations was daily collected from D1 to D21 for all animals (n = 45). This time includes the weighing of the rat, the preparation of either syringe or micropipette and the administration itself.

2.7. Statistical analysis and figures

Statistical analyses were performed using Prism (version 8.0; GraphPad Software, USA). Data that followed a Gaussian distribution (Kolmogorov-Smirnov normality test) and had equal variances are presented as mean ± Standard Error Mean (SEM) and were analyzed using parametric statistical tests (unpaired student t-test or ANOVA for repeated measures). Data sets that did not meet either of these criteria are presented as median ± interquartile range (IQ) and were analyzed using non-parametric statistical tests (Kolmogorov-Smirnov test). Statistical significance was set at $p < 0.05$.

Figures have been created with BioRender.com (agreement number: MG252P2KJK).

3. Results

3.1. Effects of the administration method on the animal welfare

3.1.1. Stress response

Anxiety-like behavior was first assessed by the EPM after a single oral administration, then by the OF test 3 weeks later after repetitive daily oral administration. No statistical group difference (OG *versus* MDA) was observed in the EPM test (Supplemental Fig. 1). Similarly, the OF test showed no major difference between groups. In fact, neither the total distance moved nor the percentage of time spent in the central zone differed between groups (Fig. 5A; $t(18) = 0.81$, $p = 0.43$; Fig. 5B; $t(18) = 0.75$, $p = 0.46$, unpaired t-test). Although, a statistically higher number of entries in the central zone was observed for MDA group, reflecting thus a lower level of anxiety-like behavior (Fig. 5C; $t(18) = 2.19$, $p = 0.04$, unpaired t-test).

In line with the behavioral results, plasma corticosterone levels did not statistically differ from one method to another (OG *versus* MDA), neither at D21 (Fig. 5D; $t(18) = 0.60$, $p = 0.56$, unpaired t-test), nor at D1 (Fig. 5E; D=0.50, $p = 0.77$, Kolmogorov-Smirnov test).

3.1.2. Impact of the use of a sweet vehicle on body weight and the carbohydrate metabolism

Analysis of body weight curve revealed a significant time effect ($F(27, 486) = 1254$, $p < 0.0001$), but neither group effect (OG *versus* MDA) ($F(1, 18) = 0.39$, $p = 0.54$), nor time x group interaction ($F(27, 486) = 0.78$, $p = 0.77$) (Fig. 6A). Thus, chronic daily consumption of the condensed milk solution required for MDA did not influence body weight gain in the MDA group (compared to the OG group).

Regarding carbohydrate metabolism, no significant group difference (OG *versus* MDA) was observed for blood glucose levels, either at D1 (Supplemental Fig. 3) or at D21 (Fig. 6B; $t(18) = 1.03$, $p = 0.32$, unpaired t-test). Besides, after 3 weeks of treatment, no group difference was found in plasma fructosamine levels at D21 (Fig. 6C; $t(18) = 0.14$, $p = 0.89$, unpaired t-test).

3.2. Effects of the administration method on the pharmacokinetics

Pregabalin plasma concentrations did not differ between groups (20.43 ± 2.26 ng/mL and 18.90 ± 0.95 ng/mL for OG and MDA, respectively) (Fig. 7A; D=0.25, $p > 0.99$, Kolmogorov-Smirnov test).

Conversely, a significant group difference was observed for aripiprazole plasma concentrations. The MDA group had a significantly

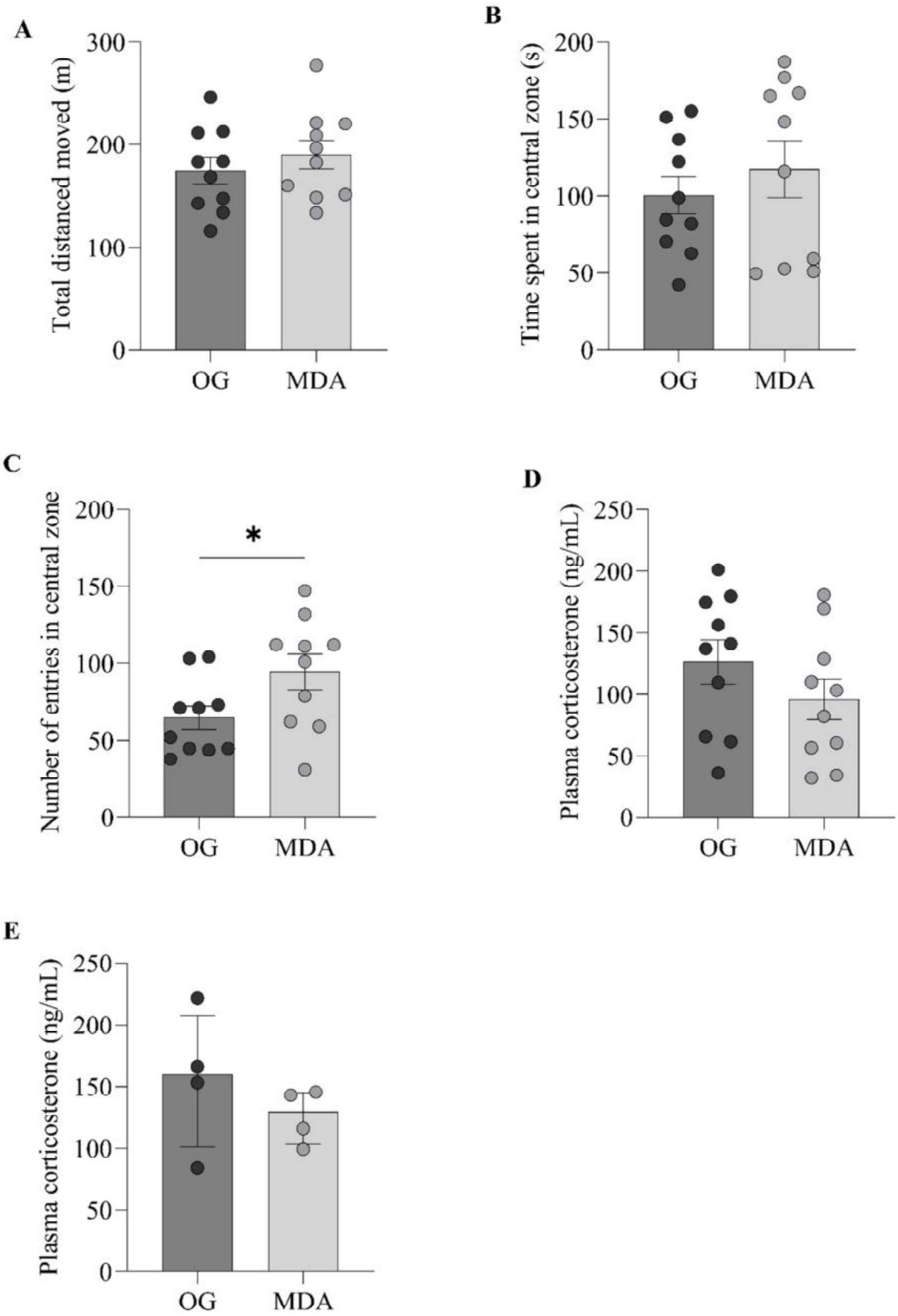

Fig. 5. Effects of 3 weeks daily oral administration (OG or MDA) on behavioral parameters in the open field test (**A**) Distance moved; (**B**) Time spent in central zone; (**C**) Number of entries in central zone; on (**D**) plasma corticosterone concentration; and on (**E**) plasma concentration after a single oral administration. *$p < 0.05$, based on unpaired t-test.

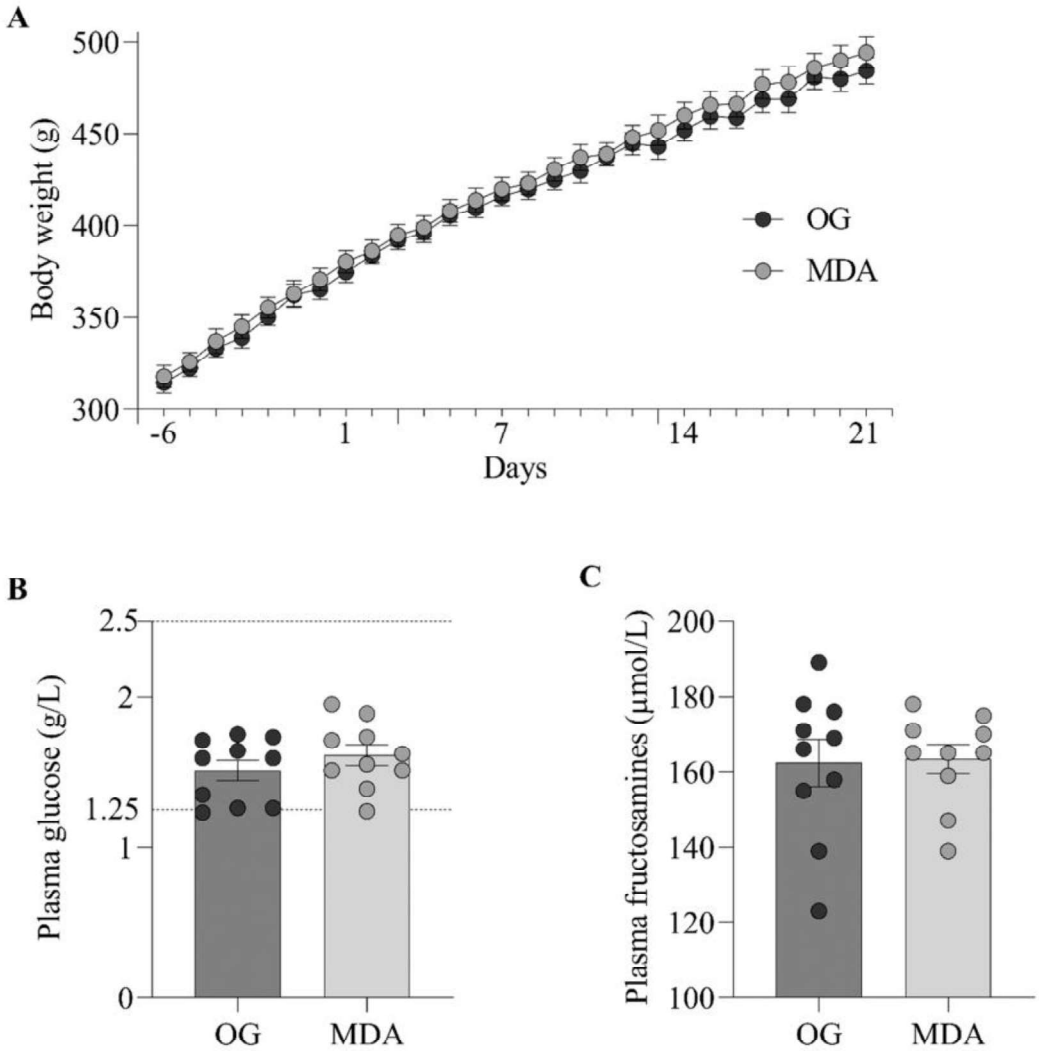

Fig. 6. Effects of 3 weeks daily oral administration (OG or MDA) on (**A**) body weight gain and serum concentration of (**B**) glucose and (**C**) fructosamines. Dotted lines reflect limits of normal values for serum glucose and fructosamines concentrations. ns=non-significant.

lower value than the OG group (4.59 ± 0.58 ng/mL and 11.55 ± 2.58 ng/mL, respectively) (Fig. 7B; D=1, p = 0.016, Kolmogorov-Smirnov test).

3.3. Applicability of the administration method to large groups

At the end of training, the average time for rats to consume the condensed milk solution from the micropipette by MDA method was 3.45 ± 0.13 s per rat. The time duration for the two procedures was measured and compared. A significant group difference was observed, with MDA being faster to realize (119.5 ± 8.35 s/rat and 68.71 ± 10.3 s/rat, for OG and MDA, respectively) (Fig. 8A; t(32.38) = 6.74, p < 0.0001, unpaired t-test). This difference may have important implications in the context of chronic dosing experiments. Analysis of the cumulative time of administration curve revealed a significant group effect (OG *versus* MDA) (F(1, 35)= 11.93, p = 0.0015), a significant time x group interaction (F(20, 700)= 13.03, p < 0.0001) and a significant time effect (F(1.05, 36.81)= 178.8, p < 0.0001) (Fig. 8B). In our hands, it has led to a gain of 5 h in favor of MDA methods for thus a 3 weeks daily administration of 18 (OG) and 19 (MDA) rats (in favor of MDA, respectively 12.55 h while MDA method lasted 7.62 h). Of note, no difference was observed according to the administration delay of pregabalin *versus* aripiprazole (Fig. 8C; t(40) = 0.59, p = 0.56, unpaired t-test). It should be noted that on two occasions (different animals in different days, *i.e.* D1 and D17), two rats (out of a total of 45) did not show any interest in the tip and thus required to be a slight restraint for administration when necessary.

4. Discussion

We described and characterized for the first time in rats, the Micropipette-guided Drug Administration (MDA) method. We have shown that this method can be adapted from mice to rats. Easy to perform and non-invasive and therefore in some way safer for the animals (compared to other existing methods), but also less time-consuming, this new oral administration method in rodents is also closer to the clinical reality. Obviously, this method appears even more valuable when oral administration has to be performed chronically.

Animal welfare was assessed after a single administration and after 3 weeks of daily administration. Quite surprisingly, no significant

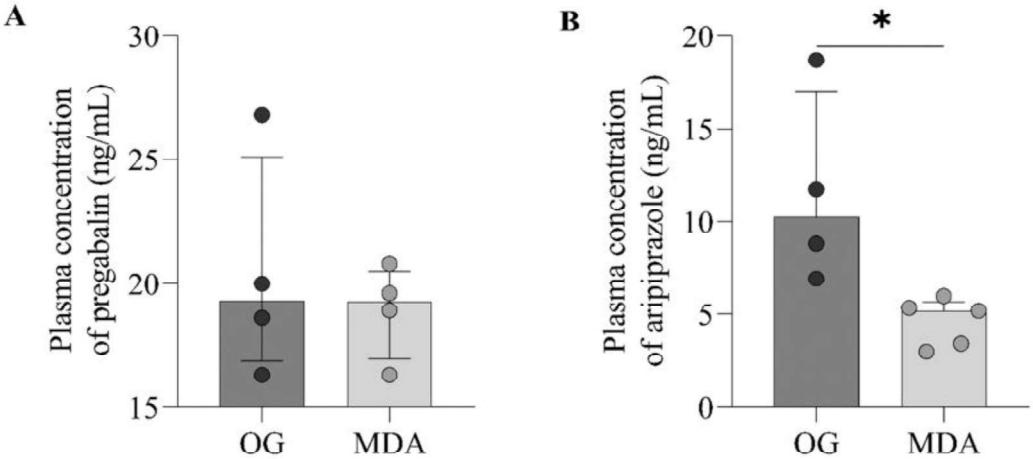

Fig. 7. Plasma concentration of (**A**) pregabalin (10 mg/kg) or (**B**) aripiprazole (3 mg/kg) administrated daily during 3 weeks, through either OG or MDA. Blood sample was realized 1 h after last administration. *$p < 0.05$, Kolmogorov-Smirnov test.

difference was observed between the two methods. In fact, both behavioral and biochemical results indicate a similar level of stress between the two methods (OG *versus* MDA). These results are unexpected as they contrast with literature data in the mouse model, where a lower level of corticosterone was observed in the MDA group compared to OG (Scarborough et al., 2020). One possible explanation could be the refinement we made of the OG method. First, in contrast to the paper in mouse, we used plastic probes for OG administration, thus minimizing animal stress (Wheatley, 2002). Second, we used an administration volume (1.5 mL/kg) well below the maximum limit (5 mL/kg) (Damsch et al., 2011), which *per se* also contributes to animal welfare. Besides, it is worth noting that for small administration volumes, the level of stress induced by the gavage is not higher than that of a simple contention (Turner et al., 2012). In addition, habituation to restraint may reduce the stress associated with the procedure (Turner et al., 2011a,2011b). This must have interfered with our data, as animal restraint was required either to place the animal in the behavioral apparatus or for animal killing before blood sample analysis (Stuart and Robinson, 2015). In particular, the killing method we used, which is quite stressful (Vahl et al., 2005), may have masked a difference in corticosterone levels between groups. In other words, the corticosterone level observed here may reflect more the stress induced by the euthanasia method rather than that due to the oral administration method used previously.

Beyond any biochemical and/or behavioral results, several arguments in favor of a higher level of anxiety in the OG group were noted and should be mentioned. First, home cages of the OG animals were dirtier than those of the MDA animals (more feces and urine). Moreover, the cardboard rolls provided as cage enrichment were chewed more by OG rats than by MDA rats. As a result, they had to be changed twice more often for OG group than MDA group. Finally, while MDA rats stopped defecating into the bowl used for daily weighing, OG rats did not (a persistent behavior that may be associated with a stressful situation relative to the subsequent contention after weighing required for oral administration).

The MDA method requires a sweetened solution, which has to be palatable enough to motivate rodents to voluntarily ingest the contents of the micropipette, or even to mask the potentially aversive taste of pharmacological compounds. Herein, we used dilute condensed milk solution as it was previously described for mice (Scarborough et al., 2020). Nevertheless, individual taste preferences may lead to palatability issues and behavioral changes over time in some animals that would not or no longer cooperate (Turner et al., 2011a,2011b). In our study, two animals did not fully acclimate to the MDA method and showed a higher consumption delay. Beyond compound palatability and/or individual taste preferences, the active substance tested may also influence the degree of adherence or cooperation of the rat to the method. For example, if the tested pharmacological compound causes rapid onset side effects, the animal may associate the oral treatment with the impending discomfort events. Whether the animals become less prone to lick and swallow the tip, the animal will then require special attention. Of note, this situation is obviously not specific to the MDA method but is related to the properties of pharmacological agents tested. Thus, such an event has already been described when administered in a sucrose solution (Atcha et al., 2010).

Since the MDA method uses a sweet vehicle, its effect was evaluated *by assessing* the animals' body weight, glycemia and fructosamine level. The evolution of the body weight as the rats treated with MDA did not differ with the OG group. The use of sweetened condensed milk has no long-term effects on body weight compared to saline. This result is consistent with the blood glucose levels, the administration of a sweetened vehicle did not affect the animals' glycemia. Glucose levels were variable because samples were not collected on an empty stomach. Fasting blood glucose should be approximately 1.27 g/L (Nowland et al., 2011) and breeding labs provide glycemia data specific to the Sprague Dawley strain: 1.5–1.7 g/L (Janvier Labs) and 2.5 g/L (Charles River). Since glycemia is an acute indicator, measuring fructosamine levels provided a reflection of glucose concentration over the last 3 weeks. As expected, no significant difference was found between the two groups and the obtained values are close to those reported in the literature (Ejdesjö et al., 2011). If these results support an absence of metabolic consequences associated with the use of a sweet vehicle, this may be a limitation in experiments using rewards.

A pharmacokinetic study was performed to investigate how much equivalent or different the OG and MDA methods are. Two pharmacological substances with different physicochemical properties were studied: aripiprazole and pregabalin.

For pregabalin study, similar plasma concentrations were observed after MDA and OG, suggesting a pharmacokinetic equivalence of the two administration methods for hydrophilic compounds. In addition, it is worth noting that we found similar concentrations that already reported using the IP method at 10 mg/kg (Lau et al., 2013).

Conversely, a statistical group difference (OG *versus* MDA) was observed in the plasma concentration of aripiprazole. Quite interestingly, the concentration we observed with the MDA method was consistent with literature data for oral administration (OG, 2.5 ng/mL after 3 mg/kg 15days of daily administration) (Raish et al., 2019).

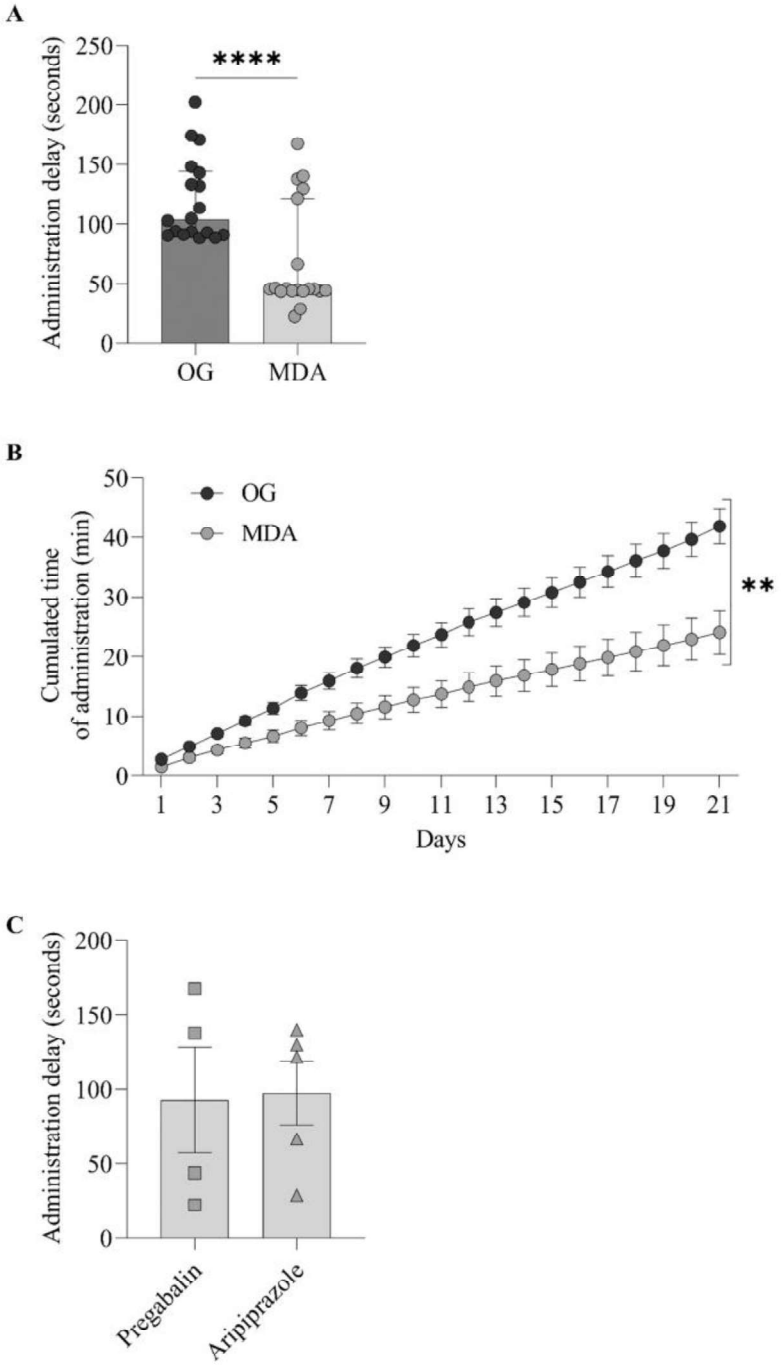

Fig. 8. Comparison of average time duration for each method (OG *versus* MDA). (**A**) Administration delay. (**C**) Cumulated time duration for administration during 3 weeks (**B**) MDA delay of pregabalin *versus* aripiprazole. * *p < 0.01, based on unpaired t-test. * ** *p < 0.0001, based on Kolmogorov-Smirnov test.

Therefore, this led us to search for an explanation for the unusually high level of plasma concentration of aripiprazole we found after OG. As suggested by the dispersion of the results observed, an issue of solubilization of the compound could explain our result. Indeed, the suspension variability of this hydrophobic compound could have affected the OG measurement. To verify this hypothesis, a sonication step could be added to the solubilization protocol of hydrophobic compounds in future experiments. Indeed, by using this method, Scarborough and colleagues demonstrated pharmacokinetic equivalence of OG and MDA for risperidone, a lipophilic compound (Scarborough et al., 2020).

One may discuss on the relevance of using the MDA method and qualify as a tedious method to perform. At a first sight, the MDA method appears to be more time-consuming than the classical OG method. In fact, one week of training prior to treatment is mandatory to accustom

the animal to the method and to overcome inter-individual preferences. However, when we looked at the time required for treatment in each group, it clearly appeared that MDA method is faster than OG one. Ultimately, the time spent before starting treatment can be quickly recovered during the treatment phase itself. Since then, the advantage of the MDA method becomes even more apparent when long-term studies are planned. Besides, while the MDA method is easy to perform and within everyone's reach, the OG method requires specific training of the experimenter, particularly with regard to the high risk of injury (lesion of the oral cavity, pharynx, larynx, trachea, esophagus and stomach…) that would result from improper positioning of the head and body (Machholz et al., 2012).

In conclusion, this study presents a non-invasive administration method that is much closer to oral administration performed in humans and is particularly suitable for long-term administration in rats. In addition to being easy to set up, this method does not require any training for the experimenter and does not entail any risk of injury to the digestive tract. This method is particularly suitable for hydrophilic compounds and allows strict control of the time and the dose administered according to the animal's body weight.

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CRediT authorship contribution statement

Marie Heraudeau: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Candice Roux:** Conceptualization. **Caroline Lahogue:** Conceptualization. **Stacy Largillière:** Conceptualization. **Stéphane Allouche:** Resources. **Véronique Lelong-Boulouard:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Thomas Freret:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

None of the author has conflict of interest to declare.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jneumeth.2023.109951.

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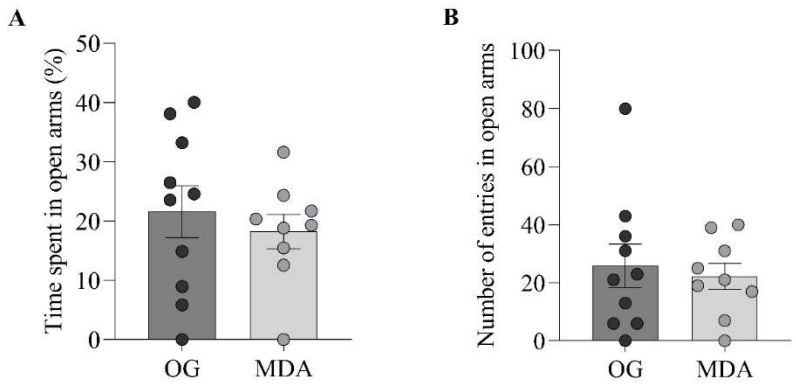
Supplemental materials

1. *Effects of a single administration on anxiety-like behavior*

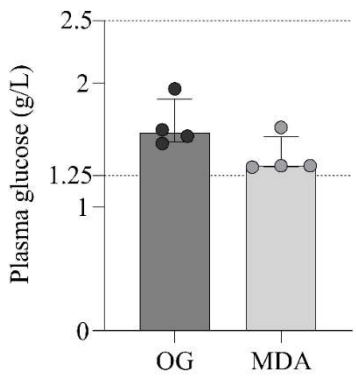
Effects of single oral administration on anxiety-like behavior was assessed through elevated plus maze test. As shown in 0, no statistical group difference (MDA-group or OG-group) was observed for either the percentage of time spent in open arms (t(17)=0.62, p=0.54, unpaired t-test) or the number of entries (t(17)=0.42, p=0.68, unpaired t-test). Note that one rat from MDA group was excluded for falling from the apparatus.



Supplemental Figure 1. Effects of a single oral administration (OG or MDA) on behavioral parameters in the elevated plus maze test (**A**) Time spent in open arms; (**B**) Number of entries in open arms.

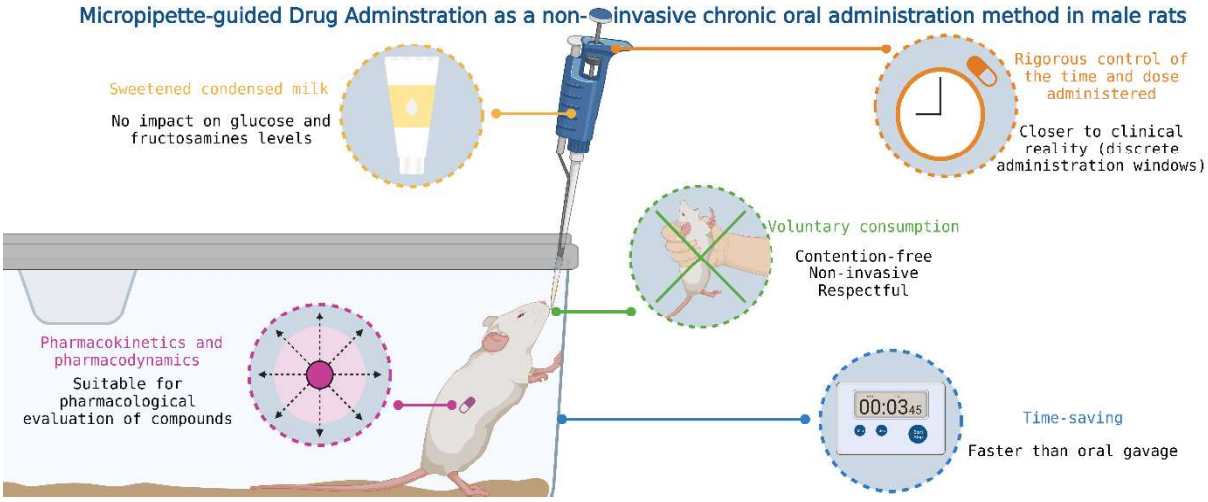
2. *Impact of the use of a sweet vehicle on glycaemia after single oral administration*

The average blood glucose levels were not significantly different in MDA from OG group after single oral administration (D=0.75, p=0.23, Kolmogorov-Smirnov test) at D1 (Supplemental Figure 3).



Supplemental Figure 3. Effects of a single oral administration (OG or MDA) on plasma glucose levels.

GRAPHICAL ABSTRACT:


Micropipette-guided Drug Adminstration as a non-invasive chronic oral administration method in male rats
Sweetened condensed milk
No impact on glucose and fructosamines levels
Rigorous control of the time and dose administered
Closer to clinical reality (discrete administration windows)
Voluntary consumption
Contention-free
Non-invasive
Respectful
Pharmacokinetics and pharmacodynamics
Suitable for pharmacological evaluation of compounds
00:03 45
Time-saving
Faster than oral gavage

DISCUSSION GÉNÉRALE

Ce travail de thèse avait pour objectif d'évaluer les effets cognitifs associés à l'exposition aiguë et chronique de 3 substances psychoactives largement utilisées en clinique chez la personne âgée : le tramadol, la paroxétine et la sertraline.

À l'**initiation** du traitement, **des comportements transitoires aspécifiques** ont été observés dans une situation de doses suprathérapeutiques, induits par l'administration de chacune des 3 substances à effets centraux testées. Ces effets, à type d'hyperactivité et de stéréotypie, ont été objectivés à un temps donné après l'initiation du traitement, sans réapparition ultérieure. Ainsi, leur caractère transitoire suggère l'hypothèse d'un **état de type confusionnel**, tel que classiquement décrits chez le sujet âgé lors de certaines prises médicamenteuses.

Après **exposition chronique**, une **accumulation systémique associée à des troubles cognitifs comportementaux** a été mise en évidence pour le tramadol et la paroxétine, administrées à des doses suprathérapeutiques.

Nos résultats suggèrent que, dans notre modèle de rat adulte surexposé, la prise prolongée de certains médicaments à effet psychoactifs peut conduire à une accumulation systémique favorisant la survenue de troubles cognitifs (**Tableau 1**).

Tableau 1 : Synthèse des résultats des Articles 1 et 2

	Tramadol (25 mg/kg)	Tramadol (50 mg/kg)	Paroxétine (10 mg/kg)	Sertraline (16 mg/kg)
Intervalle thérapeutique chez l'Homme (Schulz et al., 2020)	10-1000 ng/mL		2-65 ng/mL	10-150 ng/mL
A L'INITIATION DU TRAITEMENT				
Concentration moyenne 20h après administration	TRAM : 10 ng/mL ODM : <5 ng/mL	TRAM : 10 ng/mL ODM : 20 ng/mL	75 ng/mL	50 ng/mL
Activité locomotrice	↘	↗	↗	↗
Comportement stéréotypique	Non	**Oui**	**Oui**	Non
APRES EXPOSITION CHRONIQUE				
Concentration moyenne 20h après administration	TRAM : 30 ng/mL ODM : 25 ng/mL	TRAM : 110 ng/mL ODM : 100 ng/mL	**500 ng/mL**	40 ng/mL
Apprentissage spatial	=	=	↘	=
Flexibilité cognitive	=	=	↘	=
Mémoire spatiale	=	=	=	=
Mémoire de reconnaissance	↘	↘	=	=

1. TRANSPOSABILITÉ À L'HOMME

Bien que le modèle animal ne reproduise pas l'ensemble de la complexité du contexte clinique humain, il constitue un outil pertinent pour générer des hypothèses translationnelles, en particulier lorsque les effets comportementaux sont interprétés à la lumière de l'exposition systémique et des mécanismes pharmacologiques sous-jacents.

Transposabilité des observations comportementales à l'Homme

Bien que varié, le répertoire comportemental du rat demeure plus limité et moins nuancé que celui de l'Homme, rendant difficile la modélisation de certains états cognitifs complexes tels que la confusion. Néanmoins, certains marqueurs comportementaux peuvent constituer des analogues fonctionnels pertinents.

L'un des objectifs de ce travail était d'**explorer le potentiel confusiogène** de trois médicaments administrés à dose suprathérapeutique chez le rat adulte. La confusion est définie comme un **trouble neurocognitif aigu, transitoire et fluctuant**. Afin d'intégrer cette dimension temporelle et multidomaine (motivation, exploration et mémoire), une série de trois tests comportementaux (*buried food test, open field, Y-maze*), inspirée de modèles de confusion post-chirurgicale, avait été initialement mise en œuvre avec une évaluation avant et après exposition au facteur confusiogène (Ang et al., 2023; Peng et al., 2016) (résultats non présentés). Toutefois, ce protocole n'a pas pu être validé. Les animaux témoins ne remplissaient pas les critères de performance dès la phase basale, compliquant toute interprétation. Ces limites suggèrent que des tests principalement développés chez la souris ou le rat adulte et en contexte chirurgical nécessitent une adaptation spécifique en pharmacologie chez le rat âgé. À ce jour, seule une étude a évalué le potentiel confusiogène de substances pharmacologiques par une approche comportementale comparable, avec la scopolamine (Qiu et al., 2016), soulignant l'intérêt mais aussi les limites de ces modèles hors contexte chirurgical.

Bien que les résultats issus de cette série de tests n'aient pu être exploités, les données d'actimétrie permettant de mesurer des modifications fines de l'activité locomotrice et exploratoire en continu sur 24 heures, nous sont apparues pertinentes pour la caractérisation de comportement de type confusiogène. En effet, dans mes deux études, l'analyse du comportement locomoteur des rats traités, immédiatement après la

première administration, des trois substances psychoactives, a provoqué une **hyperactivité transitoire** associée, pour le tramadol et la paroxétine, à des **comportements stéréotypiques**. Chez le rat, un comportement stéréotypique est caractérisé par l'association d'une **désorientation spatiale et/ou d'une hyperactivité non exploratoire**. Ce comportement stéréotypique a été, à plusieurs reprises, décrits dans la littérature, comme pouvant être assimilé à un **comportement de type confusion** (Cheon et al., 2021; Jafarian et al., 2019; Qiu et al., 2016). Ces premiers résultats obtenus dans notre modèle de rat surexposé, pourraient donc correspondre à l'agitation et/ou à l'hyperactivité transitoire parfois observée en clinique, chez le sujet âgé, lors de l'initiation de traitements à potentiel confusiogène (Mart et al., 2021).

Dans la suite de mes expérimentations, les administrations ont été réalisées de façon quotidienne en fin de journée. En procédant ainsi, l'objectif recherché était de s'éloigner du moment du pic plasmatique lors de la réalisation des tests comportementaux, dans le but de s'affranchir des effets aigus et de s'assurer de caractériser les effets cognitifs liés à l'administration chronique des différentes substances étudiées. Par ailleurs, **ce choix méthodologique renforçait la validité translationnelle de notre design expérimental**, dans la mesure où, en clinique, de nombreux traitements chroniques sont administrés le soir.

Nos résultats démontrent que la consommation chronique de tramadol et de paroxétine entraine une augmentation de l'exposition systémique des rats ainsi que des altérations cognitives concernant la flexibilité et l'apprentissage pour la paroxétine, et la mémoire de reconnaissance pour le tramadol. Chez le rat, cette dernière est considérée comme un analogue de la mémoire épisodique humaine, laquelle, avec la flexibilité cognitive, fait partie des fonctions particulièrement affectées par le vieillissement (Collette, 2019; Tromp et al., 2015). Ainsi, notre étude suggère que **la surexposition continue mesurée pour ces deux substances psychoactives pourrait être à l'origine de perturbations cognitives**, de nature à aggraver celles fréquemment observées lors du vieillissement.

1. TRANSPOSABILITÉ À L'HOMME

Exposition systémique et équivalence inter-espèce

Compte tenu de la complexité du protocole, il n'a pas été possible d'évaluer plusieurs doses simultanément. Ceci constitue une limite méthodologique pour l'extrapolation des effets dose-dépendants. Cependant, le choix de la dose étudiée a fait l'objet d'une réflexion approfondie, fondée sur le calcul de l'équivalence Homme–animal (*Human Equivalent Dose*, HED) et sur les données de la littérature, afin de viser des expositions suprathérapeutiques au regard des posologies cliniques sans risque de létalité. L'interprétation des résultats de nos études a toutefois été réalisée au regard de l'exposition systémique plutôt que la dose administrée.

Chez l'Homme, les concentrations plasmatiques thérapeutiques rapportées pour la paroxétine sont comprises entre 2 et 65 ng/mL, et entre 10 et 150 ng/mL pour la sertraline (Schulz et al., 2020). Dans notre étude, après administration chronique et à l'état d'équilibre, les concentrations moyennes mesurées étaient d'environ 500 ng/mL pour la paroxétine et de 40 ng/mL pour la sertraline. **Aucune accumulation n'a été observée pour la sertraline**, dont les concentrations sont restées dans l'intervalle thérapeutique humain. En revanche, la paroxétine présentait une accumulation significative après exposition chronique conduisant à des concentrations, chez les rats adultes, **environ 7 fois supérieures aux concentrations obtenues après administration aiguë**. En outre, la concentration mesurée, chez nos rats, pour la paroxétine était également 7 fois supérieure à la fourchette haute de l'intervalle thérapeutique humain. Ce résultat confirme l'hypothèse d'une **accumulation systémique de la paroxétine en cas d'administration continue** et suggère une surexposition potentiellement fréquente pour ce médicament, chez la personne âgée, du fait des modifications pharmacocinétiques observées avec le vieillissement.

Concernant le tramadol, les concentrations plasmatiques thérapeutiques chez l'Homme sont généralement comprises entre 100 et 1000 ng/mL (Schulz et al., 2020). Dans notre étude, les concentrations moyennes mesurées à l'état d'équilibre étaient de 30 et 110 ng/mL pour les doses de 25 et 50 mg/kg respectivement, calculées comme thérapeutique et suprathérapeutique. Malgré une accumulation significative après administration chronique environ **3 fois supérieures (25 mg/kg) et 10 fois supérieure (50 mg/kg) aux concentrations obtenues après administration aiguë**, les

concentrations systémiques de tramadol sont restées dans l'intervalle thérapeutique humain. Compte tenu du large intervalle thérapeutique décrit chez l'homme, il est difficile d'établir une comparaison avec le rat. Le principal déterminant de l'activité pharmacologique du tramadol étant l'O-desméthyltramadol (ODM-tramadol), la comparaison inter-espèce doit donc également s'appuyer sur l'exposition à son métabolite actif lorsque l'on étudie le lien entre exposition systémique et effets. L'intervalle thérapeutique du ODM-tramadol n'est pas établit dans la littérature. Cependant, bien qu'il puisse varier selon le type de métaboliseur, un ratio tramadol/ODM peut être calculé. Chez l'Homme, des études sur la pharmacocinétique du tramadol et de son principal métabolite rapportent des ratio tramadol/ODM-tramadol mesurés entre 4 et 7 (Jeong et al., 2019; Skinner-Robertson et al., 2015), donc largement en faveur du tramadol. À l'inverse, dans notre étude, les concentrations d'ODM-tramadol étaient supérieures à celles de la molécule mère pour les deux doses utilisées, conduisant à un ratio tramadol/ODM-tramadol compris entre 0,7 et 0,8. Ces valeurs sont en accord avec les données publiées chez le rat, qui rapportent un ratio tramadol/ODM plasmatique compris entre 0,5 et 1,5 (Tao et al., 2002). **Le rat serait donc un métaboliseur plus efficace que l'Homme et le modèle rongeur pourrait présenter une exposition systémique suprathérapeutique en métabolite actif malgré des concentrations considérées comme thérapeutiques en tramadol chez l'Homme.** Malgré ces différences inter-espèces pour le tramadol, l'exposition chronique des rats à ce dernier, a conduit à une **accumulation systémique** de la substance active, avec des concentrations largement supérieures à celles mesurées en aigu. La demi-vie du tramadol, étant théoriquement courte en conditions d'utilisation transitoire, **nos résultats démontrent un potentiel d'accumulation en condition chronique** et une demi-vie potentiellement allongée chez le rat. Par ailleurs, il est également possible, que la pharmacocinétique du tramadol soit différente chez le rat, et que les concentrations, considérées comme thérapeutiques chez l'Homme, ne soient pas nécessairement transposables à cette espèce. Ainsi, malgré les similitudes physiologiques entre le rat et l'Homme, l'extrapolation interspécifique des concentrations plasmatiques demeure à considérer avec prudence, en raison notamment de différences métaboliques.

1. TRANSPOSABILITÉ À L'HOMME

Ces résultats soulignent les limites d'une extrapolation basée exclusivement sur la dose administrée ou sur la concentration de la molécule mère. Bien que le calcul de la HED constitue un outil pertinent pour une première estimation posologique, les différences inter-espèces de métabolisme, en particulier pour les substances à métabolite actif, peuvent conduire à une exposition pharmacologiquement supra-thérapeutique chez le rat malgré des concentrations plasmatiques apparentes dans l'intervalle thérapeutique humain.

Limites intrinsèques du modèle : influence du sexe

L'utilisation exclusive de rats mâles visait à simplifier la mise en œuvre de notre protocole déjà complexe et de réduire la variabilité liée aux fluctuations hormonales, garantissant une plus grande homogénéité des données. Toutefois, ce choix méthodologique limite la transposabilité des résultats à l'Homme. Des différences inter-sexes dans la survenue et la nature des effets indésirables ne peuvent être exclues.

Par exemple, une étude portant sur les différences de réponse à la paroxétine chez des rats mâles et femelles n'a pas mis en évidence de différence inter-sexes sur la locomotion ou la préférence sociale en conditions basales, mais a montré une augmentation de la préférence sociale chez les femelles en condition de stress (Ishaq et al., 2023). Chez l'Homme, plusieurs données suggèrent une susceptibilité accrue des femmes aux effets indésirables de certains psychotropes et opioïdes. Une étude de cohorte rétrospective sur 8 457 participants a montré que les femmes rapportaient davantage d'effets indésirables liés au tramadol, en particulier sur le système nerveux central (Lopes et al., 2021). En revanche, le principal biais de ces études épidémiologiques est que les hommes sous-déclarent les effets indésirables par rapport aux femmes. Une revue de 36 articles a également mis en évidence des différences inter-sexes dans la prévalence et la nature des effets indésirables pour plusieurs psychotropes, souvent liées à des **disparités pharmacocinétiques** (Shan et al., 2023).

De telles différences pourraient être expliquées par un ratio concentration/dose plus élevé chez les femmes que chez les hommes pour de nombreuses substances actives (Haack et al., 2009; Zucker & Prendergast, 2023). Ces observations

pharmacocinétiques sont confirmées par une étude pharmacocinétique menée chez 22 jeunes volontaires (18–45 ans) et 22 sujets âgés (>65 ans), hommes et femmes traités par sertraline (Ronfeld et al., 1997). Cette dernière a été administrée à la dose maximale recommandée (200 mg/j) pendant 21 jours, après une titration progressive de 50 mg/j sur 9 jours. L'étude a montré un effet significatif du sexe sur la constante d'élimination et sur la fraction de sertraline non liée (toutes deux plus faibles chez la femme), sans différence significative sur les concentrations plasmatiques à l'état d'équilibre.

Des différences inter-sexes peuvent également résulter de **mécanismes pharmacodynamiques**. Les systèmes dopaminergiques, sérotoninergiques et cholinergiques peuvent présenter des variations entre mâles et femelles, susceptibles de moduler la sensibilité aux psychotropes (Ishaq et al., 2023). Chez la souris, les femelles sont plus sensibles que les mâles aux effets cognitifs de la scopolamine sur des tâches spatiales, ce qui pourrait s'expliquer par une plus grande sensibilité des récepteurs muscariniques de l'hippocampe (Berger-Sweeney et al., 1995; Hall et al., 2017).

Ainsi, si des différences inter-sexes existent, il est possible que les effets indésirables soient plus fréquents et plus marqués chez la femelle, en lien avec une **susceptibilité pharmacocinétique et pharmacodynamique accrue**. Il est donc également possible que les altérations cognitives que nous avons observé chez le rat mâle dans notre étude, **auraient été plus marquées si nous avions utilisé des rates**.

Limites intrinsèques du modèle : influence de l'âge

Nos expérimentations ont été volontairement conduites chez des rats adultes pour simplifier le modèle et éviter les biais liés au vieillissement. Afin de poursuivre les investigations, le recours à des animaux âgés sera une nécessité afin de mieux comprendre les interactions entre surdosage plasmatique et fragilité cognitive. Des essais préliminaires menés au cours de ma thèse chez des rats âgés (14 mois) n'ont pas été présentés dans le manuscrit, les animaux ne satisfaisant pas aux critères comportementaux minimaux requis pour une interprétation fiable. Ces limitations peuvent s'expliquer par les effets du vieillissement, incluant le déclin progressif des fonctions cognitives (Robitsek et al., 2008), la diminution de l'activité locomotrice et

exploratoire (Altun et al., 2007), ainsi qu'une baisse de la motivation et une prise de poids excessive. Ces observations soulignent la nécessité d'adaptations méthodologiques, telles que la détermination d'un âge optimal pour la réalisation des tests comportementaux, la constitution de groupes homogènes sur le plan cognitif par une évaluation préalable, et le contrôle du poids corporel. En effet, les variations de composition corporelle peuvent modifier la distribution des médicaments, en particulier pour les composés lipophiles, rendant l'interprétation pharmacocinétique plus complexe. Dans cette perspective, le recours à un modèle de vieillissement réussi, tel que le rat LOU/c/Jall (Alliot et al., 2002; Paban et al., 2013), pourrait constituer une alternative pertinente pour de futures investigations.

L'exploitation d'un modèle rongeur âgé aurait été difficile dans le cadre du protocole utilisé. Les expérimentations ont donc été réalisées chez le rat adulte, ce qui peut constituer une limite quant à l'extrapolation des résultats au sujet âgé. Néanmoins, cette extrapolation peut être discutée et estimée à la lumière des données pharmacocinétiques et pharmacodynamiques disponibles. Les effets observés chez des rats adultes étant déjà marqués, ils suggèrent que **des conséquences au moins équivalentes, voire amplifiées, pourraient survenir chez le sujet âgé**, population chez laquelle une surexposition médicamenteuse est probablement fréquente.

Des différences liées à l'âge peuvent être dues à la **variabilité pharmacocinétique** importante retrouvée chez la personne âgée susceptibles d'augmenter l'exposition systémique. Chez l'Homme, l'effet de l'âge sur la pharmacocinétique du tramadol après une dose unique de 200 mg à libération prolongée a été étudié chez des sujets adultes (18-40 ans) et des sujets âgés (≥ 75 ans) (Skinner-Robertson et al., 2015). Les sujets âgés présentaient une élimination plus lente, un volume de distribution plus élevé et une exposition accrue à l'ODM-tramadol, principalement liée à une diminution de la clairance rénale. Bien que cette étude n'ait pas évalué l'exposition après administration chronique, des simulations à l'état d'équilibre suggéraient une demi-vie prolongée, des concentrations plus élevées et un **indice d'accumulation accru chez les sujets âgés**, indiquant un risque majoré d'accumulation. Des observations similaires ont été rapportées pour les antidépresseurs. À partir de données de suivi thérapeutique, des auteurs ont montré que

les patients âgés (≥ 65 ans) présentaient des **concentrations sériques ajustées à la dose significativement plus élevées** que les sujets de moins de 40 ans, notamment pour la paroxétine (x1,8) et la sertraline (x1,3) (Waade et al., 2012). Pour la paroxétine, plusieurs études cliniques ont mis en évidence, chez les patients âgés de plus de 60 ans, des concentrations à l'état d'équilibre plus élevées, une demi-vie prolongée et une variabilité interindividuelle accrue, tant après administration aiguë que chronique (Bayer et al., 1989; Hebenstreit et al., 1989; Lundmark et al., 1989). Ces observations seraient liées à un ralentissement du métabolisme hépatique et à une cinétique non linéaire, pouvant conduire à des augmentations disproportionnées de concentrations pour de faibles variations de dose (DeVane & Pollock, 1999). Bien que certaines études n'aient pas mis en évidence de modification significative de l'exposition systémique globale, la sertraline présente une pharmacocinétique plus linéaire, mais une clairance réduite chez le sujet âgé qui pourrait également contribuer à une exposition accrue (Ronfeld et al., 1997).

Au-delà des modifications pharmacocinétiques, le vieillissement s'accompagne de **changements pharmacodynamiques** susceptibles d'amplifier le risque iatrogène. Concernant les opioïdes, les données cliniques suggèrent une sensibilité accrue chez la personne âgée, caractérisée par des effets plus marqués pour des doses équivalentes à celles administrées chez le sujet adulte (McLachlan et al., 2011; Naples et al., 2016). Cette majoration de l'effet ne semble toutefois pas liée à une hypersensibilité des récepteurs opioïdes. Les données précliniques chez le rongeur indiquent, au contraire, une diminution du potentiel de liaison des récepteurs μ, une altération des voies de signalisation et une augmentation des mécanismes de régulation négative avec l'âge (Fullerton et al., 2022). Ces éléments suggèrent que la réponse clinique accrue observée chez le sujet âgé reflète davantage une **vulnérabilité pharmacodynamique liée au vieillissement** du système nerveux central, associant diminution de la réserve fonctionnelle, fragilisation des réseaux neuronaux et réduction de la plasticité synaptique (Bowie & Slattum, 2007; Singh et al., 2023). Concernant les ISRS, des études cliniques et épidémiologiques rapportent une sensibilité accrue chez la personne âgée, avec une fréquence plus élevée d'effets indésirables, notamment cognitifs (Coupland et al., 2011;

Jacob & Spinler, 2006). Les données précliniques montrent une diminution liée à l'âge de la densité du transporteur de la sérotonine (SERT) et de certains récepteurs sérotoninergiques (David et al., 2001; Felice et al., 2015; Herrera-Pérez et al., 2013). Ces observations sont corroborées par des études d'imagerie chez l'Homme mettant en évidence une altération globale de la transmission sérotoninergique avec l'âge (Karrer et al., 2019). Chez le sujet âgé, les modifications liées à l'âge du système sérotoninergique pourraient se traduire par une fenêtre thérapeutique plus étroite des ISRS, associant une sensibilité accrue aux effets indésirables, sans diminution systématique de l'efficacité antidépressive.

Par ailleurs, la diminution des récepteurs muscariniques liée au vieillissement réduit la réserve cholinergique (Richardson et al., 1985; Schwarz et al., 1990), ce qui **augmente la sensibilité aux médicaments anticholinergiques** et le risque d'effets cognitifs indésirables (Attoh-Mensah et al., 2020; Feinberg, 1993). Cette modification est particulièrement importante chez la personne âgée, chez qui l'intégrité des fonctions cognitives repose davantage sur les mécanismes cholinergiques. Ainsi, la sensibilité pharmacodynamique accrue aux effets anticholinergiques constitue un déterminant majeur de la survenue d'effets centraux et contribue à la relation observée entre charge anticholinergique et troubles cognitifs.

L'ensemble de ces données soutient l'hypothèse d'une **exposition systémique accrue, d'une variabilité interindividuelle augmentée et d'une sensibilité pharmacologique renforcée chez le sujet âgé**. Cette vulnérabilité est encore amplifiée par la polymédication fréquente dans cette population, susceptible de moduler à la fois la pharmacocinétique et la pharmacodynamie des traitements. Ainsi, dans la mesure où nos résultats obtenus chez le rat adulte suggèrent déjà un risque d'accumulation lors d'une administration chronique et des altérations cognitives, **il est probable que ces effets soient plus marqués chez des animaux âgés**. Cette majoration potentielle repose sur deux mécanismes principaux : (i) des modifications pharmacocinétiques liées au vieillissement, responsables d'une exposition systémique plus élevée et plus variable, et (ii) des modifications pharmacodynamiques, incluant une diminution de la réserve fonctionnelle et une sensibilité accrue de certains systèmes récepteurs.

Hypothèses mécanistiques sous-jacentes aux altérations cognitives observées

À l'**initiation** du traitement, des **comportements pouvant s'apparenter à de la confusion** ont été observés principalement pour la paroxétine et le tramadol. Ces composés partagent plusieurs mécanismes pharmacologiques communs susceptibles d'expliquer ces observations. Tous deux inhibent le transporteur de la sérotonine, entraînant une augmentation rapide des concentrations extracellulaires de sérotonine (Fuller, 1994). Ce mécanisme est également partagé avec la sertraline qui a également entrainé une hyperactivité. En revanche, le tramadol et la paroxétine possèdent une charge anticholinergique plus élevée que la sertraline (**Tableau 2**) (Mizokami et al., 2025; Rube et al., 2023). Ainsi, **l'augmentation d'activité locomotrice** observée à forte dose avec les 3 composés pourraient être reliée aux **effets sérotoninergiques** tandis que les **comportements stéréotypiques** observés avec la paroxétine et le tramadol, pourraient être plus particulièrement liés à une **activité anticholinergique** centrale plus importante.

La pathogenèse de la confusion demeure mal comprise, mais elle serait associée à des altérations structurales et fonctionnelles de régions cérébrales impliquées dans les fonctions exécutives (notamment le lobe frontal et le diencéphale), la mémoire (hippocampe et amygdale basolatérale) et l'attention (cortex pariétal et cervelet) (Gunther et al., 2012). Les principales hypothèses physiopathologiques de la confusion impliquent des perturbations de la neurotransmission, des processus inflammatoires et des mécanismes de stress chronique. Des données expérimentales chez le rongeur soutiennent ces hypothèses. Un comportement de type confusion induit par la scopolamine est associé à une augmentation des concentrations cérébrales de sérotonine et de dopamine dans des régions impliquées dans les fonctions cognitives (Qiu et al., 2016). Par ailleurs, l'administration de médicaments anticholinergiques induit une confusion chez l'Homme et l'animal et la charge anticholinergique est accrue chez les patients confus (Han et al., 2001). Enfin, les inhibiteurs de cholinestérase, tels que la physostigmine, permettent d'inverser la confusion (Bleifuss et al., 2025). L'hypothèse d'une réduction de l'activité cholinergique et d'une **implication plus importante des substances à activité anticholinergique** apparaît donc majeure dans la survenue de comportement de type confusion.

2. PROFIL PHARMACOLOGIQUE À RISQUE ?

Chez l'Homme, le tramadol et les ISRS figurent parmi les médicaments identifiés comme facteurs de risque de confusion (Haute Autorité de Santé, 2009). Bien qu'un seul facteur puisse déclencher un épisode de confusion, la plupart des cas résultent d'une interaction complexe entre une **vulnérabilité préexistante**, considérée comme un facteur prédisposant (âge, déficit cognitif, multimorbidité) et un **facteur déclenchant** (médicament psychotrope, opioïde ou anticholinergique, polymédication) (Inouye, 2006; Inouye et al., 2014). Un déficit cholinergique et/ou un excès de dopamine sont fréquemment évoqués en corrélation avec les effets indésirables des médicaments confusiogènes (Alagiakrishnan & Wiens, 2004; Hshieh et al., 2008; Lauretani et al., 2010; Young et al., 1997). Les médicaments peuvent aussi induire indirectement une confusion, par exemple via une hypoglycémie, une déshydratation, une hyponatrémie, une hémorragie cérébrale ou un syndrome sérotoninergique.

Si les effets aigus observés à l'initiation du traitement relèvent principalement de perturbations rapides de la neurotransmission, l'exposition chronique s'inscrit dans une dynamique différente, caractérisée par des **phénomènes cumulatifs et des altérations fonctionnelles plus durables, susceptibles de conduire à des troubles cognitifs persistants.**

Après exposition chronique, le tramadol et la paroxétine ont induit des troubles cognitifs, contrairement à la sertraline, suggérant un profil de risque cognitif plus défavorable pour ces deux substances. Le tramadol a entraîné une altération persistante de la mémoire de type épisodique, tandis que la paroxétine a induit une altération de la flexibilité cognitive, témoignant d'atteintes cognitives différentielles selon les profils pharmacologiques. Or ces deux substances sont celles qui se sont le plus accumulées dans le compartiment plasmatique après administration chronique. **Cette accumulation systémique est susceptible d'augmenter l'exposition cérébrale**, entraînant une majoration de l'activité pharmacologique et une potentialisation des effets indésirables centraux. Afin de tester cette hypothèse, nous avons prélevé des structures cérébrales des rats testés pour évaluer si l'accumulation plasmatique se traduit par une accumulation cérébrale. Le dosage cérébral des substances actives permettra, dans une étude ultérieure, de calculer des ratios cerveau/plasma, indicateurs

de la distribution centrale. Cette hypothèse est cohérente avec l'existence de relations concentration–effet décrites pour les troubles cognitifs induits par les médicaments psychoactifs (Reimers et al., 2025). Ainsi, **l'accumulation systémique après exposition répétée constitue un mécanisme pharmacocinétique plausible favorisant les effets cognitifs**, surtout chez des patients à risque d'accumulation (sujet âgé, insuffisant rénal ou hépatique).

Le système cholinergique joue un rôle central dans les fonctions cognitives, notamment la mémoire, l'attention et la flexibilité cognitive (Attoh-Mensah et al., 2020). Une diminution prolongée de cette neurotransmission peut altérer durablement les réseaux neuronaux impliqués dans ces fonctions. Dans ce contexte, l'altération de la mémoire de type épisodique observée avec le tramadol et la diminution de la flexibilité cognitive induite par la paroxétine pourraient refléter une atteinte différentielle de réseaux cholinergiques, en lien avec leurs profils pharmacodynamiques respectifs. Cette hypothèse est appuyée par une revue systématique montrant que l'exposition prolongée (≥ 6 mois) à des médicaments psychotropes à activité anticholinergique est associée à un déclin cognitif chez les sujets âgés (Chandramouleeshwaran et al., 2024).

Par ailleurs, une stimulation sérotoninergique prolongée pourrait également contribuer aux effets observés en perturbant la modulation fine des circuits hippocampiques impliqués dans la cognition, notamment lorsqu'elle s'associe à une diminution de la réserve fonctionnelle neuronale (Bombardi et al., 2021). Toutefois, son implication dans les troubles cognitifs chroniques demeure moins documentée que pour les mécanismes anticholinergiques. De plus, nos résultats ont montré que la sertraline, qui agit en stimulant le système sérotoninergique de façon prolongée, n'a pas eu d'effet néfaste sur les processus cognitifs. Ce mécanisme pourrait donc constituer un **facteur aggravant** la composante anticholinergique.

Tableau 2 : Mécanismes pharmacologiques communs aux substances étudiées selon leur constante d'inhibition Ki (Grond & Sablotzki, 2004; Raffa et al., 1992; Sanchez et al., 2014) et leur charge anticholinergique, calculée selon diverses échelles (Boustani et al., 2008; Briet et al., 2017; Mizokami et al., 2025; Rube et al., 2023). L'intensité de la couleur est représentative de l'intensité de l'inhibition (Ki) ou de la charge anticholinergique.

	Tramadol	Sertraline	Paroxétine
Ki (nmol/L) : inhibition de recapture de la sérotonine	0,9-1	0,2-0,4	0,07-0,2
Ki (nmol/L) : inhibition de recapture de la noradrénaline	0,79	420-820	40-85
Ki (nmol/L) : inhibition de recapture de la dopamine	Pas de données	25	490
Charge anticholinergique (synthèse des scores des différentes échelles)	Faible à modérée	Faible	Modérée à forte

Dans l'ensemble, **la convergence de mécanismes pharmacocinétiques (accumulation systémique) et pharmacodynamiques (activité anticholinergique, modulation sérotoninergique) apparaît comme déterminante dans la survenue des troubles cognitifs** induits par le tramadol et la paroxétine après exposition chronique.

Charge anticholinergique chez la personne âgée

La charge anticholinergique individuelle ou cumulée des médicaments apparait donc importante à prendre en compte dans la surveillance et la prévention des effets indésirables centraux de type cognitifs, qu'ils soient aigus ou chroniques.

La charge anticholinergique correspond à l'**effet cumulatif de substances actives possédant une activité anticholinergique**, principalement antimuscarinique, et reflète l'exposition globale d'un patient à ces effets. Chez la personne âgée, souvent polymédiquée, l'association de plusieurs médicaments présentant des propriétés

anticholinergiques est à l'origine d'une additivité des effets et peut donc entrainer la survenue d'effets indésirables (Briet et al., 2017; Gray et al., 2015).

Son évaluation repose sur différentes approches méthodologiques. Historiquement, elle a d'abord été estimée par des méthodes biologiques, notamment la *Serum Anticholinergic Activity*, qui mesure in vitro la concentration sérique d'une substance nécessaire à l'inhibition de la liaison d'un antagoniste puissant muscarinique radiomarqué (Carnahan et al., 2006; Tune et al., 1993). Cette méthode présente cependant des limites importantes incluant variabilité interindividuelle, faible reproductibilité, influence de facteurs non médicamenteux et difficulté d'accès en pratique clinique. En parallèle, des approches pharmacologiques fondées sur les données de liaison aux récepteurs muscariniques, telles que les constantes d'inhibition (Ki) ou d'affinité, ont permis d'estimer le potentiel anticholinergique intrinsèque des molécules.

Ces données, issues de modèles expérimentaux, ont ensuite été transposées en outils cliniques sous forme d'échelles attribuant à chaque médicament un score d'activité anticholinergique généralement gradué de 0 à 3 (Boustani et al., 2008; Briet et al., 2017; Carnahan et al., 2006; Rudolph et al., 2008). Parmi ces échelles, l'OMEDIT a retenu l'*Anticholinergic Burden* (Boustani et al., 2008) et le *Coefficient d'Impregnation Anticholinergique* (Briet et al., 2017) pour le développement d'un calculateur, évaluant respectivement les effets centraux et périphériques. La charge anticholinergique est le plus souvent estimée par la somme des scores de plusieurs médicaments.

L'intérêt d'un **calcul adapté au contexte clinique** est majeur afin d'**identifier et de prévenir les situations à risque**, notamment en cas de polymédication (effets cumulatifs des anticholinergiques et associations à risque avec les psychotropes), **d'âge avancé** (sensibilité accrue des récepteurs muscariniques), **ainsi que dans certains contextes physiopathologiques** (démence, déclin cognitif).

3. IMPLICATIONS ET RECOMMANDATIONS CLINIQUES

Intérêt du Suivi Thérapeutique Pharmacologique en pratique clinique chez le sujet âgé

Les deux substances présentant une accumulation après exposition chronique sont également celles pour lesquelles des effets indésirables cognitifs chroniques ont été observés, suggérant l'existence d'un **lien entre la concentration plasmatique et la survenue d'effets cognitifs** à long terme.

Chez la personne âgée, la variabilité pharmacocinétique et pharmacodynamique est importante : pour une même posologie, les concentrations plasmatiques peuvent varier considérablement en fonction de l'âge, du contexte physiopathologique, de la fonction rénale et hépatique, des comorbidités et de la polymédication (Roberts & Gibbs, 2018). **Dans ce contexte, les relations concentration–effet apparaissent plus pertinentes que les relations dose–effet pour prédire le risque d'effets indésirables et notamment de troubles cognitifs.**

En pratique clinique, **le Suivi Thérapeutique Pharmacologique (STP)** des médicaments à effet psychoactif, comme les antidépresseurs, est actuellement peu réalisé. Pourtant, il pourrait constituer un **levier essentiel de prévention des effets indésirables, notamment cognitifs chez le sujet âgé**. Son utilisation, intégrant les facteurs individuels tels que l'âge, les co-prescriptions à risque, les interactions médicamenteuses, le profil métabolique et les fonctions d'élimination, permettrait d'optimiser l'exposition médicamenteuse. Dans certaines circonstances, l'utilisation de la **pharmacogénétique** pourrait également constituer une prédiction fine et personnalisée de la réponse d'un patient à un médicament (Picard et al., 2026). Chez le sujet âgé, le STP permettrait non seulement l'ajustement individualisé des posologies pour prévenir les accumulations toxiques de médicaments, mais également constituerait un outil d'accompagnement à la déprescription raisonnée. Intégré dans une approche multidisciplinaire, il participerait à une **prescription personnalisée**, sécurisée et adaptée à l'évolution des paramètres pharmacocinétiques et pharmacodynamiques liés au vieillissement.

Prescription de médicaments chez la personne âgée : principe et recommandations

La prescription d'un médicament chez la personne âgée requiert une vigilance particulière en raison des modifications physiologiques liées au vieillissement. Avant toute initiation de traitement, il est indispensable d'évaluer les fonctions rénales et hépatiques car une altération de ces paramètres peut majorer l'exposition systémique et, par conséquent, la toxicité de nombreux médicaments. Selon l'indication, plusieurs référentiels identifient des **médicaments inappropriés chez le sujet âgé**. La première liste de ce type a été proposée dans les années 1990 par le gériatre américain M. H. Beers (Beers et al., 1991). En France, la liste de Laroche recense les médicaments ou classes médicamenteuses à éviter chez les patients de plus de 75 ans (Laroche et al., 2009). Les médicaments à propriétés anticholinergiques y figurent en raison de leur profil de sécurité défavorable. Lorsque leur prescription s'avère néanmoins nécessaire, il est essentiel de limiter la charge anticholinergique globale, en particulier chez les patients présentant des troubles cognitifs ou à risque d'en générer, afin de réduire la survenue d'effets indésirables cognitifs.

Chez la personne âgée, il est recommandé d'initier les traitements à faibles doses, avec une augmentation progressive de la posologie (« start low, go slow »), correspondant à une **titration lente et prudente**, en particulier pour les médicaments à effets sérotoninergiques ou anticholinergiques (Gurwitz, 1995). Les recommandations de la Société Française de Gériatrie et Gérontologie (SFGG) préconisent, pour le tramadol, l'utilisation préférentielle des formes à libération prolongée, débutées à 50 mg toutes les 12 heures, afin d'améliorer la sécurité et l'observance. Pour les antidépresseurs, la SFGG recommande une prescription vespérale avec des posologies initiales réduites de moitié par rapport à l'adulte jeune est recommandée, avec une titration lente. Une surveillance étroite des patients est nécessaire durant les premiers jours suivant l'initiation du traitement, afin de prévenir l'apparition d'une confusion.

Si la prescription d'un médicament est nécessaire à long terme, la SFGG recommande de réviser systématiquement l'ensemble du traitement régulièrement, au moins une fois par an et lors de tout événement intercurrent ou diagnostic d'une nouvelle pathologie qui peut modifier les objectifs et les modalités de l'ensemble du traitement.

L'**optimisation thérapeutique** apparaît donc comme une priorité et peut être réalisée en utilisant des méthodes validées comme OPTIMEDOC (Guerin et al., 2025). L'apparition d'un nouveau symptôme chez la personne âgée (anorexie, malaise, chute, confusion) doit faire évoquer en priorité un effet indésirable médicamenteux, en particulier en cas de modification thérapeutique récente. Dans ce cas, et avant de décider de la déprescription du médicament en cause, envisager de réaliser une mesure des concentrations plasmatiques afin de s'assurer qu'il n'y ait pas d'accumulation de la substance active, pourrait être pertinent. Le STP pourrait également être réalisé lors d'événements susceptibles de modifier l'exposition systémique du médicament (retrait/ajout d'un traitement, nouvelle situation physiopathologie) afin d'adapter les posologies et limiter la survenue d'effets indésirables concentration-dépendants.

Toutes ces arguments vont dans le sens d'une **médecine personnalisée et d'un suivi des concentrations permettant d'adapter la prise en charge en fonction du contexte clinique**, notamment chez le patient âgé à risque cognitif. Les **approches multidisciplinaires**, associant médecins, pharmaciens, infirmiers et aidants, sont donc essentielles pour accompagner les patients dans une gestion sécurisée et adaptée des traitements.

CONCLUSION

Cette étude chez le rat adulte a permis d'évaluer les effets spécifiques de la prise chronique de trois médicaments très prescrits chez la personne âgée et souvent administrés sur de longues périodes, sur les fonctions cognitives. Une telle investigation est difficile à réaliser directement chez le sujet âgé, en raison des biais liés au vieillissement, à la multimorbidité, à la polymédication et au déclin cognitif. Dans notre étude, les effets délétères observés dans notre modèle de rat surexposé, pourraient être **amplifiés dans une population de rats âgés**, en particulier en raison des modifications pharmacocinétiques et pharmacodynamiques liées à l'âge.

A son échelle, cette étude chez le rat rappelle la nécessité de **prudence quant à la prescription chronique de substances psychoactives chez le sujet à risque de troubles cognitifs**, tant au début du traitement qu'en exposition prolongée. Lors de l'initiation, ces médicaments peuvent provoquer des épisodes de confusion, tandis qu'une utilisation prolongée peut conduire à l'accumulation de la substance active et à des altérations des fonctions cognitives. Le choix des molécules et le suivi régulier des concentrations plasmatiques semblent donc essentiels dans le but de mieux prévenir la survenue ou l'aggravation de troubles cognitifs existants.

Au-delà de la polymédication, la variabilité interindividuelle de la réponse pharmacocinétique et pharmacodynamique chez la personne âgée, ainsi que la présence fréquente de comorbidités, contribuent de manière additive ou synergique au risque de troubles cognitifs. **Une vigilance particulière s'impose donc non seulement chez les personnes âgées, mais également chez les sujets à risque de déficit cognitif ou d'accumulation plasmatique (insuffisance rénale ou hépatique).**

Pour compléter cette étude, des approches complémentaires pourraient être développées chez l'animal, notamment en intégrant des cohortes de femelles ou de rats âgés. Par ailleurs, l'étude des relations concentration-effet pourrait être approfondie en utilisant différentes doses d'administration. Afin d'identifier les mécanismes sous-jacents, de nouvelles études utilisant des outils tels que l'imagerie cérébrale, l'électrophysiologie, la quantification des neurotransmetteurs et de leurs récepteurs ou l'analyse des voies de signalisation, permettraient de corréler les données pharmacocinétiques et pharmacodynamiques.

Chez l'Homme, au-delà des approches pharmaco-épidémiologiques souvent limitées par les biais, des études cliniques prospectives seraient nécessaires. Celles-ci devraient associer des mesures de concentrations plasmatiques, des évaluations cognitives standardisées, la prise en compte de la durée d'exposition et des covariables comme la polymédication, afin de tenter d'établir une relation exposition-effet pour ces médicaments.

La **pharmacologie expérimentale reste un outil essentiel** pour explorer des mécanismes difficilement accessibles chez l'Homme, en permettant un contrôle strict de l'exposition aux substances et en limitant les biais inhérents aux études cliniques. Il est donc essentiel de maintenir une **approche translationnelle**, en effectuant des allers-retours entre recherches chez l'Homme et chez l'animal. Par exemple, un signalement d'effet indésirable chez l'Homme pour un médicament donné peut être étudié chez l'animal afin de vérifier si ce médicament, pris isolément, peut provoquer le même type d'effet. Inversement, les observations réalisées chez l'animal peuvent permettre de prédire la survenue d'effets cognitifs chez l'Homme, contribuant ainsi à une meilleure prévention et à un usage plus sûr des médicaments psychoactifs chez les populations vulnérables.

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ANNEXES

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RESEARCH Open Access

Association between antidepressant use and delirium in older adults: an analysis of the World Health Organization's global pharmacovigilance database

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Abstract

Background Psychoactive drugs frequently cause delirium adverse events in older adults. However, few data on the relationship between antidepressants and delirium are available. Here, we investigated the association between antidepressant prescription and pharmacovigilance reports of delirium in older adults.

Methods Using the World Health Organization's VigiBase® global pharmacovigilance database from 1967 to 2022, we performed a disproportionality analysis in order to probe the putative associations between each antidepressant class (non-selective monoamine reuptake inhibitors (NSMRIs), selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), monoamine oxidase inhibitors (MAOIs), alpha-2-adrenergic receptor antagonists, and other antidepressants) and reports of delirium in people aged 65 or over. We calculated the reporting odds ratios (r-OR) and their 95% confidence interval ([95%CI]) with logistic regression models before and after adjustment for confounding factors. Secondary analyses were performed for each drug and within each class by age group (65-74, and 75 and over). We also studied the reports of concomitant delirium and hyponatremia.

Results Our main analysis included 87,524 cases of delirium. After adjustment for confounders, a significant association was found between delirium and all antidepressant classes other than SNRIs. Intraclass disparities were found for the association between the most frequently prescribed antidepressants and reports of delirium. An elevated risk of reports of concomitant delirium and hyponatremia was found for SSRIs (4.46 [4.01-4.96]), SNRIs (1.25 [1.07-1.46]), MAOIs (1.72 [1.41-2.09]), and the "other antidepressants" class (1.47 [1.30-1.65]).

Conclusions There was a significant association between reports of delirium and antidepressant classes (other than SNRIs). However, this association varied from one drug to another within a given antidepressant class. Moreover, this association could not always be explained by antidepressant-induced hyponatremia.

Keywords Antidepressants, Delirium, Older adults

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Background

The reported incidence of delirium in hospitalized older adults ranges from 25 to 56% [1], and the occurrence of delirium is associated with elevated morbidity and mortality rates and higher health costs [2]. However, it has been shown that 30-40% of cases of delirium could be prevented [2]. Thus, the establishment of preventive measures is a key public health objective that could help to improve the mental health of older adults and reduce the costs associated with managing delirium.

Adverse drug events (ADEs) are major risk factors for the occurrence of delirium. It is estimated that 12-39% of cases of delirium are attributable to an ADE in general and to psychoactive drugs in particular [3]. In contrast to the well-documented association between delirium and the use of antipsychotics and benzodiazepines [3], the association with antidepressant use has been seldom studied. In fact, some antidepressants (such as non-selective monoamine reuptake inhibitors (NSMRIs)) have anticholinergic properties [4] and others (such as selective serotonin reuptake inhibitors (SSRIs) and serotonin norepinephrine reuptake inhibitors (SNRIs)) can trigger hyponatremia [5]; both of these properties are risk factors for delirium [4, 5]. Due to multimorbidity, polypharmacy (including drug interactions), and aging-related changes in drug pharmacokinetics and pharmacodynamics, antidepressants might accentuate the risk of delirium in older patients [6].

Depression is known to predispose to delirium [1] and so is a public health issue [7]. Furthermore, depression is common in patients with cognitive impairment, which itself is a major risk factor for delirium [1]. Untreated depression, the wrong choice of antidepressants, or poorly conducted treatment might impede the resolution of concomitant somatic problems, increase the time spent in hospital, augment the risk of suicide, and/or worsen treatment compliance among older patients [7]. Therefore, the treatment of depression in older adults is often challenging; the goal is to effectively relieve the depression while avoiding ADEs like delirium.

To the best of our knowledge, there are no literature data on the risk of delirium associated with the use of various antidepressant classes in older adults. We hypothesized that delirium is more strongly associated with antidepressants with anticholinergic properties (e.g. NSMRIs) or hyponatremic properties (e.g. SSRIs and SNRIs) than with other antidepressant classes. We then studied these putative associations by analyzing data from the World Health Organization (WHO)'s VigiBase® global pharmacovigilance database.

Methods

Study design, setting, and population

We conducted an international, retrospective, pharmacovigilance disproportionality analysis using the WHO's VigiBase® pharmacovigilance database. VigiBase® contains more than 30 million individual case safety reports received from 160 members countries since 1967.

We used included VigiBase® data from the database's inception to March 1st, 2022. We restricted our analysis to the individual case safety reports on people aged 65 or over. The study protocol was registered at ClinicalTrials.gov (NCT05356078). In line with the French legislation on retrospective, anonymized studies of routine medical practice (MR-004), and in accordance with the European regulation of April 27, 2016 on the protection of individuals with regard to the processing of data to personal character, the study protocol was approved by a hospital committee (C.L.E.R.S Comité Local d'Ethique de la Recherche en Santé) with competency for research not requiring authorization by an Institutional Review Board (University of Caen Normandy (Caen, France); reference: 2646, dated July 15th, 2021).

Variables

Each individual case safety report include administrative data (country, type of report, and type of reporter), sociodemographic data (age and sex), the time to the onset of the ADE, the outcome (coded according to the Medical Dictionary for Regulatory Activities (MedDRA) version 24.0), the WHO causality assessment, and the drug(s) involved (drug name, start and stop dates, time to onset, indication, dose, dechallenge, and rechallenge).

In VigiBase®, drugs are coded using the WHODrug Global dictionary. Antidepressant classes were based on the Anatomical Therapeutic Chemical (ATC) hierarchical classification and classified into NSMRIs, SSRIs, SNRIs, monoamine oxidase inhibitors (MAOIs), alpha-2-adrenergic receptor antagonists, and other antidepressants (for details, see Supplementary Table 1). Most studies included SNRIs and alpha-2-adrenergic receptor antagonists in an "other antidepressants" class; however, in view of their specific pharmacodynamic properties and their frequency of use, we decided to consider these two classes in their own right [5, 8]. We also noted the most frequently prescribed antidepressant drugs within each class, defined as those mentioned in more than 1000 reports (regardless of the type of ADE) in VigiBase®.

ADEs were coded according to the MedDRA terminology. In the present study, the event "delirium" encompassed the MedDRA terms "Delirium", "Confusional state" and "Disorientation". A concomitant delirium-hyponatremia event was defined as a report in which

delirium (as defined above) and hyponatremia at the same time were reported.

We selected all types of reports, regardless of whether the antidepressant was suspected to be responsible for delirium, concomitantly prescribed with another suspected drug, or thought to be interacting with another drug.

Outcomes

The primary outcome was the association between antidepressant use and reports of delirium among people aged 65 or over. The secondary outcomes included (i) the association between the most frequently prescribed antidepressants and reports of delirium, (ii) the association between antidepressant classes and concomitant delirium-hyponatremia events, and (iii) the same associations by age class (65 to 74 vs. 75 and over).

Statistical methods

A case/non-case disproportionality analysis was used to probe the effect of antidepressant prescription on reports of delirium; this type of analysis has been described in detail previously [9]. The case/non-case disproportionality method is recommended for detecting a signal for an association between a drug and an adverse event in a pharmacovigilance database. A signal is present when the number of reports of an adverse event is greater than expected. This is referred to as a disproportionate reporting rate of an adverse event, relative to others. In the present study, a signal corresponded to a statistically significant difference in the distribution of cases of delirium related to antidepressants or classes of antidepressant vs. cases of delirium related to drugs other than antidepressants. The strength of the disproportionality was quantified as the reporting odds ratio (r-OR) and their 95% confidence interval ([95%CI]) estimated with univariate and multivariate logistic regression models. An r-OR was considered statistically significant if it was higher than 1 and if the lower boundary of its 95%CI did not include 1. An r-OR lower than 1 was considered statistically unsignificant, as the method was unable to detect the absence of a signal.

Disproportionality analyses typically include positive controls (i.e. drugs or drugs classes established to trigger the ADE of interest) and negative controls (i.e. drugs that are not known to trigger the ADE of interest); if the obtained results and the expected results are consistent, major sources of bias are likely to be absent. In the present multivariate analysis, we chose natural opium alkaloids (ATC N02AA) as positive controls and bisphosphonates (ATC M05BA) as negative controls [3].

Our models were adjusted for potential confounders: age class (65-74, 75 and over), sex, geographic region, and the major potentially associated prescriptions of drugs and illnesses reported in the literature as inducing delirium (opioids (ATC N02), antipsychotics (ATC N05A), anxiolytics (ATC N05B), and hypnotics (ATC N05C), constipation, acute urinary retention, alcohol use, unspecified infections, drug misuse, dementia, dehydration, hyponatremia ("hyponatraemia", according to the spelling used in MedDRA), anticholinergic syndrome, hypoglycemia ("hypoglycaemia", according to the spelling used in MedDRA), seizure disorder, drug abuse, drug dependence, drug withdrawal, central nervous system vascular disorders, hearing impairment, and visual impairment [2, 3, 10–12]; for details, see Supplementary Table 2. We studied the collinearity of our final models by computing the variance-inflation factors.

Sensitivity analyses were conducted on a multivariate model for the primary outcome by using three different definitions of delirium: (i) "Delirium" or "Confusional state"; (ii) "Delirium" or "Confusional state" or "Disorientation" or "Circadian rhythm sleep disorder"; and (iii) "Delirium" or "Confusional state" or "Disorientation" or "Circadian rhythm sleep disorder" or "Hallucinations".

In analyses of pharmacovigilance databases, it is sometimes not possible to assess certain variables because the latter data are missing or because the patient did not meet certain clinical or pharmacologic criteria. In the present study, the person's age was specified for all reports; hence, sex was the only variable for which missing data could have been imputed. As less than 1% of the data for this variable were missing, we decided not to impute them and so performed a complete case analysis.

All statistical analyses were performed using R software (v 4.0.2, R Studio v1.4.1717) and its packages rlang, dplyr, stats, base, fst, data.table, magrittr, openxlsx, carData, car, grid, and checkmate [13].

Results

Descriptive analysis of the study population

In VigiBase®, we found 87,524 reports of delirium in people aged 65 and over (Table 1). The 65-74 and 75+age groups accounted for 41% and 59% of the reports, respectively. 54% of the reports concerned women. Of the people presenting delirium, 22% were being treated with antidepressants; SSRIs: 7%; other antidepressants: 7%; NSMRIs: 3%; SNRIs: 3%; alpha-2-adrenergic receptor antagonists: 2%; MAOIs: <1%. It is also noteworthy that 34% of patients with delirium were being treated with opioids, and 7% suffered from an unspecified infection.

Primary outcome

The associations between antidepressant classes and delirium are shown in Table 1 and Fig. 1. No collinearity was found in our final models (Supplementary

Table 1 The risk of reports of delirium in older adults, and characteristics of the study population

A)

		Main analysis			
		Number of reports of delirium	Number of total reports	Univariate analysis r-OR[95%CI]	Multivariate analysis r-OR[95%CI]
Total number of reports in patients aged 65 or over		87524	6048742		
Antidepressant classes	Non-selective monoamine reuptake inhibitors	2658 (3.0%[1])	55646 (< 1%[2])	3.49[3.36-3.63]	1.37[1.32-1.43]
	Selective serotonin reuptake inhibitors	6475 (7.4%)	148194 (2.4%)	3.28[3.20-3.37]	1.14[1.11-1.18]
	Serotonin-norepinephrine reuptake inhibitors	2247 (2.6%)	54171 (< 1%)	3.00[2.87-3.13]	0.88[0.84-0.93]
	Monoamine oxidase inhibitors	158 (< 1%)	2256 (< 1%)	5.14[4.37-6.04]	2.76[2.33-3.27]
	Alpha-2-adrenergic receptor antagonists	1553 (1.8%)	31470 (< 1%)	3.58[3.40-3.77]	1.37[1.30-1.45]
	Other antidepressants	5850 (6.7%)	133197 (2.2%)	3.28[3.19-3.37]	1.23[1.20-1.27]

[1] 3% of adults aged 65 and over, for whom a report of delirium was made in Vigibase®, were using non-selective monoamine reuptake inhibitors
[2] < 1% of adults aged 65 and over, for whom a report of adverse drug event was made in Vigibase®, were using non-selective monoamine reuptake inhibitors

B)

		Variables introduced into the multivariate analysis[3]		
		Number of reports of delirium	Number of total reports	Univariate analysis r-OR[95%CI]
Sex[4]	Men	39655 (45%)	2552052 (42%)	1.13[1.12-1.15]
	Women	47123 (54%)	3427187 (57%)	Ref
Age	65-74	36155 (41%)	3401357 (56%)	Ref
	75+	51369 (59%)	2647385 (44%)	1.84[1.82-1.87]
Potentially associated illnesses known to induce delirium	Constipation	1626 (1.9%)	66587 (1.1%)	1.72[1.63-1.81]
	Acute urine retention	643 (< 1%)	12283 (< 1%)	3.78[3.49-4.10]
	Alcohol	26 (< 1%)	327 (< 1%)	5.88[3.94-8.78]
	Infections unspecified	6442 (7.4%)	308170 (5.1%)	1.49[1.45-1.53]
	Drug misuse	2641 (3.0%)	191303 (3.2%)	0.95[0.92-0.99]
	Dementia	1318 (1.5%)	10994 (< 1%)	9.40[8.87-9.96]
	Dehydration	2174 (2.5%)	34166 (< 1%)	4.72[4.52-4.93]
	Hyponatremia	3532 (4.0%)	39553 (< 1%)	6.92[6.68-7.17]
	Anticholinergic syndrome	36 (<1%)	162 (< 1%)	19.47[13.44-28.20]
	Hypoglycemia	1576 (1.8%)	45506 (< 1%)	2.47[2.35-2.60]
	Seizure disorder	2064 (2.4%)	34392 (< 1%)	4.43[4.23-4.63]
	Drug abuse, dependence, withdrawal	896 (1.0%)	29000 (< 1%)	2.18[2.04-2.33]
	Central nervous system vascular disorders	3734 (4.3%)	141280 (2.3%)	1.89[1.83-1.95]
	Hearing impairment	934 (1.1%)	37396 (< 1%)	1.75[1.64-1.87]
	Visual impairment	530 (< 1%)	43516 (< 1%)	0.84[0.77-0.91]
Potentially associated prescriptions of drugs known to induce delirium	Opioids	30134 (34%)	1175087 (19%)	2.22[2.18-2.25]
	Antipsychotics	9659 (11%)	173605 (2.9%)	4.39[4.29-4.48]
	Anxiolytics	16860 (19%)	415752 (6.9%)	3.33[3.27-3.38]
	Hypnotics	9126 (10%)	181304 (3.0%)	3.91[3.83-4.00]

[3] including geographic region
[4] 746 NA (1% missing data for sex variable)

C)

		Controls		
		Number of reports of delirium	Number of total reports	Univariate analysis r-OR[95%CI]
Positive controls	Natural opium alkaloids	5203 (5.9%)	113314 (1.9%)	3.35[3.25-3.44]
Negative controls	Bisphosphonates	1805 (2.1%)	117483 (1.9%)	1.04[0.99-1.09]

Table 3). There was a significant association between each antidepressant class and delirium, with the exception of the SNRIs (Fig. 1).

Secondary outcomes

Associations between the most frequently prescribed antidepressants and reports of delirium in patients aged 65

Antidepressant classes	Number of reports of delirium	Number of total reports	r-OR	[95%CI]
NSMRIs	2658	55646	1.37	[1.32-1.43]
MAOIs	158	2256	2.76	[2.33-3.27]
SSRIs	6475	148194	1.14	[1.11-1.18]
SNRIs	2247	54171	0.88	[0.84-0.93]
Alpha-2-adrenergic-receptor-antagonists	1553	31470	1.37	[1.30-1.45]
Other antidepressants	5850	133197	1.23	[1.20-1.27]

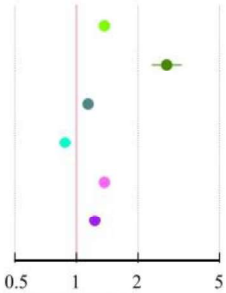


Fig. 1 Multivariate analysis of the association between antidepressant classes and reports of delirium in older adults

Antidepressant classes	Number of reports of delirium	Number of total reports	r-OR	[95%CI]
NSMRIs				
Amitriptyline	1465	32233	1.20	[1.13-1.27]
Clomipramine	251	3214	1.61	[1.41-1.85]
Dosulepin	112	2399	2.16	[1.78-2.63]
Doxepin	190	4483	0.81	[0.70-0.94]
Imipramine	171	2631	2.82	[2.40-3.31]
Lofepramine	65	1359	1.87	[1.45-2.42]
Maprotiline	82	1305	2.71	[2.14-3.42]
Nortiptyline	239	5950	1.42	[1.24-1.63]
Trimipramine	63	1428	1.62	[1.24-2.10]
MAOIs				
Moclobemide	87	1214	2.97	[2.35-3.74]
SSRIs				
Citalopram	1501	32965	1.49	[1.41-1.58]
Escitalopram	1052	26846	0.85	[0.80-0.91]
Fluoxetine	926	22376	1.01	[0.94-1.08]
Fluvoxamine	119	2551	1.72	[1.42-2.08]
Paroxetine	1386	26281	1.29	[1.22-1.37]
Sertraline	1604	39213	0.92	[0.87-0.97]
SNRIs				
Venlafaxine	1161	23223	1.00	[0.94-1.07]
Desvenlafaxine	69	2537	1.08	[0.85-1.38]
Duloxetine	1009	27649	0.79	[0.74-0.84]
Milnacipran	38	1186	0.99	[0.71-1.37]
Alpha-2-adrenergic receptor antagonists				
Mianserin	309	5855	1.68	[1.49-1.89]
Mirtazapine	1250	25676	1.31	[1.23-1.39]
Other antidepressants				
Bupropion	459	13066	1.14	[1.04-1.26]
Nefazodone	61	1155	2.27	[1.74-2.95]
Selegiline	315	3625	4.55	[4.03-5.13]
Tianeptine	85	1925	1.55	[1.24-1.94]
Trazodone	988	23250	1.13	[1.06-1.21]
Vortioxetine	66	2057	1.17	[0.90-1.51]

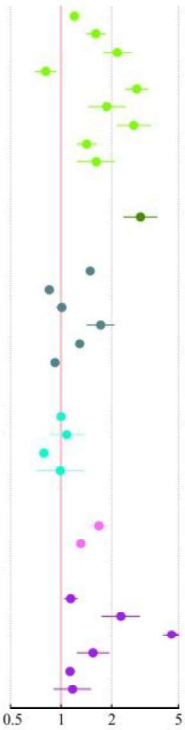


Fig. 2 Multivariate analysis of the association between the most frequently prescribed antidepressants and reports of delirium in older adults

or over

We observed a significant, positive association with reports of delirium for all NSMRIs other than doxepin (Fig. 2). Moclobemide was the only MAOI to be studied; we observed a significant association with reports of delirium (2.97 [2.35-3.74]). When considering the SSRIs, reports of delirium were significantly associated with citalopram (1.49 [1.41-1.58]), fluvoxamine (1.72 [1.42-2.08]),

and paroxetine (1.29 [1.22-1.37]) but not fluoxetine, escitalopram, or sertraline. Within the SNRI class, no associations with reports of delirium were found. Within the alpha-2-adrenergic receptor antagonist class, mianserin (1.68 [1.49-1.89]) and mirtazapine (1.31 [1.23-1.39]) were associated with reports of delirium. Within the "other antidepressants" class, a significant association with reports of delirium was found for all the studied drugs other than vortioxetine.

Associations between antidepressant classes and concomitant delirium and hyponatremia

All the antidepressant classes other than NSMRIs were significantly associated with reports of concomitant delirium and hyponatremia (Fig. 3 and Supplementary Table 4). The strongest association was found for SSRIs (4.46 [4.01-4.96]).

Associations by age group

The associations between antidepressant classes and delirium reporting for the 65-74 and 75+age groups were similar to those of the main analysis (Fig. 4 and Supplementary Tables 5 and 6).

Sensitivity analyses

The results of sensitivity analyses using the various definitions of delirium were consistent with the those of the primary analysis (Supplementary Table 7).

Positive and negative controls

As expected, the positive control was significantly and positively association with reports of delirium (3.35 [3.25-3.44]) and the negative control was not (1.04 [0.99-1.09]) (Table 1).

Discussion

The overall objective of the present study was to investigate the association between antidepressant classes and reports of delirium in VigiBase®. To the best of our knowledge, the present study population of older adults, for whom an adverse drug event had been reported, is the largest yet studied in this respect. After adjusting for a large number of potential confounders, we found that all antidepressant classes other than SNRIs were associated with an elevated risk of delirium reporting in older patients. These results by age class did not differ markedly from the overall results. We also observed that within a given antidepressant class, the risk varied from one drug to another. All antidepressant classes other than NSMRIs were associated with reports of concomitant delirium and hyponatremia.

NSMRIs are known to have anticholinergic and sedative properties via their affinities for muscarinic acetylcholine receptors and histamine H1 receptors, respectively [14]. Both of these properties are known to be precipitating risk factors for delirium [4, 15]. Here, we indeed observed a significant, positive association between NSMRIs and reports of delirium. This association was found for all NSMRIs other than doxepin, which has a similar mechanism of action but a lower affinity for muscarinic receptors (and thus a weaker anticholinergic effect) than other drugs in this class [16]. Due to its high affinity for a subtype of histamine H1 receptor, doxepin is also used to manage chronic urticaria [17]. Thus, the characteristics of patients treated with doxepin might differ from those of other patients treated with NSMRIs – particularly in terms of psychiatric comorbidities like depression, which is associated with a greater likelihood of reports of delirium.

We also found a significant association between the use of alpha-2-adrenergic receptor antagonists and reports of delirium. Although alpha-2-adrenergic receptor

Antidepressant classes	Number of reports of delirium	Number of total reports	r-OR	[95%CI]
NSMRIs	143	55646	1.03	[0.87-1.23]
MAOIs	9	2256	3.09	[1.60-5.99]
SSRIs	857	148194	4.46	[4.01-4.96]
SNRIs	200	54171	1.25	[1.07-1.46]
Alpha-2-adrenergic-receptor-antagonists	111	31470	1.72	[1.41-2.09]
Other antidepressants	397	133197	1.47	[1.30-1.65]

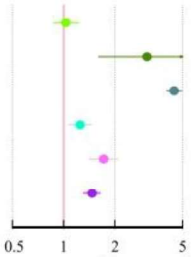
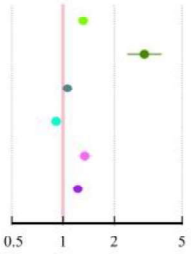


Fig. 3 Multivariate analysis of the association between antidepressant classes and reports of concomitant delirium and hyponatremia in older adults

A) 65-74 age group

Antidepressant classes	Number of reports of delirium	Number of total reports	r-OR	[95%CI]
NSMRIs	1172	32342	1.31	[1.23-1.40]
MAOIs	82	1340	3.00	[2.38-3.79]
SSRIs	2436	76193	1.06	[1.01-1.11]
SNRIs	1082	32792	0.91	[0.85-0.97]
Alpha-2-adrenergic-receptor-antagonists	507	13137	1.34	[1.22-1.48]
Other antidepressants	2543	73503	1.22	[1.17-1.28]



B) 75+ age group

Antidepressant classes	Number of reports of delirium	Number of total reports	r-OR	[95%CI]
NSMRIs	1486	23304	1.42	[1.34-1.51]
MAOIs	76	916	2.49	[1.95-3.18]
SSRIs	4039	72001	1.20	[1.16-1.25]
SNRIs	1165	21379	0.86	[0.81-0.92]
Alpha-2-adrenergic-receptor-antagonists	1046	18333	1.39	[1.30-1.48]
Other antidepressants	3307	59694	1.24	[1.19-1.29]

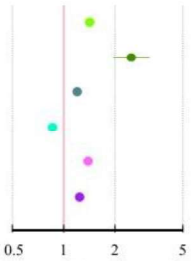


Fig. 4 Multivariate analysis of the association between antidepressant classes and reports of delirium, by age group

antagonists do not have any anticholinergic activity [14], antagonism of histamine H1 receptors should also be considered for this class of antidepressant. mianserin and mirtazapine have a high affinity for histamine H1 receptors (as do first-generation antihistamines) [18, 19]. Thus, the sedative properties of histamine H1 receptor antagonists might also explain the elevated risk of reports of delirium associated with alpha-2-adrenergic receptor antagonists [15].

Most SSRIs lack anticholinergic or antihistaminic properties [14], although paroxetine and fluvoxamine both have anticholinergic activity [4]. It is noteworthy that in the present study, both paroxetine and fluvoxamine were associated with the reports of delirium. This finding strengthens the hypothesis whereby the elevated risk of delirium reporting associated with antidepressants is partly explained by anticholinergic mechanisms. Moreover, the results for escitalopram vs. citalopram were discordant. It should be borne in mind that citalopram is a 1:1 racemate of R- and S-enantiomers, whereas escitalopram is S-citalopram [20]. It has been shown that R-citalopram limits the therapeutic efficacy and delays the onset of action of S-citalopram; this explains why the required doses are higher for citalopram than for escitalopram [20]. It has also been shown in animals that the presence of the R-enantiomer in citalopram can produce a paradoxical anxiogenic effect above a certain concentration threshold [20]. The R- and S-enantiomers therefore differ in their pharmacokinetic, pharmacodynamic and clinical properties. The difference between citalopram and escitalopram observed in our study might also be due to the preferential use of citalopram in severe forms of depression, for which parenteral administration is perhaps superior to oral administration [21]. One can hypothesize that the people taking citalopram had more severe depression, refused oral administration, or had other behavioral disturbances; this would constitute a confounding bias and might explain the significant association between reports of delirium and citalopram [22] but not escitalopram.

Interestingly, SNRIs were the only antidepressant class not associated with reports of delirium in our study. Although the r-OR for SNRIs was < 1 with its higher 95%CI boundary < 1, it cannot be interpreted as a protective signal, in the setting of disproportionality analysis. This lack of an association might be due to

the drugs' pharmacodynamic properties. Indeed, SNRIs have a more selective action on the reuptake of serotonin and noradrenaline and are not linked to adverse effects caused by cholinergic and histamine receptor blockades [23]. One can hypothesize that these properties of the SNRIs can explain (at least in part) the lack of an association with the occurrence of delirium in older people with high inter-individual pharmacodynamic variability [6].

Here, we found that concomitant delirium and hyponatremia were associated with the use of SSRIs, SNRIs, alpha-2-adrenergic receptor antagonists, and MAOIs. These findings are consistent with the literature data on an association between SSRIs/SNRIs and hyponatremia [5]. The literature data on alpha-2-adrenergic receptor antagonists are somewhat contradictory but tend to show an association with hyponatremia [24]. Significant associations with SSRIs, SNRIs and alpha-2-adrenergic receptor antagonists might be due to the drugs' action on certain serotonin receptors, which would lead to inappropriate antidiuretic hormone secretion and then hyponatremia [24]. Our results for MAOIs are consistent with an analysis of the French national pharmacovigilance database [25]. However, the number of reported events was small in both studies (only 9 here, for example); this lack of statistical power and the conflicting literature results make it hard to draw firm conclusions in this respect [5]. One can nevertheless suspect that the occurrence of delirium is mediated partly by the induction of hyponatremia by antidepressant drugs from these four classes.

In contrast, we did not find a significant association between NSMRIs and concomitant delirium and hyponatremia in our study. Nevertheless, an association between some NSMRIs and the occurrence of hyponatremia has been reported [26]. Even though NSMRIs are less likely to cause hyponatremia than SSRIs [5], they can still induce inappropriate antidiuretic hormone secretion [26]. Hence, hyponatremia is unlikely to be the main mechanism in NSMRI-induced delirium. Likewise, it would have been interesting to study the association between antidepressant classes and anticholinergic syndrome. However, the very small number of cases reported in VigiBase® prevented us from performing a robust analysis.

The "other antidepressants" class was also associated with reports of delirium and reports of concomitant delirium and hyponatremia. The antidepressants classified in this class have very different pharmacodynamic properties [8] and so the results for each drug should be interpreted with caution, due to a possible lack of statistical power. There are only a few literature data (from case reports and a study of the French national pharmacovigilance database) in support of our present findings [25, 27].

The present study had a number of strengths, most of which were due to its statistical methodology. Firstly, VigiBase® includes 30 million reports on ADEs from more than 160 countries and thus provides a large sample of older adults with reported delirium. Secondly, disproportionality analysis is a well-established method for detecting signals in pharmacovigilance safety research and post-marketing surveillance [28]. Lastly, our multivariate analysis and our use of positive and negative controls reduced potential sources of bias and enhanced the validity of our hypothesis [9].

Study limitations

Although pharmacovigilance analysis can detect safety signals and generate hypotheses, it cannot alone provide evidence of causal associations; this constituted a first limitation [28]. Moreover, the diagnosis of delirium can be challenging, due to a variety of presentations and the probable under-reporting of hypoactive forms [12]. However, in order to limit possible classification bias in our study, we defined cases of delirium by reference to the MedDRA terms "Delirium", "Confusional state" and "Disorientation". Indeed, our chosen definition was shown to be robust in comparative sensitivity analyses.

Secondly, we were not able to check the diagnoses reported in VigiBase®. Thirdly, the individual case safety reports did not all have details of the age, drug dose, time of onset, comorbid conditions, and concomitant medications [28]. For example, the prevalence of dementia in Vigibase® for adults aged 65 and over was 0.2% (10994 reported cases out of 6048742 adults aged 65 and over) but (according to the WHO) is about 5-8% in people aged 60 and over [29]. Lastly, our focus on delirium prevented us from drawing conclusion about the overall iatrogenic burden of antidepressant drugs or each antidepressant class.

Conclusions

We observed a significant association between most antidepressant classes and reports of delirium in VigiBase®. This likelihood of reports was greater for antidepressants with high affinity for histamine H1 receptors and/or muscarinic receptors than for antidepressants lacking these properties. Our results might suggest that the use of SNRIs (venlafaxine, desvenlafaxine, duloxetine, milnacipran) and certain SSRIs (escitalopram, sertraline, fluoxetine) as well as doxepin and vortioxetine might be safer in older people at a high risk of delirium, such as those with cognitive impairment. However, further preclinical and clinical studies (e.g. animal models, and functional imaging in humans) of the antidepressants' mechanisms of action in older people will be necessary to consider practical applications of our results.

Abbreviations

NSMRIs	Non-selective monoamine reuptake inhibitors
SSRIs	Selective serotonin reuptake inhibitors
SNRIs	Serotonin-norepinephrine reuptake inhibitors
MAOIs	Monoamine oxidase inhibitors
r-OR	Reporting odds ratios
[95%CI]	95% Confidence interval
ADEs	Adverse drug events
WHO	World Health Organization
MedDRA	Medical Dictionary for Regulatory Activities
ATC	Anatomical Therapeutic Chemical

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

EM.M., C.V., C.D., V. LB.: Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work. EM.M., C.V., C.D., V. LB.: drafting the work or revising it critically for important intellectual content. EM.M., C.V., C.D., V. LB, S.B., B.C., P.D., M.H., M.S., M.P., O.M.: Final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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The study data are the property of the World Health Organization, the corresponding author has access to the data extraction and the R script used to analyse it.

Availability of data and materials

The study data are the property of the World Health Organization, the corresponding author has access to the data extraction and the R script used to analyse it.

Declarations

Ethics approval and consent to participate

In line with the French legislation on retrospective, anonymized studies of routine medical practice (MR-004), and in accordance with the European regulation of April 27, 2016 on the protection of individuals with regard to the processing of data to personal character, the study protocol was approved by a hospital committee (C.L.E.R.S Comité Local d'Ethique de la Recherche en Santé) with competency for research not requiring authorization by an Institutional Review Board (University of Caen Normandy (Caen, France); reference: 2646, dated July 15th, 2021).

Competing interests

The authors declare no competing interests.

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Pharmacokinetic validation of a new protocol of chronic oral administration of aripiprazole in male C57 Bl/6 mice through drinking solution

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ABSTRACT

Schizophrenia is a chronic mental illness, with a prevalence of about 1%. The symptoms are classified in three categories: positive symptoms, negative symptoms and deficits in cognitive function. Regarding the pharmacological treatment, current antipsychotics mainly improve positive symptoms while negative and cognitive symptoms remain inadequately treated. Among them, aripiprazole, through its pharmacological properties results in regulation of positive symptoms and, to a lesser extent, to negative and cognitive ones. Drug treatment studies in animals typically employ administration methods such as injection or gavage, which is time-consuming over long periods, does not reflect route of administration used in patients, and cause substantial stress in animals. We assessed herein the pharmacokinetic properties and behavioural effects of aripiprazole after chronic administration via drinking solution in C57Bl/6 mice. Results show that subchronic administration of 1 and 10 mg/kg doses of aripiprazole via drinking solution present significant blood concentrations, without any significant effect on the spontaneous activity of mice (between 10 a.m. and 3 p.m.). Furthermore, we described for the first time to our knowledge the main pharmacokinetic parameters of aripiprazole after gavage acute administration (10 mg/kg). Collectively, all of these data will be very useful in future projects including chronic administrations (several months) of this antipsychotic.

1. Introduction

Schizophrenia, with a prevalence of approximately 1%, is a common chronic mental illness. Its symptoms are classified into three categories: positive, negative and deficits in cognitive function (Davidson and McGlashan, 1997). Current pharmacological treatment, namely antipsychotics, are characterized by severe limitations. Firstly, they are effective in only approximately 70% of patients (Diniz et al., 2023). Secondly, they mainly target positive symptoms (*e.g.* hallucinations and thought disorders), while both negative symptoms (*e.g.* flat affect and social withdrawal) and cognitive deficits (*e.g.* learning, memory and attention disorders) remain inadequately addressed (Maroney M., 2022).

Aripiprazole is a third-generation antipsychotic, due to its partial agonist activity on dopamine D_2, D_3 receptor and serotonin 5-HT_{1A} receptor as well as its antagonist activity on serotonin 5-HT_{2a} receptor (Preda and Shapiro, 2020). Its pharmacological properties allow it to regulate positive schizophrenic symptoms and, to a lesser extent, negative and cognitive ones with minimal metabolic and extrapyramidal side effects (Hori et al., 2017). Currently, aripiprazole is approved for use in patients with various psychotic disorders, including schizophrenia, bipolar disorder, depression, and autism (Kirino, E., 2012; Pae C.U., 2009; Pae et al., 2011) and some clinical studies showed its beneficial effects on certain symptoms encountered in Alzheimer disease, such as aggressivity and anxiety (Streim et al., 2008), or even as a potential therapy for Alzheimer disease (Gupta et al., 2023; Jeong et al., 2021). Thus, among atypical antipsychotics, aripiprazole can be considered as a clinical reference (widely used), a pharmacological reference (first dopamine receptor partial agonist approved) and a potential innovative treatment for certain neurodegenerative pathologies such as Alzheimer's disease. This increases the interest in its use and a more in-depth characterization of its properties in preclinical studies.

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The oral route is the preferred method of drug administration (accounting for 80% of pharmaceutical forms distributed) primarily because it is less invasive and less expensive than the parenteral route. In preclinical research on rodents, oral gavage is commonly used to facilitate the transition to clinical use. However, oral gavage has several drawbacks. Like intra-peritoneal or subcutaneous methods, it is highly stressful and requires animal restraint. Oral gavage demands a high level of technical skill from experimenters to ensure safe administration (Stuart and Robinson, 2015). Even with extensive training, oral gavage can cause a significant level of animal discomfort, posing a major limitation in psychiatric research. Less invasive alternatives include adding the drug to food (Hovard et al., 2015) or drinking water (Zhan et al., 2019). However, these methods provide continuous or long-term access to the drug, potentially leading to a different plasma profile compared to the clinical situation where drugs are typically administered at specific intervals (Turner et al., 2011). Our laboratory has just reported another non-invasive chronic administration method in rats: micropipette-guided drug administration (Heraudeau et al., 2023). The results of this study suggest that this method would be more suitable for hydrophilic compounds than for lipophilic derivatives such as aripiprazole.

In this context, the main objective of the present study was to assess the consistency of subchronic administration of aripiprazole via drinking solution (DS) in C57Bl/6J mice (compared to oral gavage). This was carried out in several stages. Given the few pharmacokinetic studies of aripiprazole described in the literature (Kirschbaum et al., 2010; Nagasaka et al., 2014; Wang et al., 2009), especially in the C57Bl/6 mouse (Balog et al., 2022), we first realized an in-depth investigation of the main pharmacokinetic parameters of acute oral route administration of aripiprazole. The highest dose used in literature data was chosen (10 mg/kg) to avoid any issues with limit of detection/quantification. In a second step, a range of aripiprazole doses (0.1, 1, and 10 mg/kg) was used and peak blood concentrations were measured (1h after oral gavage) together with behavioural locomotor profile (study 2). Finally, we compared two modes of oral administration (oral gavage *versus* drinking solution) in a subchronic protocol of 9 days. Investigations released no comparisons in both locomotor activity and blood concentrations.

2. Materials and methods

2.1. Animals and ethical concerns

Male C57Bl/6J mice, 6–10 weeks of age were used (Janvier Labs, France) and housed in groups of 5 within standard polycarbonate cages, with food and water *ad libitum*. The animal facility was maintained under a reversed 12h light-dark cycle (light on at 7 a.m.), with controlled temperature (22 ± 1 °C) and humidity (50 ± 10%). Animals were acclimatized to the facility and daily handled during one week before the beginning of the experiments. All experiments were approved by the regional ethical committee (Comité d'Ethique Normandie en Matière d'Expérimentation Animale, CENOMEXA; agreement number: n°54) in compliance with the European directive (2010/63/UE) for animal experiments.

As mentioned, this study was performed in only one sex of mice (male mice), but would reasonably be generalized to female since literature data indicate no significant sex in pharmacokinetics of aripiprazole (Aichhorn et al., 2006; Molden et al., 2006)

2.2. Experimental setting

Study 1: Pharmacokinetic parameters after acute oral gavage of aripiprazole (10 mg/kg): To avoid any food-drug interactions, mice (n = 25) were fasted 12 h prior the administration of aripiprazole, but had free access to water. A single oral 10 mg/kg dose of aripiprazole was given to mice and blood samples were collected at different time point (0.5, 1, 2, 4, 8, 12 and 24 h) (Fig. 1).

Study 2: Effect of acute oral administration of ARZ (OG at 0.1, 1 and 10 mg/kg) at 1 h (peak) blood concentration: One hour after oral gavage (OG) administration of either vehicle (control group) or aripiprazole (0.1, 1, and10 mg/kg), mice were subjected to locomotor activity test (n − 6 per group) or to a blood sample collection (for aripiprazole treated mice, n = 6 per group) (Fig. 2).

Study 3: Effect of subchronic oral administration: The two routes of subchronic (9 days) oral administration of aripiprazole (0.1, 1 and 10 mg/kg) were compared, *i.e.* the common oral gavage (OG, n = 15/dose) and administration through drinking solution (DS, n = 15 mice/dose) (Fig. 3). On day 8, all mice were subjected to a locomotor activity measurement test. For drinking solution and oral gavage groups of mice, three different time points of the day were set (10 a.m., 12 p.m., 3 p.m.). All drinking solution mice had access to the bottle with aripiprazole solution until the behavioral test being started. On day 9, the same schedule was implemented to recover of blood samples at 24 h (Fig. 3).

2.3. Pharmacological treatment

2.3.1. Formulation

Aripiprazole chlorhydrate (ARZ) (Thermo Fischer Scientific©, Waltham, USA) was dissolved in water with 0,5 % polysorbate 20 % (Tween 20®, Sigma-Aldrich©, Saint-Louis, USA) and acetic acid (2 drops for 100 ml) (Kirschbaum et al., 2009). The different doses of aripiprazole used, based on literature data (Flood et al., 2007; Gil et al., 2018; Kim et al., 2017; Park et al., 2019; Rajagopal et al., 2022; Stephan et al., 2022) were obtained through successive dilution (0.1, 1 and 10 mg/kg).

2.3.2. Routes of administration

For oral gavage administration, aripiprazole and vehicle were administered in a volume of 10 ml/kg at 9 a.m. For drinking solution administration, daily water consumption was first measured in each cage during 8 days before initiating the treatment. No statistical difference of drinking solution consumption was detected between different cages. Then, the concentrations of drug solutions (in mg/ml) were prepared according to the mean amount of consumed solution (3,8 ml/mouse/day). Of note, neither the amount of drinking solution consumed, nor the body weight of treated animals did significantly vary across the 9 days of subchronic administration (data not shown). All bottles were changed every three days. Importantly, the stability of aripiprazole solution for 3 days at room temperature was previously ensured (Wilcoxon test samples paired: p = 0.6250, see below for detail of liquid chromatography-mass spectroscopy/mass spectroscopy detection method used).

2.4. Biochemical analysis

For blood collection, animals were anesthetized with isoflurane (5 % bolus and then 2.5 % for maintenance) in a mixture N_2O/O_2 (70 %/30 %). Blood was collected by intracardiac puncture into heparinized tubes and stored at −20 °C until analysis. The mice were then killed by cervical dislocation after collection. As previously published (Heraudeau et al., 2023), aripiprazole concentration was measured by a liquid chromatography method coupled to tandem mass spectrometry in accordance with the Food and Drug Administration's Bioanalytical Method Validation guideline. (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry). Aripiprazole was extracted from blood using a liquid-liquid extraction method as follow: to 100 µL of blood, were added 25 µL of internal standard (d6-Venlafaxine, stock: 100 µg/mL, Sigma Aldrich; final concentration: 250 ng/mL), 500 µL of NaOH buffer solution (0,25 M) and 1 mL of extraction solution (mix of ethyl acetate/dichloromethane/hexane 40/22/38 v/v/v). Thereafter, organic phase was removed and dried with a concentrator. Extract was reconstituted with 100 µl of A mobile phase. The lower limit of quantification

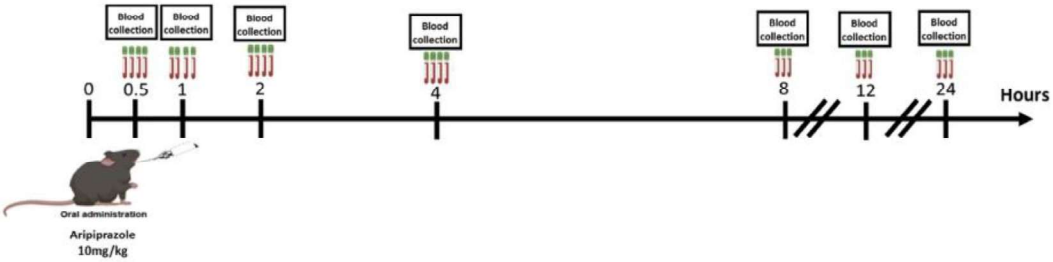

Fig. 1. Timing of blood sample collection after acute oral gavage administration of aripiprazole (10 mg/kg).

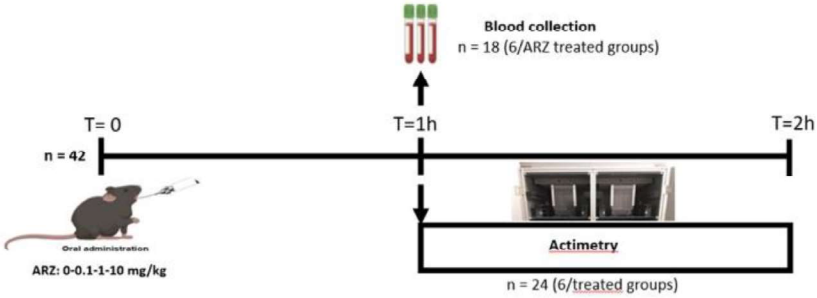

Fig. 2. Experimental design of the acute oral gavage administration study (Study 1) with the time schedule of locomotor activity and blood concentration. ARZ = Aripiprazole; h = hours.

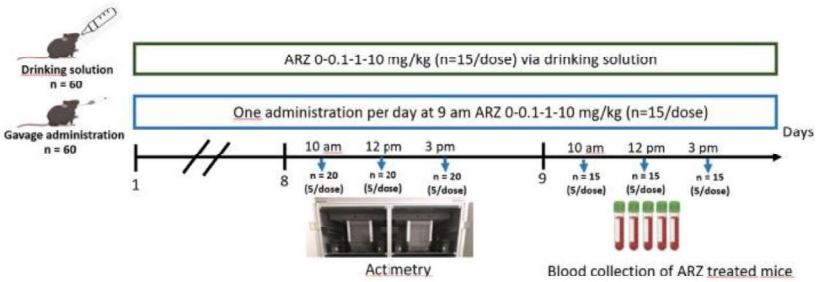

Fig. 3. Experimental design of subchronic study with the time schedule of locomotor activity and blood concentration. ARZ = Aripiprazole.

was of 5 ng/mL.

2.5. Locomotor activity

Spontaneous locomotor activity was recorded during the active (dark) phase of animals. Mice were placed in the dimly lit testing room (30 lux), 30 min prior to start experiment. Motor activity was measured in Plexiglas cages (19 × 11 × 14 cm) by an automated actimeter containing six lines of infrared beams (IMETRONIC®) (Bouet et al., 2021). The total locomotor activity represents the activity in the front, in the back and the coming and going for 1 h (crossed beam/1h).

2.6. Data analysis

Statistical analyses were performed with Rstudio® or GraphPad® Prism (Version 8.3.0, La Jolla, USA) softwares. The homogeneity of variances (Levene's test) and normality of the data (Shapiro-Wilk test) were first assessed. Accordingly, data were then analysed either with two-way parametric or non-parametric ANOVA (followed by the post hoc Tukey's test when necessary), or the two-way non-parametric statistics tests for longitudinal data with R package "nparLD" (f1.ld.f1 plan) (followed by the pairwise comparisons test).

Main pharmacokinetic parameters: maximum concentration (C_{max}), time to peak drug concentration (T_{max}), area under the plasma drug concentration-time curve ($AUC_{0\text{-}24}$, $AUC_{0\text{-}\infty}$) the non-compartmental half-life time ($T_{1/2}$), were calculated by non-compartment analysis with the "PK" package of Rstudio® (R Core Team) software equipped.

Differences were considered significant when the associated p-value was below 0.05.

3. Results

3.1. Study 1: Pharmacokinetic parameters observed after single oral administration of aripiprazole (Oral gavage 10 mg/kg)

Maximal blood concentration of aripiprazole was found at 1 h (time to peak drug concentration (Tmax)) equal to 567.7 ng/ml (Fig. 4 and Table 1). The half-life ($T_{1/2}$) was equal to 4.84 h.

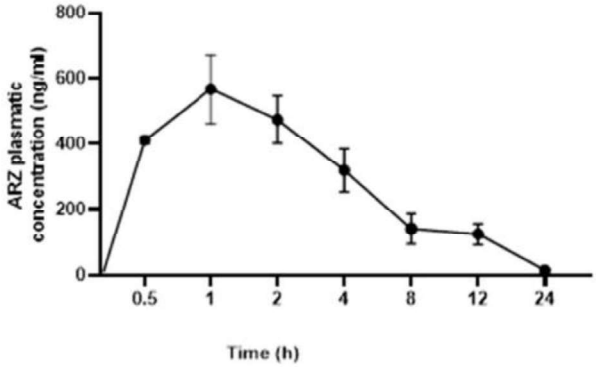

Fig. 4. Time profile of whole blood concentrations after a single oral gavage administration of aripiprazole (ARZ) to C57Bl/6 mice at the dose of 10 mg/kg. Each point represents the mean ± SEM of data from 4 mice for 0.5-1-2-4 h and 3 mice for 8-12-24 h.

Table 1
Non-compartmental analysis of aripiprazole after a 10 mg/kg oral gavage administration from 0.5 to 24 h in mice. Data are shown in mean (±SEM).

C_{max} (ng/mL)	T_{max} (h)	AUC $_{(0-24h)}$ (ng.h/ml)	AUC $_{(0\ to\ inf)}$ (ng.h/ml)	$T_{1/2}$ (h)
567.7 *(± 106)*	1	3737 *(± 413.3)*	3831.9 *(± 403.9)*	4.84

3.2. Study 2: Effect of a single aripiprazole oral administration (Oral gavage at 0.1, 1 and 10 mg/kg) observed at 1 h

Analysis of total locomotor activity revealed a dose effect (ANOVA; respectively $F_{3,20} = 20.44$, $p < 0.0001$). Thus, aripiprazole at highest doses (1 and 10 mg/kg) significantly decreased locomotor activity in comparison with the control group (Pairwise comparison post hoc, $p < 0.0001$) (Fig. 5A).

When aripiprazole blood concentrations were assessed, a significative dose effect was observed (ANOVA; $F_{2,15} = 48.04$, $p < 0.0001$). The post hoc Tukey's test indicated a significative difference between 0.1 mg/kg and 10 mg/kg ($p < 0.0001$) and between 1 and 10 mg/kg ($p < 0.0001$) (Fig. 5B).

3.3. Study 3: Effect of subchronic aripiprazole oral administration (0.1, 1 and 10 mg/kg) through oral gavage (OG) or drinking solution (DS)

3.3.1. Locomotor activity after oral gavage administration

Total locomotor activity was analysed with a parametric two-way ANOVA with the "day-time" (at 10 a.m., 12 p.m. and 3 p.m.) effect and the "dose" effect as main factors. Analysis revealed a dose effect ($F_{3,48} = 31.55$, $p < 0.0001$), a day-time effect ($F_{2,48} = 16.34$, $p < 0.0001$) and interaction between the two factors ($F_{6,48} = 1.983$, $p = 0.0866$).

The post hoc Tukey's test showed significant differences for the control group between 10 a.m. and 12 p.m. ($p = 0.0019$) and between 10 a.m. and 3 p.m. ($p = 0.0075$). For the 0.1 mg/kg group, differences were found between 10 a.m. and 3 p.m. ($p < 0.0001$) and between 12 p.m. and 3 p.m. ($p = 0.04969$). For the 1 mg/kg group, a difference was found between 10 a.m. and 3 p.m. ($p = 0.0358$). No differences were found for 10 mg/kg group (Fig. 6A).

Post hoc Tukey's test also shows dose effects on locomotor activity at the three measurement times of the day for the 10 a.m. day-time with decrease in locomotor activity for 1 mg/kg ($p = 0.0095$) and 10 mg/kg ($p = 0.0159$) in comparison with "10 a.m. control group". For 12 p.m. day-time, significative differences were also found for the 0.1 mg/kg group ($p = 0.0221$), for the 1 mg/kg group and 10 mg/kg group ($p < 0.0001$) versus "12 p.m. control group". And at 3 p.m., locomotor activity significatively decreased) for the 1 mg/kg group ($p = 0.0016$) and for the 10 mg/kg group ($p < 0.0001$) versus "3 p.m. control group" (Fig. 6A).

3.3.2. Locomotor activity after drinking solution administration

For drinking solution administration, we found no dose effect ($F_{3,48} = 2.494$, $p = 0.0711$), no day time effect ($F_{2,48} = 1.970$, $p = 0.1506$) and no interaction between the two factors ($F_{6,48} = 0.9732$, $p = 0.4535$) (Fig. 6B).

3.3.3. Blood concentration after oral gavage administration

After 9 days of treatment, the blood concentration of ARZ at 10 a.m., 12 p.m. and 3 p.m. after the last single oral gavage administration (at 9 a.m.) were collected. ANOVA of aripiprazole blood concentration revealed a dose effect ($F_{2,12} = 201.784$; $p < 0.0001$), a time effect ($F_{2,24} = 39.801$; $p < 0.0001$), and dose × time interaction ($F_{4,24} = 20.307$; $p < 0.0001$). The post hoc Tukey's test showed significative differences between 10 a.m. and 3 p.m. for the three groups ($p = 0.00394$; 0.0188 and 0.04068 for respectively, 0.1, 1 and 10 mg/kg group). Another

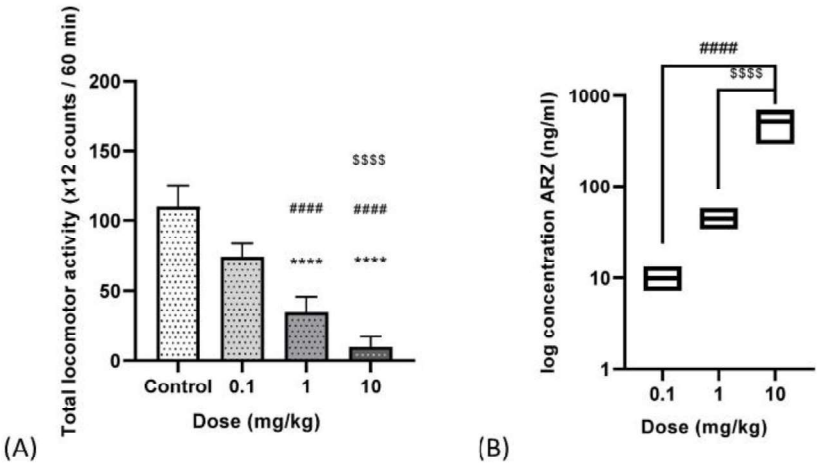

Fig. 5. Effect of acute oral gavage administration of aripiprazole (0.1, 1 or 10 mg/kg) observed at 1 h. **(A)** 60 min locomotor activity and **(B)** blood sample concentration. Data are means values ± SEM (n = 6 per group). Analysis with two-way non-parametric statistics tests for longitudinal data with R package "nparLD" (f1.ld.f1 plan) and the pairwise multiple comparisons test: $p < 0.0001$: **** (versus control group), #### (versus 0.1 mg/kg group), $$$$ (versus 1 mg/kg group).

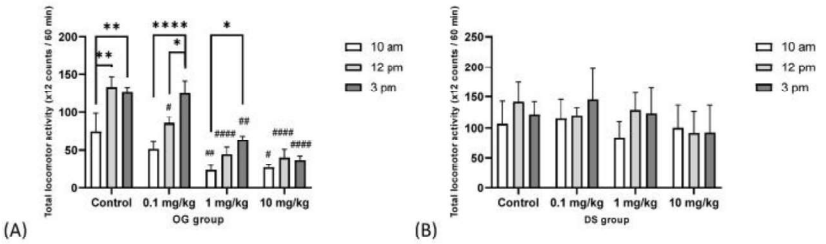

Fig. 6. Effects of subchronic oral administration of aripiprazole (0.1, 1 or 10 mg/kg) – given either via *oral gavage* **(A)** or drinking solution method **(B)** on locomotor activity at the three times of the day. Data are presented as the mean (±S.E.M) of total locomotor activity (n = 5 per group). Two-way parametric ANOVA followed by post hoc Tukey's test. *p < 0.05, **p < 0.01, ****p < 0.0001; #p < 0.05, ##p < 0.01, ####p < 0.0001 vs day time control group.

difference was found for the 10 mg/kg group between 12 p.m. and 3 p.m. (p = 0.000609) (Fig. 7A).

3.3.4. Blood concentration after drinking solution administration

When aripiprazole was administered through drinking solution with the lowest dose, *i.e.* 0.1 mg/kg, the blood concentration was not detectable (below the limit of quantification of the method (<5 ng/ml). For the two remaining doses, the parametric ANOVA showed a significant dose effect ($F_{1,8} = 406.2$, $p < 0.0001$), a time effect ($F_{2,16} = 5.208$, $p = 0.0181$), and a significant interaction between the two ($F_{2,16} = 5.316$, $p = 0.0170$).

The post hoc Tukey's test showed a significative increase of concentration for the 10 mg/kg group between 10 a.m. and 3 p.m. (p = 0.0256) and a significative decrease between 12 p.m. and 3 p.m. (p = 0.0083). For the 1 mg/kg group, the only differences found was between 10 a.m. and 12 p.m. (p = 0.0447) (Fig. 7B).

4. Discussion

Our study describes the main pharmacokinetic parameters after acute oral administration of 10 mg/kg aripiprazole in C57Bl/6 mice. To our knowledge, very few studies previously assessed pharmacokinetic properties of this antipsychotic in mice, especially after oral administration, and none was found using this mouse strain (C57Bl/6). In FVB mice, after a single oral administration of 2 mg/kg of ARZ, authors reported a maximum concentration (Cmax) equal to 272 ng/ml observed 30 min after administration (Nagasaka et al., 2014). Besides, the half-life ($T_{1/2}$) was determined as equal to 4.1 h, with an area under the curve at infinity (AUC_{inf}) value of 1810 ng h/ml). Due to the differences mouse strains used, our results are not strictly comparable. Despite, it can be noted that the half-life calculated in our study with a single administration of 10 mg/kg is comparable to those measured by Nagasaka et al. with a dose of 2 mg/kg (4.84 h for us *versus* 4.1 h). Moreover, the inter-strain differences underline the importance of carrying out pharmacokinetic studies before considering behavioural studies evaluating the effects of pharmacological substances in mice.

Concerning behavioral approach, in our study, mice treated with aripiprazole by oral gavage administration displayed a dose-dependent decrease in total locomotor activity, at 1 mg/kg, 10 mg/kg and modestly at 0.1 mg/kg. To our knowledge, no study has so far reported the effects of aripiprazole on locomotor activity after a single oral administration in C57Bl/6 mice. Interestingly, our results are however near to those obtained on the same strain after intra-peritoneal administration of similar doses of aripiprazole (Zocchi et al., 2005). Likewise, the same hypolocomotor effects has been observed in Swiss mice after a single administration of 1 mg/kg of aripiprazole, either intraperitoneally or orally (Boulay et al., 2010; Marinho et al., 2014), in NMRI mice after a single intraperitoneal administration of 1.25 mg/kg (Jerlhag, 2008) and in SR-1 mice after a single intraperitoneal administration of aripiprazole ranging from 1 to 10 mg/kg (Picada et al., 2011). However, there are also conflicting data which did show no changes in locomotor activity in Swiss mice treated with single intraperitoneal doses ranging from 0.1 to 10 mg/kg of aripiprazole (Almeida-Santos et al., 2014; Viana et al., 2013). Several elements could explain these differences reported in the effects on spontaneous activity which are related to the variations between studies: the route of administration, the strain of mice, the housing conditions of the animals (normal versus reversed light/dark cycle), the time between aripiprazole administration and the behavioral test (although often similar), the methods of measuring activity (for example, counting the number of interrupted photocell beams versus measuring the distance traveled in an open field) and the duration of the test.

Our study allowed to determine the blood concentrations at steady state after 9 days of treatment with aripiprazole solubilized in drinking solution. The concentrations could be determined at 1 and 10 mg/kg, but not 0.1 mg/kg due to very low concentrations, below the limit of quantification of the method. However, these results validate the drinking solution administration as a method of administration suitable to assess the chronic effects of pharmacological substances in mice. We also demonstrated that the concentrations remain relatively stable during the period of behavioral experimentation (*i.e.* during 6 h). Interestingly, a weak inter-individual variability in concentrations was observed after this mode of administration. Thus, despite the absence of control of the volumes actually consumed by each mouse (house-grouped animals), this method of administration, based on the average water consumption for each group of mice, allows to achieve satisfactory

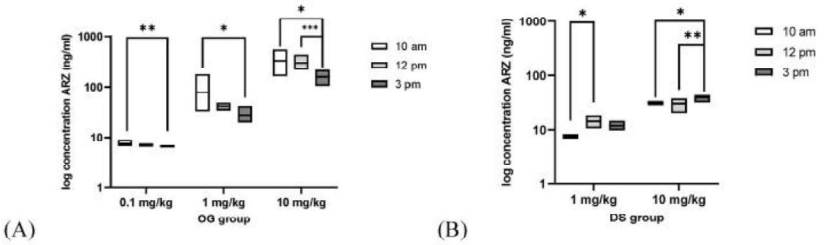

Fig. 7. Blood concentration of aripiprazole (ng/ml) collected at 10 a.m., 12 p.m. and 3 p.m. for the 3 doses of aripiprazole administered via oral gavage **(A)** or drinking solution **(B)**. Data are shown in mean, n = 5 per group. (post hoc Tukey's test. *p < 0.05; **p < 0.01; ***p < 0.001).

systemic exposure levels of aripiprazole for each group.

To our knowledge, only one study has measured the concentration of aripiprazole in C57Bl/6J male mice after subchronic administration (Balog et al., 2022). In this study, blood concentrations vary between 100 and 125 ng/ml after a daily intraperitoneal injection at 8 a.m. for 8 days of 2.5 mg/kg of aripiprazole. The blood samples were taken 4–6h after the last treatment. Given methodological differences concerning the route of administration and the time of blood collection after the last administration, the concentrations measured in this study are difficult to compare to ours. However, we can note that the concentration values (100–125 ng/ml) obtained by Balog is between those obtained 3 h and 6 h after the last oral gavage administration of doses of 1 mg/kg and 10 mg/kg, respectively. Moreover, no accumulation of aripiprazole was revealed in subchronically oral gavage treated mice compared to acutely injected animals (Cmax = 567.7 (±106) ng/ml 1 h after 10 mg/kg oral gavage acute administration versus 336.8 (±82.5) ng/ml after oral gavage subchronic administration of the same dosage of aripiprazole (last data not shown in results part). This result is in agreement with that obtained after subchronic intraperitoneal administration or aripiprazole in FVB mice (Kirschbaum et al., 2010).

In our experimental conditions, the hypoactivity observed after oral gavage administration of ARZ was not observed after drinking solution administration. This probably results from the lower blood concentrations found after drinking solution administration. Moreover, this method of administration makes it possible, for the doses of 1 and 10 mg/kg, to obtain stable levels of spontaneous activity during the period from 10 a.m. to 3 p.m.

5. Conclusion

This study aimed to characterize the pharmacokinetic data of acute and subchronic administration of aripiprazole by different oral administration route. It allowed for the first time to describe the main pharmacokinetic parameters of an oral administration of 10 mg/kg of aripiprazole in male C57Bl/6 mice. Moreover, despite lower blood concentrations than after oral gavage administration, it also allowed to validate, from a pharmacokinetic point of view, a method of chronic drinking solution administration of aripiprazole. Taking into accounts the lower blood concentrations obtained for a given dose than that obtained by oral gavage, administration of aripiprazole via drinking solution appears, therefore, relevant to study the chronic or subchronic behavioral effects of aripiprazole on C57Bl/6 mice. Finally, knowing these blood concentrations at the end of such a chronic administration protocol will allow better extrapolation of preclinical to clinical data (calculation of equivalent concentration between mice and humans).

CRediT authorship contribution statement

Cécile Chofflet: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Marie Héraudeau:** Investigation. **Pierre-Marie Morice:** Supervision, Investigation. **Imane Mouffok:** Conceptualization. **Véronique Lelong-Boulouard:** Supervision, Conceptualization. **Valentine Bouet:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Thomas Freret:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Michel Boulouard:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Conceptualization.

Data availability

All the data supporting the findings of this study are available on request from the corresponding author.

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Declaration of competing interest

The authors have no conflict of interest to report.

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Data availability

Data will be made available on request.

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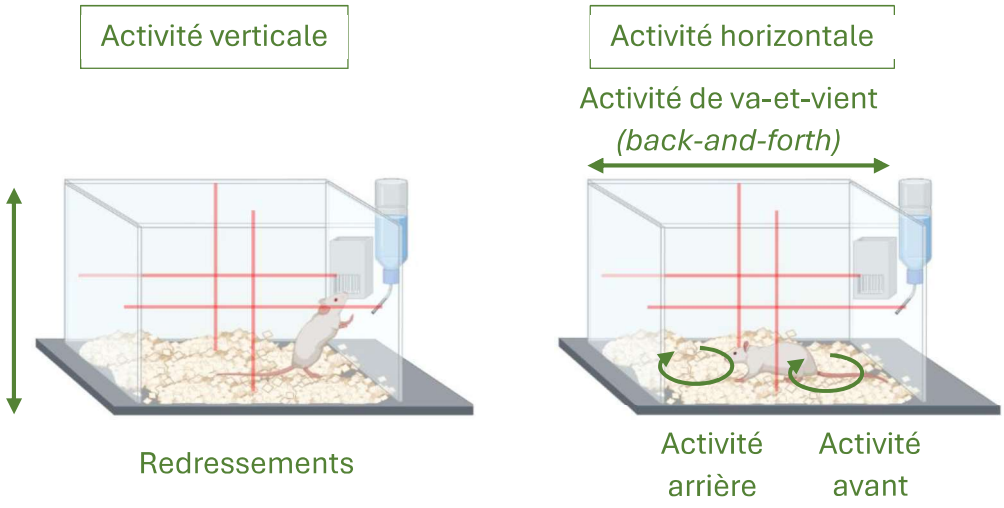
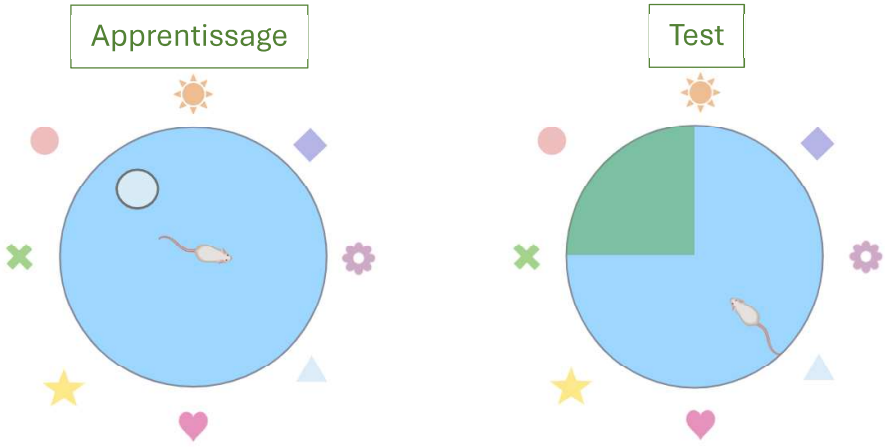
ANNEXE 3 : Le test d'actimétrie

Le test d'**actimétrie** mesure l'activité locomotrice spontanée de l'animal. Il repose sur le comportement naturel de déplacement et d'exploration. Des capteurs infrarouges détectent les mouvements horizontaux et verticaux dans une cage individuelle. Ce test évalue la locomotion, l'activité générale.

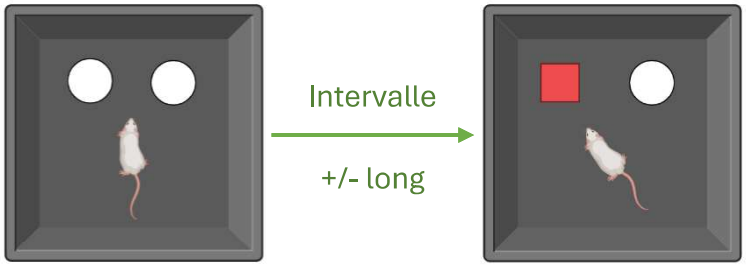


ANNEXE 4 : Le labyrinthe aquatique de Morris

Le **labyrinthe aquatique de Morris** évalue l'apprentissage spatial et la mémoire. Il repose sur le comportement naturel de fuite de l'animal, qui cherche à sortir de l'eau. L'animal apprend à localiser une plateforme immergée à l'aide d'indices visuels présents dans l'environnement. Lors de la phase de test, la plateforme est retirée et la mesure du temps passé dans le cadran où était anciennement placée la plateforme est un indicateur de mémoire spatiale.



Le **test de reconnaissance d'objet** évalue la mémoire de reconnaissance chez le rongeur. Il est basé sur la tendance naturelle de l'animal à explorer davantage un objet nouveau qu'un objet familier. Après une phase d'exposition à deux objets identiques, un objet est remplacé par un nouveau. Le temps d'exploration du nouvel objet par rapport au familier permet d'évaluer la mémoire à court ou long terme, impliquant notamment le cortex et l'hippocampe.



Impact de la consommation aiguë et chronique de médicaments à effets psychoactifs sur les fonctions cognitives : étude comportementale et pharmacocinétique chez le rat

RÉSUMÉ

Les antalgiques opioïdes tels que le tramadol et les antidépresseurs inhibiteurs sélectifs de la recapture de la sérotonine, notamment la paroxétine et la sertraline, sont largement prescrits au long cours chez les sujets âgés. En raison d'importants facteurs de variabilité pharmacocinétique et pharmacodynamique liés à l'âge, les risques de surexposition à ces médicaments sont fréquents dans cette population. Parallèlement, l'utilisation de ces substances psychoactives est souvent associée à des risques de troubles neurocognitifs aigus et chroniques, sans qu'un lien de causalité formel puisse être établi en clinique, du fait de nombreux facteurs confondants liés à la polypathologie et à la polymédication. Les modèles animaux constituent une approche pertinente pour étudier les effets propres d'un médicament isolement et explorer la relation entre exposition médicamenteuse et altérations cognitives. L'objectif de cette thèse était d'évaluer les conséquences de l'administration aiguë et chronique de tramadol, paroxétine et sertraline, sur plusieurs processus cognitifs et de les mettre en relation avec l'exposition systémique des rats. Afin d'évaluer spécifiquement les effets de la surexposition souvent fréquente chez le sujet âgé, des rats adultes ont reçu des doses suprathérapeutiques. L'administration aiguë de tramadol et de paroxétine a induit une hyperactivité locomotrice et des comportements stéréotypés, suggérant un état de type confusionnel des animaux. L'administration chronique de tramadol a entrainé des déficits persistants de la mémoire de type épisodique et celle de paroxétine a généré une altération de la flexibilité cognitive. Dans les deux cas, une accumulation systémique était associée à ces perturbations comportementales. En dehors d'une hyperactivité après administration aiguë, la sertraline n'a pas provoqué de déficit cognitif. Les propriétés pharmacocinétiques et pharmacodynamiques de ces substances pourraient expliquer ces résultats différentiels. Ces résultats soulignent l'importance de prendre en compte ces données lors de la prescription de ce type de médicaments et plus particulièrement chez le sujet âgé, à risque de troubles cognitifs. Le suivi thérapeutique par la mesure de concentrations sanguines dans cette population âgée, pourraient permettre d'éviter la surexposition et le risque de développer des troubles cognitifs.

Mots clés : comportement, pharmacocinétique, cognition, tramadol, paroxétine, sertraline

ABSTRACT

Opioid analgesics such as tramadol and selective serotonin reuptake inhibitor antidepressants, particularly paroxetine and sertraline, are widely prescribed for long-term use in elderly patients. Due to significant age-related pharmacokinetic and pharmacodynamic variability, the risks of overexposure to these drugs are common in this population. At the same time, the use of these psychoactive substances is often associated with risks of acute and chronic neurocognitive disorders, without a formal causal link being established in clinical practice due to numerous confounding factors related to multiple pathologies and polypharmacy. Animal models are a relevant approach for studying the specific effects of a drug in isolation and exploring the relationship between drug exposure and cognitive impairment. The objective of this thesis was to evaluate the consequences of acute and chronic administration of tramadol, paroxetine, and sertraline on several cognitive processes and to relate them to systemic exposure in rats. In order to specifically evaluate the effects of overexposure, which is often frequent in elderly subjects, adult rats were given supratherapeutic doses. Acute administration of tramadol and paroxetine induced locomotor hyperactivity and stereotypical behaviors, suggesting a state of confusion in the animals. Chronic administration of tramadol led to persistent deficits in episodic memory, and chronic administration of paroxetine led to impaired cognitive flexibility. In both cases, systemic accumulation was associated with these behavioral disturbances. Apart from hyperactivity after acute administration, sertraline did not cause cognitive deficits. The pharmacokinetic and pharmacodynamic properties of these substances could explain these differential results. These results highlight the importance of taking these data into account when prescribing this type of medication, particularly in elderly patients at risk of cognitive impairment. Therapeutic monitoring by measuring blood concentrations in this elderly population could help avoid overexposure and the risk of developing cognitive impairment.

Keywords: behavior, pharmacokinetics, cognition, tramadol, paroxetine, sertraline