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**INSTITUT  
PASTEUR**



**SORBONNE  
UNIVERSITÉ**

Ecole doctorale Cerveau, Comportement, Cognition  
*Institut Pasteur – Unité Perception et Mémoire – UMR3571*

# Cortical inputs onto adult-born neurons in the Olfactory Bulb: Plasticity and role in experience- dependent perception

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*Aux souris, aux pigeons et aux abeilles.*



*En fait, il n'y a pas de perception qui ne soit imprégnée de souvenirs.*

Henri Bergson – Matière et Mémoire

# Résumé

L'olfaction permet à la plupart des animaux de percevoir les molécules volatiles de leur environnement. Un réseau neuronal complexe – le système olfactif – est nécessaire pour détecter, identifier et discriminer les odeurs, mais aussi pour les associer à des situations particulières et déclencher une réponse comportementale adaptée. Le bulbe olfactif est le premier relai de l'information provenant de l'organe de détection, mais intègre également de nombreuses afférences, les fibres centrifuges, provenant d'autres régions cérébrales comme le cortex olfactif. Parmi ces connections, nous avons récemment découvert des connections inhibitrices à longue distance entre le cortex et le bulbe olfactifs.

De plus, au cours des dernières décennies, un mécanisme auparavant réfuté a été démontré : la neurogenèse adulte. Ce processus, présent dans l'hippocampe, l'hypothalamus et le bulbe olfactif des rongeurs, permet l'intégration de nouveaux neurones dans les réseaux cérébraux tout au long de la vie d'un individu. Il a également été montré que ces neurones sont particulièrement impliqués dans les phénomènes d'apprentissage et de mémoire. Nous avons donc cherché à déterminer si les connections entre les projections corticales inhibitrices et les nouveaux neurones dans le bulbe olfactif sont modifiées par l'apprentissage olfactif, suggérant une modulation du signal entrant par les expériences passées.

En combinant un apprentissage olfactif à de l'électrophysiologie *ex vivo*, nous avons montré que les neurones générés à l'âge adulte sont spécifiquement plus inhibés par les fibres du cortex après l'apprentissage, mais aussi que l'inhibition accrue est due en partie à une augmentation du nombre de synapses entre ces cellules.

De plus, des manipulations optogénétiques de l'inhibition corticale entraînent une altération de la détection d'odeurs proches et l'association de celles-ci à des valeurs, mais n'ont pas d'effet sur la mémoire de ces associations.

Nous avons donc démontré un mécanisme où les neurones inhibiteurs à longue-portée, ici ceux du cortex, jouent un rôle majeur dans l'apprentissage sensoriel grâce à une inhibition accrue, spécifiquement sur les neurones générés à l'âge adulte.

# Abstract

Olfaction allows most animals to perceive volatile molecules filling the environment they roam. A complex neuronal network – the olfactory system – is required for an animal to detect, identify, and discriminate odors, as well as associate them with contextual cues, leading to adapted behavioral responses. The olfactory bulb is the first relay for information coming from the odor detection organ, but also integrates considerable inputs from various brain regions such as the olfactory cortex. Among them, we have recently uncovered a direct long-range inhibitory connection between the olfactory cortex and the olfactory bulb.

In addition, the past decades shed light on a previously refuted mechanism, adult neurogenesis. This process has been demonstrated in the hippocampus, hypothalamus, and olfactory bulb of rodents, and allows the integration of new functional neurons in the brain networks throughout the whole life of an individual. Moreover, it was shown that neurons born in the adult brain are uniquely involved in learning and memory processes. We thus sought to determine whether the inhibitory projections from the cortex to the adult-born neurons in the olfactory bulb were modified after olfactory learning, underlying entering signal modulation by previous experiences.

Combining an olfactory learning task with *ex vivo* electrophysiology, we showed that adult-born granule cells in the olfactory bulb exclusively received more inhibition from the olfactory cortex after learning. Furthermore, we demonstrated that this increase in inhibition was partly due to a greater number of synapses between cortical fibers and adult-born granule cells.

Furthermore, optogenetic manipulation of GABAergic top-down activity bidirectionally modulates fine odor detection and impairs olfactory learning, but not memory, revealing their significant impact on behavior.

Thus, we unraveled a mechanism where top-down projections might play a major role in sensory learning through increased inhibition, specific to adult-born neurons, and highlighted the relevance of this inhibitory feedback for olfactory processing.



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Ce travail n'aurait pas été ce qu'il est sans toutes les personnes qui y ont participé, celles qui m'ont conseillé, et toutes celles qui m'ont soutenu au cours de ces 4 années.

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## List of abbreviations

AON: Anterior Olfactory Nucleus

BF: Basal Forebrain

CoA: Cortical Amygdala

EPL: External Plexiform Layer

OB: Olfactory Bulb

GC: Granule Cell

nnGC: Neonatal Granule Cell

abGC: Adult-born Granule Cell

GCL: Granule Cell Layer

GL: Glomerular Layer

HPC: Hippocampus

HDB: Horizontal limb of the Diagonal band of Broca

IPL: Internal Plexiform Layer

LEC: Lateral Entorhinal Cortex

LOT: Lateral Olfactory Tract

MC: Mitral Cell

MCL: Mitral Cell Layer

OSN: Olfactory Sensory Neuron

PC: Piriform Cortex

PGC: PeriGlomerular Cells

TC: Tufted Cell

eTCs: external Tufted Cells

# Introduction

## Chapter I. Olfactory perception: overview of the canonical pathway

Olfactory perception allows most animal organisms, from nudibranch to mammals, to perceive environmental olfactory stimuli and generate, if needed, an appropriate behavioral response. An odor is a molecule that is defined by its structure, its concentration, and its ability to vaporize and be transported in the air. Indeed, for terrestrial animals, olfaction is tightly dependent on the air they breathe, and thus cannot detect molecules that are imprisoned in a condensed state of matter.

To decode this ambient chemical information, animals developed an intricate, specialized system that enables them to make “sents” of the omnipresent stimuli they receive (see Figure I for a simplified overview).

### 1. From an odor to action potentials

#### a. Detection of volatile chemicals in the main olfactory epithelium

In the nasal cavity of most mammals resides the main olfactory detection organ, the olfactory epithelium (Figure IIA-B). Despite being constituted of only a few cell types, this organ is essential to the detection of volatile chemicals. It is mainly composed of Olfactory Sensory Neurons (OSNs) and support cells including sustentacular cells, basal and horizontal globose cells, and olfactory ensheathing cells. While support cells mainly secrete mucus and sustain the integrity of the epithelium and mucosa, OSNs are bipolar neurons responsible for the detection of odorants. Their apical dendrite rises from a small soma to reach the MOE surface through the mass of supporting cells, allowing several membrane protrusions, the cilia, to bathe in the mucus, exposed to passing inhaled molecules. Although several hypotheses emerged, such as the vibrational theory (Wright, 1972) or the swipe cards theory (Brookes,

Horsfield and Stoneham, 2012), the results finally showed that odorant molecules were binding on the surface of OSNs through G-Protein Coupled Receptors (GPCRs), hereby called Olfactory Receptors (ORs) (Buck and Axel, 1991). However, debate on previously suggested theories still continues today with further theories emerging that seek to complete the known canonical mechanisms (Willeford, 2023).

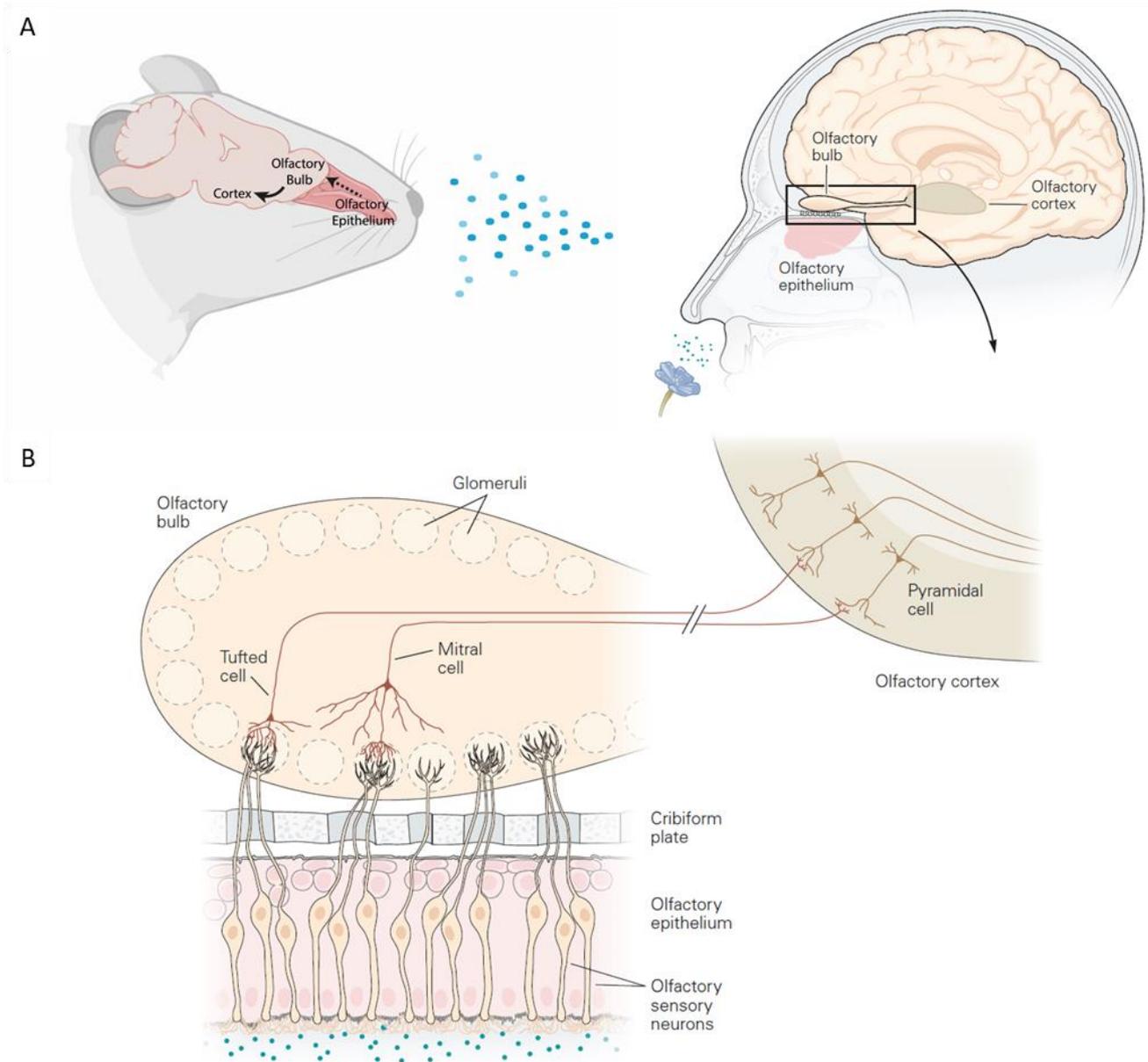


Figure I. General presentation of the mammalian olfactory system. A. Comparative view of rodent and human olfactory system. Volatile chemicals are detected by the olfactory epithelium which transmits an electrical message to the olfactory bulb. The integrated olfactory message then travels to the olfactory cortex. B. Simplified view of the sensory pathway. Odorants are detected by olfactory sensory neurons in the olfactory epithelium, which lines part of the nasal cavity. The axons of these neurons project to the olfactory bulb where they terminate on mitral and tufted cell relay neurons within glomeruli. The relay neuron axons project to the olfactory cortex where they terminate on the dendrites of pyramidal neurons whose axons project to other brain areas.

ORs (Figure IIC) are the result of the expression of the largest mammalian gene family known to date, first estimated to contain a few hundred different genes. We know today that between 1300 and 1500 odorant-receptor-encoding genes are present in the mouse genome (Young *et al.*, 2002; Zhang and Firestein, 2002), and greater than 900 genes in humans (Glusman *et al.*, 2001). Each of these receptors recognizes a specific range of molecular patterns, from simple chemical functional groups (e.g. alcohol, aldehydes) to specific molecular lengths, and only one specific OR gene is expressed by a given OSN (Serizawa *et al.*, 2003).

#### b. Signal transduction in the MOE, from chemistry to electricity

Once an odorant molecule diffuses in the superficial mucus, each molecule can bind to various ORs located on the cilia of the OSNs according to its chemical structure, concentration and conformation (Malnic *et al.*, 1999), triggering the chemical transduction that will lead to the rise of an olfactory message. Each molecule binds to various receptors with respective affinities, which in combination will create an activation code unique to a given odorant (Figure IID).

The signal transduction is a typical GPCR-mediated response: binding of the odorant onto the corresponding receptor activates the olfactory G-protein  $G_{olf}$ , resulting in the exchange of GDP for GTP, then activating an adenylyl cyclase III producing cAMP (Figure IIE). This well-known second messenger is responsible for the opening of Cyclic-nucleotide dependent ion channels, causing the influx of  $Ca^{2+}$  and  $Na^{+}$  ions into the cell. Activation of the Golf protein in response to odorants is also eased by classical GTP exchange factors (Dannecker, Mercadante and Malnic, 2005), thus amplifying the response and the subsequent opening of ion channels. The signal transduction eventually gives rise to a membrane depolarization, with entrance of  $Ca^{2+}$  triggering the opening of efflux chloride channels that amplify this mechanism (Reisert *et al.*, 2003; Takeuchi and Kurahashi, 2005).

Despite these seemingly intense responses to single molecules, it seems that one odorant binding to a receptor is not sufficient to activate the Golf protein, which alone is also unlikely to successfully trigger a response (Bhandawat, Reisert and Yau, 2005). Thus, contrary to other sensory pathways, such as vision, where a single photon can trigger an effective response, a

high number of odorant-bindings is required for proper detection. Many parameters modulate the ability of to elicit a single response, such as the density of expression of the given receptor or the ion channels (Reisert *et al.*, 2003), binding of the odorants to Olfactory Binding Proteins (Mastrogiacomo *et al.*, 2014), sniffing frequency (Connelly *et al.*, 2015) or allosteric effectors (Trimmer *et al.*, 2023).

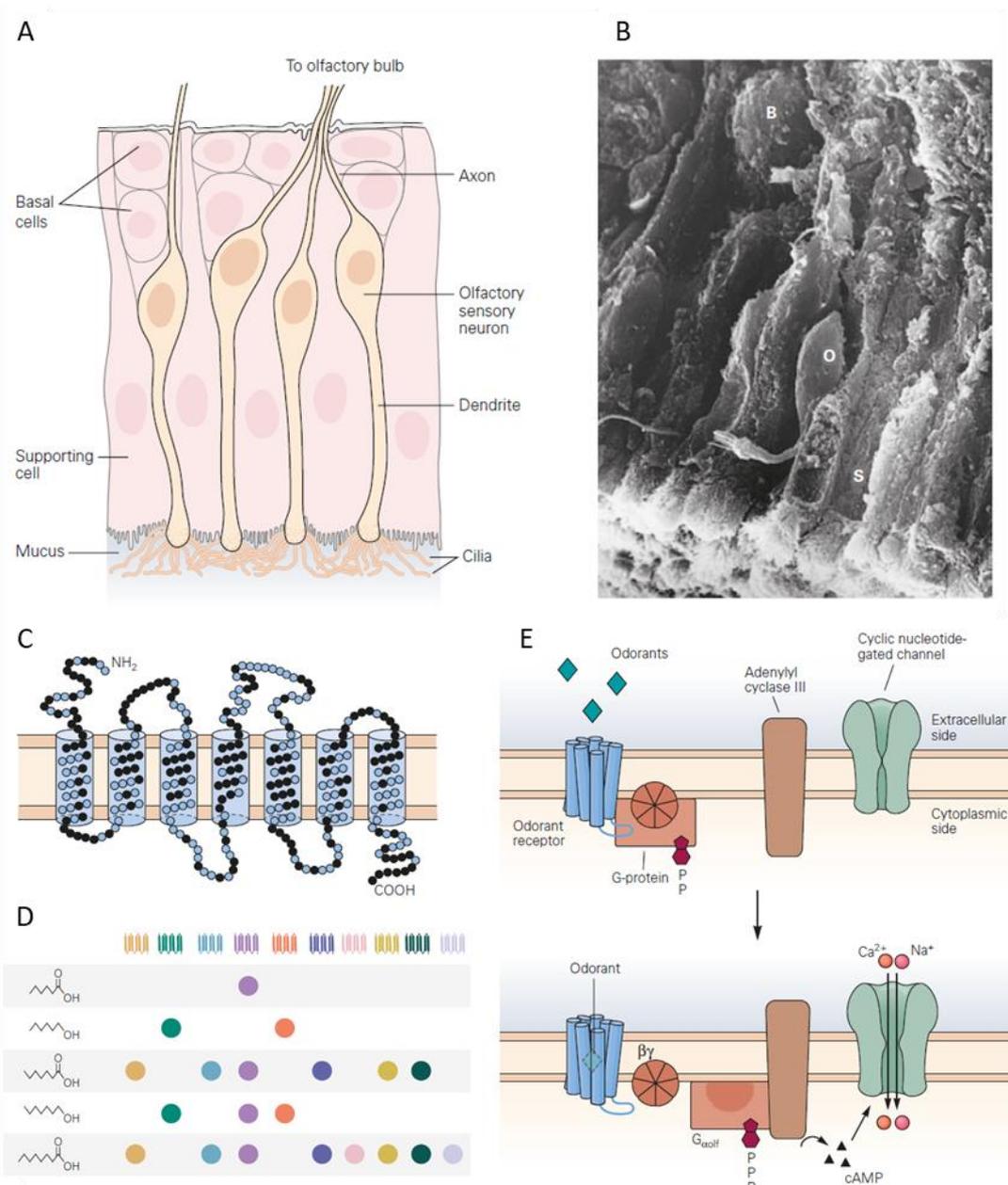


Figure II. The main olfactory epithelium and the transduction of the olfactory message. A. Schematic representation of the olfactory epithelium. B. Scanning electron micrograph of the olfactory epithelium. C. Structure of odorant receptors. Odorant receptors have the seven transmembrane domains characteristic of G protein-coupled receptors. They are related to one another but vary in amino acid sequence (positions of highest variability are shown here as black balls). D. A single odorant receptor can recognize multiple odorants, and one odorant is recognized by a specific combination of different receptors. Different odorants are detected by different combinations of receptors. This combinatorial coding of specific odorants explains how mammals can distinguish odorants with similar chemical structures. E. Message transduction. Binding of an odorant causes the odorant receptor to interact with G<sub>olf</sub>, the subunit of a heterotrimeric G protein. This causes the release of a GTP-coupled G<sub>olf</sub>, which stimulates adenylyl cyclase III, leading to an increase in cAMP. The elevated cAMP in turn induces the opening of cyclic nucleotide-gated cation channels, causing cation influx and a change in membrane potential in the ciliary membrane. (cAMP, cyclic adenosine monophosphate; GTP, guanosine triphosphate.)

However, once all the conditions are met, the depolarization passively spreads to the cell body of OSNs, as with any electrochemical potential, which results in the generation of an action potential (AP) transmitted along the axon. Finally, receptors that are spread through the surface of the epithelium will be activated by the passage of the odorant, which will result in the sparse activation of various OSNs, all more or less specific to the considered odorant. The encoded message will subsequently travel along the axons of the OSNs to reach the first central relay of the olfactory system: the main Olfactory Bulb (OB).

### c. Other means of odorants detection

Prior to delving into the OB circuit, we need to look at odor detection that is not restricted to the Main Olfactory Epithelium (MOE). Indeed, accessory olfactory organs exist in some mammal species that allow perception of specific types of molecules.

The vomeronasal organ (VNO) is a tubular structure at the base of the nasal cavity that is distinct from the MOE. Its cul-de-sac structure makes the entrance of air, and subsequently odorants, relatively difficult, and mechanisms of arousal-induced vasodilatation have been poised to intervene in the detection of odorants in this organ (Meredith, 1994). Despite anatomical differences compared to the MOE, odor detection passes through seemingly identical processes and results in sending an electrical message to a secondary olfactory relay, the accessory olfactory bulb (AOB), which is located on the most postero-dorsal part of the MOB. Specific receptor families were identified in the VNO (Dulac and Axel, 1995) that are strongly responsive to social cues, especially pheromones, giving the VNO a specific role in processing this information (Dulac and Torello, 2003). The VNO is present in numerous clades including amphibians and most mammals but seems to have reduced to a vestigial region in bats and apes. For more details on the VNO, see (Tirindelli, 2021).

Other accessory olfactory organs include the rodent's Septal organ of Maserà (SoM) and the Grueneberg ganglion (GG), which respond to odorants and mechanical stimuli for the SoM

(Ma *et al.*, 2003; Tian and Ma, 2004; Grosmaître *et al.*, 2007), and alarm pheromones for the GG (Brechtbühl, Klaey and Broillet, 2008).

Since this thesis is primarily focused on the principal canonical pathway of odor processing, these however fascinating systems will not be further detailed.

## 2. Convergence and computation of the olfactory message in the MOB

### a. Spatial organization of the main olfactory bulb

The main OB is a prominent structure at the front end of the brain, with its relative size varying across species according to their respective dependence on olfaction as a means of environment perception (Ribeiro *et al.*, 2014). However, distinct relative size of the OB is not clearly a strong approximation for olfactory capacity. Despite the common opinion that humans have poor olfactory performance, humans are able to discriminate approximately greater than a trillion different odorant mixtures (Buschdid *et al.*, 2014).

Despite the differences in sizes across species, OBs show a common layered concentric organization (Nagayama, Homma and Imamura, 2014). From here on, we will specifically describe the rodent olfactory system. From the superficial to the deep layers, the OB contains (Figure III):

- The glomerular layer (GL): the most superficial layer of the MOB, which in rodents constitutes of approximately 2000 small sub-spherical structures called glomeruli, that receive direct input from the MOE through OSN axons. It is in these glomeruli that the main feedforward excitatory synapse of the OB exists, where OSN axons connect with the dendrites of the bulb projection neurons, mitral and tufted cells.

Surrounding the glomerular structures, Juxtglomerular Cells (JCs) serve as modulatory effectors. JCs are a category of neurons containing **PeriGlomerular Cells (PGCs)**, **superficial Short-Axon Cells (sSACs)** and **external Tufted Cells (eTCs)** (PINCHING and POWELL, 1971).

- In the External Plexiform Layer (EPL) reside the somas of **Tufted Cells (TCs)** that form synapses with OSN terminals in the glomeruli (Mori, Kishi and Ojima, 1983). TCs are one of the two types of projection neurons in the OB, presenting a large apical dendrite that projects to the GL. These cells are spread across the deeper part of the EPL, and each of them contacts a single glomerulus. The EPL also contains a subset of interneurons involved in modulating odor-evoked responses of the OB principal cells (Nagayama, Homma and Imamura, 2014; Liu *et al.*, 2019).
- A bit deeper, **Mitral Cells (MCs)** are located in the eponym Mitral Cell Layer (MCL). Although their morphology is similar to TCs with a greatly developed single apical dendrite extending through the superficial layers to the glomeruli, MCs are more densely packed along a thin layer that encircles within the whole OB. However, like TCs, each of these cells is connected to only one glomerulus. A few **Granule Cell (GC)** somas have also been detected in the MCL, although most of these cells are restricted to the GC layer (see below).
- The Internal Plexiform Layer (IPL) is a thin layer mainly composed of the axons of MCs and TCs, which converge and form the Lateral Olfactory Tract (LOT). This layer also contains a sparse population of deep Short-Axon Cells (Burton *et al.*, 2017), as well as GCs dendrites and afferent axons from top-down cortical projections (detailed in chapter 2).
- Finally, just below the IPL, in the central region of the OB, is the place where mostly GC somas are found, extending their extensive dendritic arborescence in all the upper layers (PRICE and POWELL, 1970). This layer, the granule cell layer (GCL), also contains deep short-axon cells (dSACs) that participate in the message integration.

The layered organization of the OB underlies another level of topography. Specifically, glomeruli do not receive random inputs coming from various OSNs, instead, early labeling experiments showed that all OSNs expressing the same OR converge to only two separate glomeruli in each bulb (Vassar *et al.*, 1994), four in total. Thus, the olfactory message is functionally segregated before its arrival to the OB, allowing a label-line organization prior to the further transmission of the message (Willhite *et al.*, 2006), although a clear functional correlate of columnar organization hinted at by viral tracing studies is still missing (Murthy, 2011). Moreover, each glomerulus allows the connection between nearly 25,000 OSN terminals

to roughly one hundred M/T cell dendrites (estimated in rabbits, (Allison, 1953)), allowing tremendous olfactory signal convergence in the OB. It is important to note that the topographical map of the glomerular distribution on the surface of the OB is conserved between individuals and can be predicted according to RNA expression of various OSNs (Wang *et al.*, 2022).

Finally, a third level of organization is noticeable with the existence of a map, reproduced between the MOE and the MOB, of OR regional expression. In the MOE, the expression of the ORs is segregated into four zones, from I to IV in a dorsoventral to ventrolateral manner (Sullivan, Ressler and Buck, 1995). In each of these zones, only a subset of ORs is expressed by the OSNs, with no other expression pattern discernible inside them. This map is also approximately conserved in the glomerular layer of the MOB.

Interestingly, this third level of organization might have behavioral relevance. Indeed, the rodent ventral OB appears to have a larger response to pleasant odorants while the dorsal part shows greater aversive odorant related activity (Kermen, Mandairon and Chalençon, 2021). Moreover, ablation of dorsal glomeruli impaired avoidance behavior to innate aversive cues (Kobayakawa *et al.*, 2007). Thus, innate and acquired hedonics could be spread along the dorsoventral axis of the OB, in relation to the MOE topography, and the respective connectivity of these bulbar subregions (Miyamichi *et al.*, 2011).

All the olfactory system cell types which are carefully organized into different zones, layers, and columns, together participate in processing olfactory information (Lodovichi, 2021).

#### b. Integration of the olfactory message

Now that we have a clear idea of the OB anatomical and topographic organization, we need to look upon the journey of the electrical olfactory message, from its arrival at the OSN terminals to the axonal output of the OB projection cells.

*i. Message computing in the glomerular layer*

Considering the activation of only a single type of OR, we now know that activated OSNs will project to two glomeruli in each bulb, one medial and one lateral. In the glomerulus, OSNs form direct excitatory synapses on both M/TCs and eTCs (Hayar *et al.*, 2004), the latter causing excitation of M/TCs. Thus, MCs receive both direct and indirect excitation from OSNs in the glomerulus (Najac *et al.*, 2011). It is important to note that eTCs are more likely to be activated by OSNs than M/T cells (De Saint Jan *et al.*, 2009), making odorant concentration a crucial feature of the odor response in the glomeruli since a higher activation of OSNs (by highly concentrated odors) will also allow direct activation of M/TCs.

However, PG and SA cells also receive excitatory inputs from the same glomerulus eTCs, resulting in the inhibition of M/T cells through a singular type of synapse extensively found in the OB: dendrodendritic synapses. Indeed, PG cells are inhibitory interneurons that lack an axon, and thus do not form classical synapses onto other cells. However, they make connections with M/T cells between respective dendrites, where they form inhibitory synapses.

PG cells receive both direct excitation from OSNs and indirect excitation from eTCs, just as M/T cells. When activated by these inputs, they release GABA and provide feedforward inhibition to M/T cells, but also feedback inhibition to eTCs and inhibition on neighboring PGCs (Murphy, Darcy and Isaacson, 2005). Because PGCs are innervating only one glomerulus, it is thought they allow fine tuning of glomerulus transmission of the olfactory signal (Najac *et al.*, 2015).

Finally, PGCs also provide presynaptic inhibition of OSNs through secretion of GABA and dopamine, binding to GABA<sub>B</sub> and D2 receptors, respectively, which prevents glutamate release of OSN terminals (Aroniadou-Anderjaska *et al.*, 2000).

Superficial short axon cells, however, do not live up to their name. These cells instead have very long axons that can activate PGCs up to 30 glomeruli away from their location, which provides another mechanism for lateral inhibition (Aungst *et al.*, 2003), as observed in other sensory systems. It is suggested that this level of inter-glomerular inhibition helps to enhance contrast in odor-evoked responses in the GL.

Lastly, M/TCs were shown to be capable of self-activation, thus depolarizing themselves and other projection cells in the same glomerulus (Isaacson, 1999), through both AMPA receptor activation (Schoppa and Westbrook, 2002) and direct GAP-junction connections (Christie *et al.*, 2005), allowing electrical coupling in one glomerulus after activation.

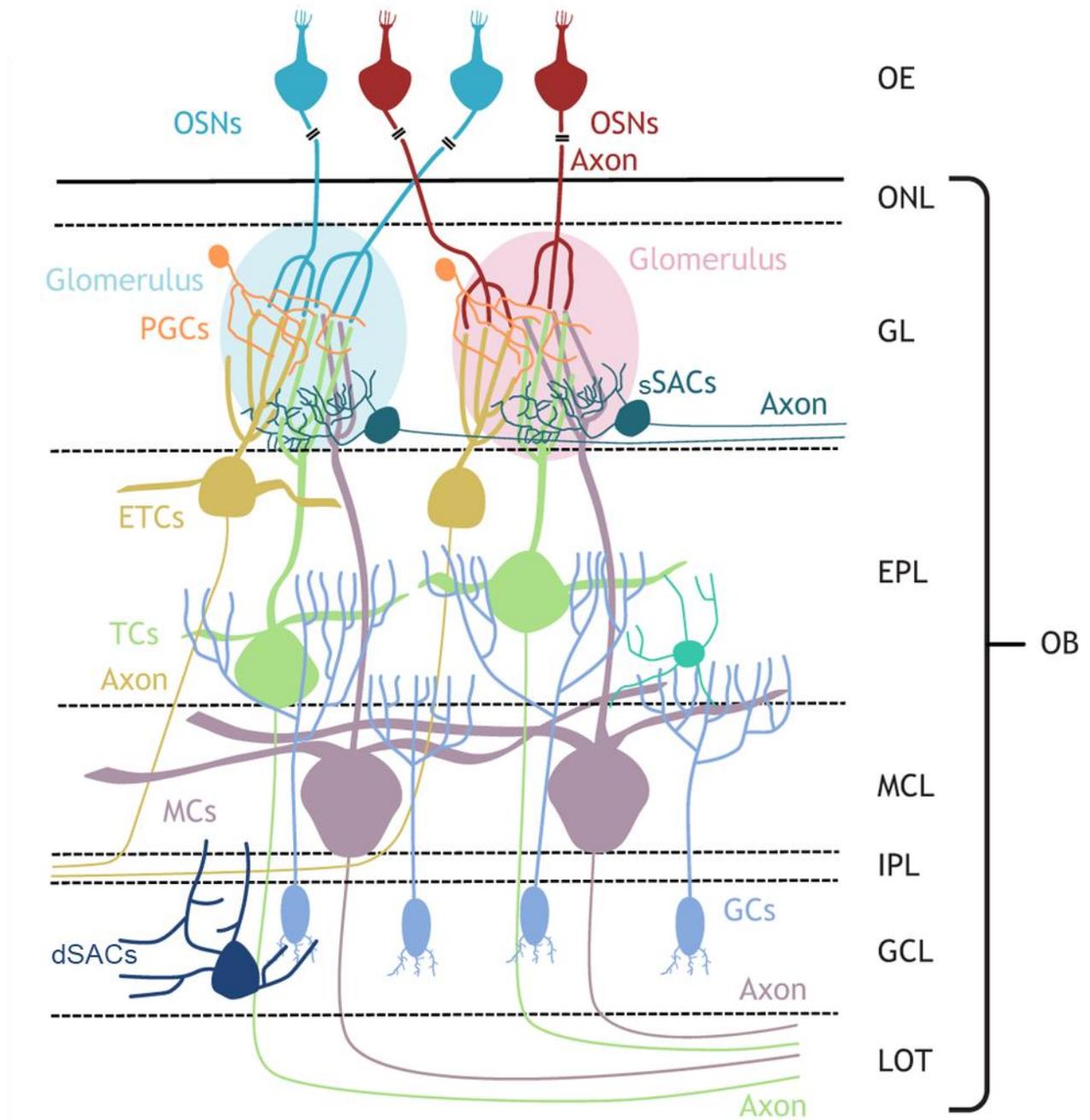


Figure III. The olfactory message in the mammalian main olfactory bulb circuitry. Different color OSNs represent populations expressing different olfactory receptors, axons of which coalesce into discrete glomeruli. dSACs, deep short-axon cells; EPL, external plexiform layer; ETCs, external tufted cells; GCs, granule cells; GCL, granule cell layer; GL, glomerular layer; IPL, internal plexiform layer; LOT, lateral olfactory tract; MCs, mitral cells; MCL, mitral cell layer; OE, olfactory epithelium; ONL, olfactory nerve layer; PGCs, periglomerular cells; sSACs, superficial short-axon cells; TCs, tufted cells. Adapted from Tufo *et al.*, 2022.

Integration in the glomerular layer is the result of all these processes, with some components still remaining to be fully understood. Once the signal exits the glomeruli, if the threshold is surpassed, action potentials are generated at the base of M/T cells axons. However, a last (but not least) gating is performed by GCs.

*ii. The granule cells, major actors in olfactory message integration*

After being described in the early work of Golgi as soon as 1875, the GC, despite its abundance in the OB, remained an enigma because of its lack of axon. However, electrophysiological data showed that these cells could in fact be excited by M/TC activation and in return suppress this very activation (Shepherd, 1963).

GCs are small, axonless cells that constitute the most represented cell type in the OB (Parrish-Aungst *et al.*, 2007). They display long spiny apical dendrites that expand and branch through the IPL, MCL and EPL, where they make connections with other cell types.

GCs are very diverse, depending on their location in the OB and their structure (Orona, Scott and Rainer, 1983), but also in their gene expression (Naritsuka *et al.*, 2009; Nagayama, Homma and Imamura, 2014), resulting in several classes of GCs. Their distinct features suggest various roles in message processing.

The connection between M/T and GCs was found to be a peculiar feature of the nervous system, a bidirectional dendrodendritic synapse (Figure IVA-B), which was first anatomically described thanks to electron microscopy (Hirata, 1964). These reciprocal synaptic connections allow inhibitory feedback directly on the M/T cells secondary dendrites: the glomeruli-generated action potential back propagates from the cell body to the dendrites in the EPL, where glutamate vesicles are accumulated. Once the depolarization arrives, glutamate is released into the synaptic cleft (Wellis and Kauer, 1993) on GCs dendritic spines, triggering depolarization of GC dendrites, which in return release GABA, resulting in the inhibition of the activated M/TC in a quick feedback loop (Figure IVC) (Rall *et al.*, 1966; Jahr and Nicoll, 1982a).

Moreover, the propagation of the depolarization in the GC dendrite also results in the inhibition of close M/T cells contacting the same GC, thus ensuring some additional lateral inhibition mechanism (Rall and Shepherd, 1968).

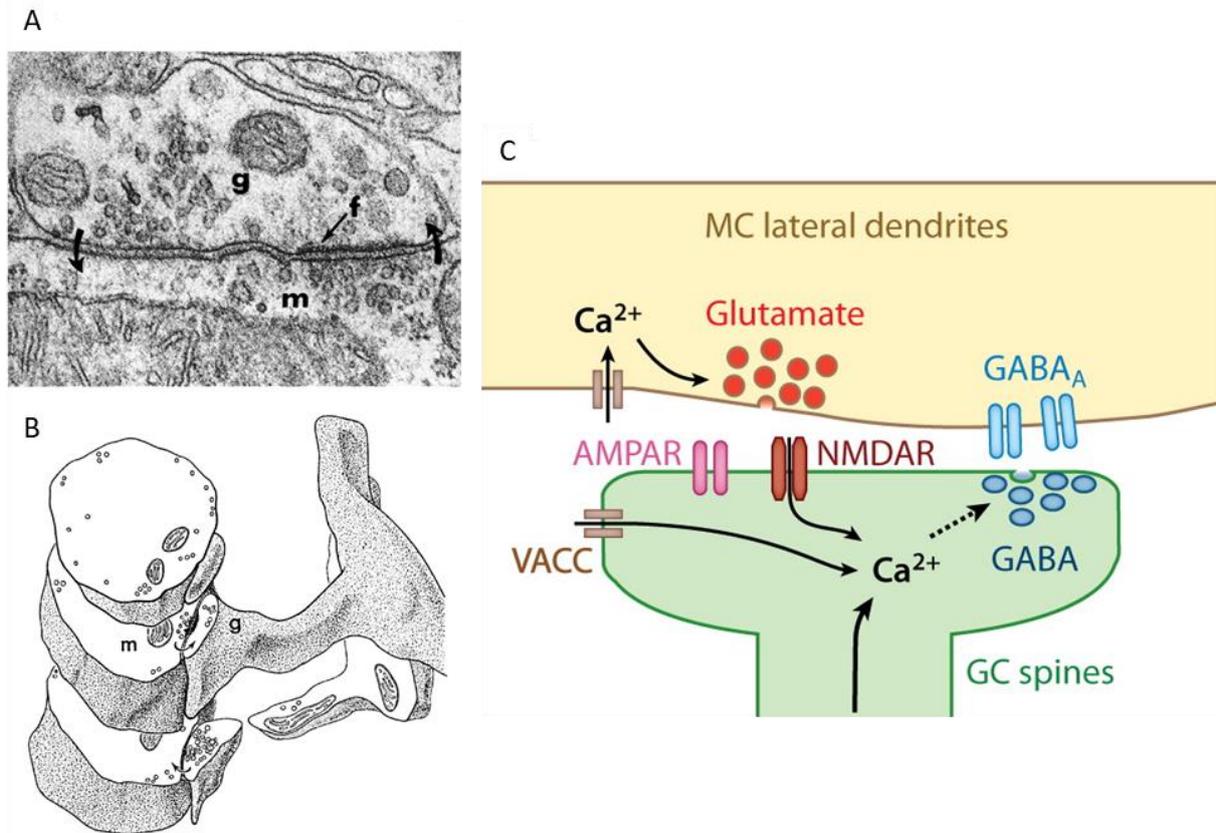


Figure IV. The dendrodendritic reciprocal synapse. A. Electron micrograph of a mitral secondary dendrite (m) making a mitral-to-granule asymmetric synapse (f and upward arrow) onto a granule cell spine (g), which makes a reciprocal granule-to-mitral symmetric synapse onto the same mitral cell dendrite (downward arrow). B. Serial reconstruction of the mitral (m) and granule (g) cell reciprocal synapses (see arrows) From Rall et al, 1966. C. Schematic representation. In this synapse (inset at left), local calcium entry within MC lateral dendrites triggers glutamate release, which activates both AMPA and NMDA receptors on GC spines. This postsynaptic activation can directly, or indirectly via voltage-activated calcium channels (VACC), increase local calcium concentration in the GC spines. This calcium increase, in turn, triggers GABA release and postsynaptic inhibition of MC dendrites via GABA<sub>A</sub> receptors. From Lepousez *et al*, 2013.

Finally, GCs can also be activated by MC axon collaterals. In this second mode of MTC/GC interaction, axodendritic synapses in the IPL and GCL generate depolarization of GCs (Schoppa, 2006) which can result in an action potential originating from GC soma (Pressler and Strowbridge, 2019). This results in inhibition the M/TCs connected to the stimulated GC. These two types of GC activation have different properties (amplitude, kinetics, short-term plasticity) and probably different computational functions (Pressler and Strowbridge, 2020).

GCs-mediated inhibition seems to be useful in enhancing the contrast between two odor responses, as shown by Yokoi and colleagues (Yokoi, Mori and Nakanishi, 1995). The inhibition provided by the GCs synchronizes M/TC activity, allowing for spatiotemporal fine-tuning of the responses of principal cells to an odor (Urban, 2002; Arevian, Kapoor and Urban, 2008), and to participate in odor discrimination (Nunes and Kuner, 2018).

GCs can self-inhibit through GABA<sub>B</sub> receptor if recurrent activation occurs, allowing another level of tuning if the odorant stimulation is repeated (Isaacson and Vitten, 2003), and can also undergo a feed-forward inhibition directly from the M/T cells through activation of local dSACs (Burton and Urban, 2015).

GCs have been extensively reviewed (Shepherd *et al.*, 2007), as well as their role in olfactory processing (Burton, 2017). Recent findings showed that proper inhibition through reciprocal synapses, which are NMDA-dependent, tightly rely on coincidental activation by principal cell axons, which enables GCs to participate in lateral inhibition and olfactory coding (Egger and Kuner, 2021). We also need to note that GCs also receive consequent inputs from other regions of the brain, which will be discussed in chapter 2.

Taken together, GC characteristics point to an incredible tuning power in the later transmission of the olfactory message. Combined with the early processing in the GL, we can affirm that the message convergence to duets of glomeruli and transmission through granular processing enhances the message salience and contrast before further processing in other brain areas.

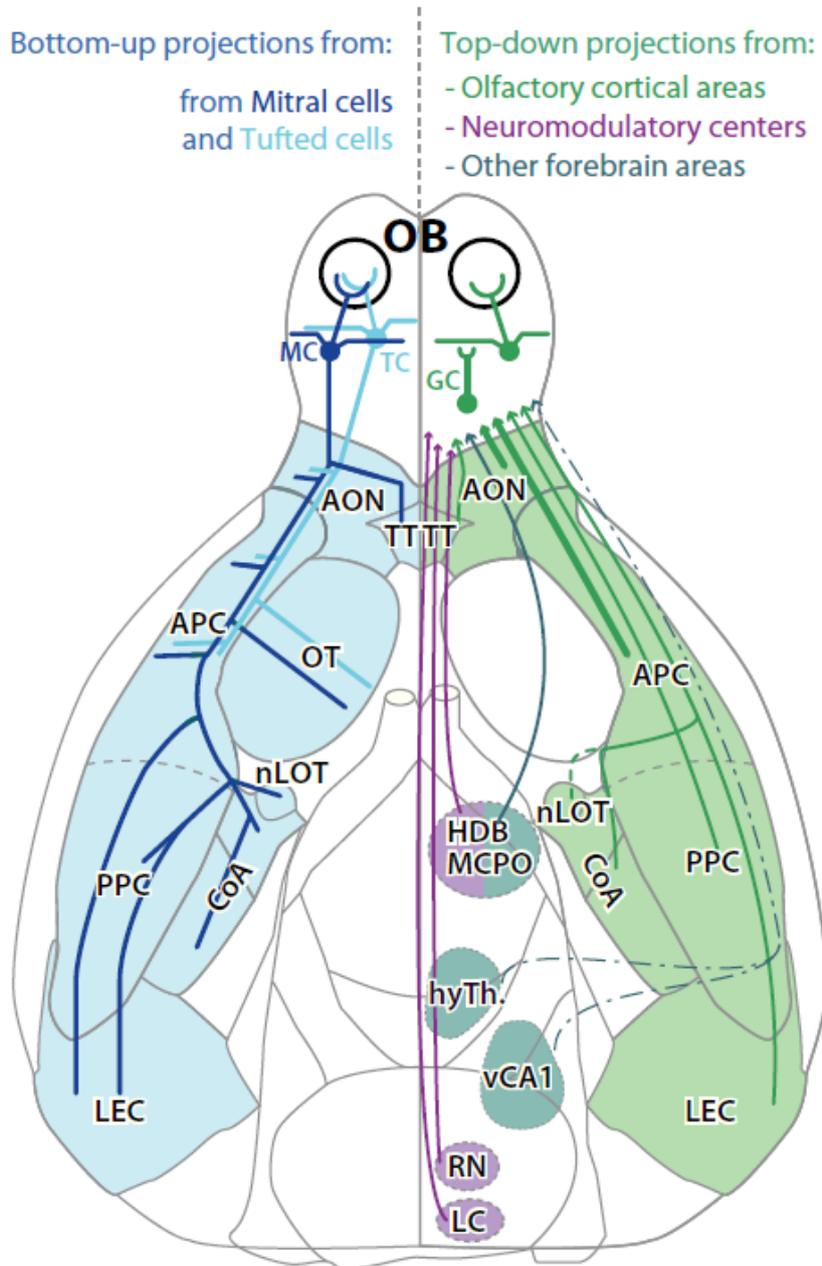


Figure V. Outputs and inputs to the Olfactory Bulb. (Left) Diagram showing the axonal projections of Mitral Cells (MCs) and Tufted Cells (TCs). MCs project axons to nearly all areas of the olfactory cortex, whereas TCs project axons to anterior areas of the olfactory cortex, but apparently spare the tenia tecta (TT). (Right) Diagram showing the origin and the diversity of the inputs to the olfactory bulb. Olfactory cortical areas are reciprocally connected to the olfactory bulb, except for the olfactory tubercle (OT). Neuromodulatory brain regions also project to the olfactory bulb. Other forebrain regions were occasionally reported to send a few projections to the olfactory bulb. Note that the basal forebrain region HDB/MCPO sends GABAergic projections in addition to neuromodulatory projections. AON: Anterior olfactory nucleus; TT: Tenia tecta; APC: Anterior piriform cortex; OT: Olfactory tubercle; PPC: posterior piriform cortex; nLOT: nucleus of the lateral olfactory tract; CoA: Cortical amygdala; LEC: Lateral entorhinal cortex; HDB/MCPO: Nucleus of the horizontal limb of the diagonal band of Broca / Magnocellular preoptic nucleus; RN: Raphe nuclei; LC: Locus coeruleus; hyTh.: hypothalamus; vCA1: ventral cornus ammonis region 1.

Another aspect of GCs (and PGCs) is their generation during adult life through adult neurogenesis, bringing even more diversity and complexity to the OB network. This will be detailed in chapter 2.

### 3. To the cortex and beyond

Most sensory systems, after a first relay of integration, send their message to the thalamus, from where it is spread across relevant areas (McCormick and Bal, 1994). However, in the olfactory system, the sensory message is directly transmitted to cortical regions after integration in the OB. Numerous areas receive direct inputs from the OB, of which we will give an overview here. Early tracing studies in hedgehogs and rats showed that OB directly projects to the Anterior Olfactory Nucleus (AON), Piriform Cortex (PC), Nucleus of the Lateral Olfactory Tract (nLOT), Horizontal limb of the Diagonal band of Broca (HDB), Cortical Amygdala (CoA) and Dorsal Raphe (DR) (Figure V, Left) (De Carlos, López-Mascaraque and Valverde, 1989). More recent studies also showed direct connections to the Olfactory Tubercle (OT), Tenia Tecta (TT) and Lateral Entorhinal Cortex (LEC) (Sosulski *et al.*, 2011). It is interesting to note that Tufted cells project to the most anterior structures, especially AON and anterior PC, while MCs send collaterals to all the regions above (Igarashi *et al.*, 2012). For the sake of clarity, only the main projection areas relevant to the understanding of this study will be extensively described, namely AON, PC, and some other examples useful for our demonstration.

#### a. The anterior olfactory nucleus

The most anterior part of the olfactory primary cortex, the anterior olfactory nucleus, extends from the very end of the OB to the beginning of the PC. Despite anatomical divisions that are more or less arbitrary, from classical Nissl labeling to actual cell organization (Haberly and Price, 1978), the AON contains two main regions: pars Externa (AONpE) and pars Principalis (AONpP), itself subdivided in several subregions.

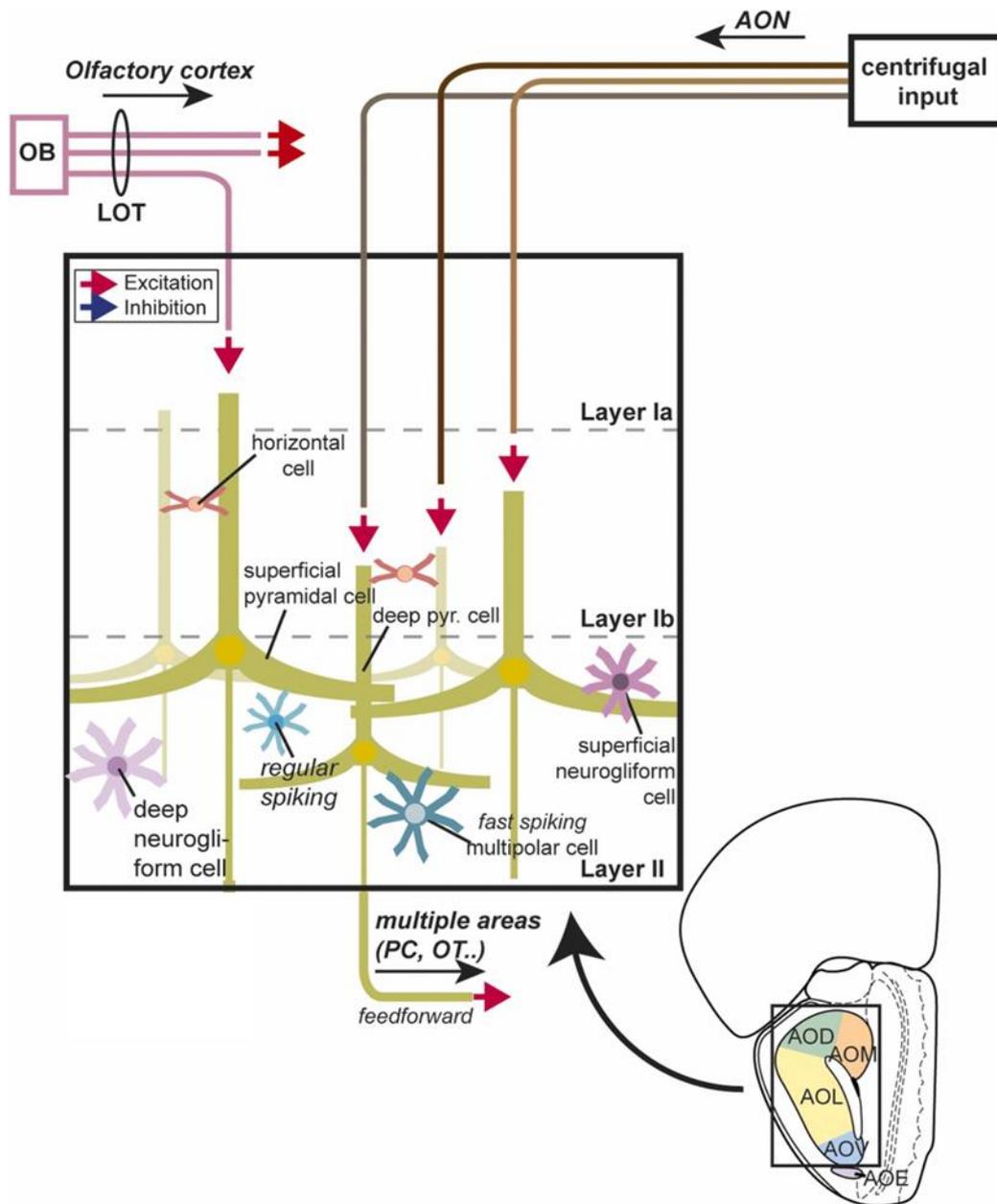


Figure VI. Cytoarchitecture of the Anterior Olfactory Nucleus. Though the neuronal composition of different pars principalis zones seems to be homogenous, there are differences in the composition of cortical layers and even within these layers. Two types of principal neurons have been identified that can be separated according to position within layer II but not according to their projection target. Furthermore, 4–5 morphologically and electrically distinguishable types of interneurons could be identified but immunohistochemical characterization suggests that the interneuron composition is far more complex. Abbreviations: AOD: AON dorsal part, AOL: AON lateral part, AOM: AON medial part, AOE: AON pars externa, AOV: AON ventral part, PC: piriform cortex, OT: olfactory tubercle. From Brunert *et al*, 2023.

This region contains mostly pyramidal cells with various morphologies, and diverse interneurons. The pyramidal cells show an extensive neurochemical diversity (Brunjes, Illig and Meyer, 2005), with distinct subpopulations expressing either calbindin or calretinin (García-

Ojeda *et al.*, 1998), while at least five types of interneurons have been identified in the AONpP (Kay and Brunjes, 2014).

Around 100,000 axons from the OB travel through the LOT and a portion of them arrives on the outer layer of the AONpE and AONpP, more precisely in layer Ia (Figure VI) (Scott *et al.*, 1985). M/TC axons arrive in the external part of the AON, which possesses a cortex-like organization with layer 2 pyramidal cells extending their apical dendrites to layer Ia, receiving MOB inputs.

Pyramidal cells in the AON exhibit responses to many of odors compared to single MCs, which indicates that numerous projection cells coming from various OB glomeruli have overlapping activation patterns in the AON (Lei, Mooney and Katz, 2006; Cousens, 2020). Moreover, while cells in AONpE display odor-specific activation patterns, those in AONpP exhibit sparser, non-specific activation similar to PC cells (see below) (Kay *et al.*, 2011). Electrophysiologically, AON cells fire in short bursts of 2 to 20 spikes and some cells respond strongly to social odors (Tsuji *et al.*, 2019).

This nucleus receives inputs from several other regions such as PC, prefrontal cortex, hippocampus, hypothalamus, striatum and neuromodulatory regions, while itself projecting notably to the Nucleus Accumbens (NAc), the PC (Russo *et al.*, 2020) and hippocampus, but also to contralateral OB and AON (Brunert, Medinaceli Quintela and Rothermel, 2023).

These numerous connections suggest an important integration role of the AON, most of which are linked to the projections that are sent back to the OB and will be detailed in chapter 2. However, other roles have been shown, depending on AON input regions, such as social odor processing (Oetl *et al.*, 2016), social transmission of food preference (C. Y. Wang *et al.*, 2020), and episodic odor memory (Aqrabawi and Kim, 2018a, 2018b; Levinson *et al.*, 2020).

## b. The piriform cortex

The PC is located on the ventral part of the brain, just posterior to the AON, and is divided into anterior (APC) and posterior PC (PPC), separated by the end of the LOT. It is a paleocortex composed of 3 layers, the first one receiving inputs from the OB, while layers 2 and 3 contain

respectively superficial and deep pyramidal cells, along with numerous inhibitory interneurons. Because of the difference in the LOT density between APC and PPC, the first one receives more input from the OB (Rennaker *et al.*, 2007), while the other is predominantly innervated by associative inputs (Luna and Morozov, 2012). Recent retrograde tracing experiments confirmed this segregation and showed extensive projection patterns to the PC from the whole brain (L. Wang *et al.*, 2020), with high input from the MOB directly onto GABAergic cells of the APC. This study also showed that the APC receives substantial inputs from the contralateral AON, reinforcing its potential role for interhemispheric coordination, while the PPC receives extensive connections from contralateral PC and nLOT.

But what is the function of the PC? This question has been reviewed regularly (Wilson and Sullivan, 2011; Bekkers and Suzuki, 2013), but we will provide a brief updated overview of major findings in this matter.

Functionally, PC neurons seem unable to respond to single glomeruli activation. Indeed, stimulation of at least 4 glomeruli seemed necessary to elicit APs in PC pyramidal cells. Moreover, specific combinations of glomeruli activation showed stronger responses in the PC, suggesting that PC cells could act as odor identity decoders (Apicella *et al.*, 2010; Davison and Ehlers, 2011). It is well known that M/TCS receiving inputs from the same glomerulus expand their axon extensively in the APC (Ghosh *et al.*, 2011), which could explain that the topography seen in MOE, OB and in a weaker manner in the AON, is not found in the APC. Rather, activation patterns in this region are scattered across space, although consistent across stimulation and concentration-dependence (Stettler and Axel, 2009). Despite its apparent scarcity, odor representations in the PC enable encoding of odor identity, concentration and even valence (Blazing and Franks, 2020), and are modified by afference from other regions such as the Basolateral Amygdala (BLA) (Sadriani and Wilson, 2015).

Sparse activation patterns are also the result of strong feed-forward inhibition. Indeed, a study showed that general inhibition in the APC is a major component of the sparse responses measured in response to odor presentation (Poo and Isaacson, 2009).

More recent work also showed that concentration-independent responses were predominant in the PC through internal feedback inhibition (Bolding and Franks, 2018), supposedly allowing higher responses to fast-arriving odors. Authors hypothesize that the most specific odor-to-

receptor combination will trigger faster activation, thus being predominant compared to other environmental stimuli, despite differences in concentrations. All these observations show strong differences with other sensory cortices where topography is usually robustly conserved and stereotyped (in the barrel cortex or V1 cortex for example).

Overall, the PC presents itself as the place where – as proposed in early works and coherently with other sensory systems – odors are discriminated and identified (Roland *et al.*, 2017). Importantly, other important functional roles have been associated with this region, including olfactory learning and memory (Courtiol and Wilson, 2017).

These two main regions of olfactory output allow, after processing in the OB, the creation and transformation of odor representations in the brain that is specific to each odor. The various activation patterns in the OB, AON and PC can be considered as a code that enables the identification and discrimination of odorants.

### c. The olfactory code and its representation in the brain

Building on the fact that all OSNs expressing the same receptor converge to two specific glomeruli in each OB, we can easily admit that the stimulation by a single molecule will induce activation of a precise and reproducible combination of glomeruli in each bulb. This has been visualized *in vivo* in several studies, confirming the existence of an olfactory activation map in the bulb underlying the olfactory code at this point of the story (Meister and Bonhoeffer, 2001; Wachowiak and Cohen, 2001; Murthy, 2011). These observations have led to hypotheses about the existence of a chemotopic representational map in the OB, as can be observed in other sensory system, where stimuli features are topographically arranged in thalamic and cortical areas (spatial map in vision and proprioception, frequency map in auditory system, etc). This chemotopic map has been drafted by carefully studying glomeruli responses to various classes of odorants (Johnson and Leon, 2007), with other parameters such as molecular length influencing bulbar activation as much as chemical groups (Johnson and Leon, 2000).

However, this view has been challenged by several studies arguing that chemical representation in the bulb is sparser than previously claimed and molecules displaying the

same chemical groups can have distinct activation patterns (Ma *et al.*, 2012), although this work has been contested (Yablonka, Sobel and Haddad, 2012). Nonetheless, the authors of this study still observed similar maps for structurally similar odors, giving hints on how similarity is topographically encoded in the OB, thus presenting a tonotopy that has further been observed by other teams (Soelter *et al.*, 2020). Moreover, recent work showed that activation maps, which were believed to be quite wide even for single molecules, are in fact narrower than expected (Burton *et al.*, 2022).

However, this does not recapitulate physiological stimuli in the sense that most environments contain several to hundreds of different odorants. Often taken as an example, coffee contains usually more than 800 distinct volatile molecules.

In multi-odor perception, computation in the bulb needs great integration capacity. In these situations, even the detection of each odorant in the MOE can get trickier, especially if several molecules in the mix possess the same chemical functions and thus compete for binding to the same receptors. An allosteric effect has also been demonstrated, with odors that do not bind to certain receptors are still able to influence their affinity for other molecules (Xu *et al.*, 2020; Trimmer *et al.*, 2023). Thus, complex interactions in odor-complex environments can drastically reshape the activation pattern in the MOE, and subsequently strongly influence integration in the OB and further interpretation.

In the OB, simultaneous detection of several odorants results in a specific activation map that is not the simple addition of single molecules signatures. As we already saw, lateral inhibition across glomeruli, allowed by various interneurons, can enhance the activation of certain glomeruli against others, and induce the predominance of certain odorants in the resulting representations. Moreover, temporal activation of various glomeruli plays a predominant role in odor recognition, as shown in recent work using precise single-spot optogenetic activation (Chong *et al.*, 2020).

Furthermore, the transmission of the electrical message to the cortex seems to completely abolish the spatial organization of odor representation. Supporting this, activation patterns in the AON and PC are scattered, although reproducible across same-odor expositions. It is thought that representational code in the PC is linked to temporal activation rather than spatial

clustering, allowing, for instance, odor discrimination (Stopfer *et al.*, 1997; Lepousez and Lledo, 2013a).

Interestingly, perception of a mixture of odors does not result in the addition of individual odor respective cortical representations, due to both feed-forward and feedback inhibition controlling the spatio-temporal features of perception (Yaksi *et al.*, 2009; Bolding and Franks, 2018). The PC is also able, on top of odor identification, to categorize similar odorants, and transforms bulbar representation according to experience-dependent associative networks (Pashkovski *et al.*, 2020). It is important to note that despite the scattered pattern of activation, the cortical representation of odors is highly conserved across individuals. However, cortical maps appear to drift across time when repeatedly presented the same odors, although the differences between different odors was maintained, thus still allowing discrimination (Schoonover *et al.*, 2021).

Thus, precise representation in the primary olfactory regions allows perception and interpretation of complex odor environments and is the substrate giving rise to odor-induced behavioral responses, which will be detailed in chapter 3.

However, the sensory message is also travelling to many other cortical regions that we need to consider.

#### d. Other regions involved in olfactory processing

Recent advances in molecular tools allow more and more precise anatomical studies of olfactory tracts. In their latest work, Chen *et al* used recently-developed Barcode sequencing to precisely assess the targets of OB projection neurons (Chen *et al.*, 2022). They showed that while MCs projected strongly to all aforementioned cortical regions, their major targets were the PC and AON, while TCs essentially project to the AON and OT. However, M/T cell projections to the CoA and LEC were clearly depicted (Figure VII).

Interestingly, they also identified a third population of deep projecting cells, maybe corresponding to deep short-axon cells in the GCL that are putatively sending GABAergic projections to extra bulbar areas (Eyre, Antal and Nusser, 2008), mainly innervating the AON and PC.

Most cortical regions of the olfactory system receive, additionally to those emerging from the OB, inputs from the AON or PC, and themselves send connections to layer II of the two parts of primary olfactory cortex. While projections to AON and PPC seem to constitute what we could call a perception pathway, allowing precise identification and representation of odors, those that innervate CoA or LEC may have roles in other components of olfactory processing. Indeed, it has been demonstrated for example in the visual cortex that pathway segregation allows processing of various characteristics of the message (Glickfeld and Olsen, 2017).

The CoA is a ventral part of the amygdala, thus included in the limbic system, known to encode and process valence in the brain. Connections between OB, PC and CoA would then logically translate the emotional value of a perceived odor and support associated behaviors (attraction/repulsion), as shown in various papers (Root *et al.*, 2014; Kondoh *et al.*, 2016).

In a more caudal part of the brain, the Lateral Entorhinal Cortex (LEC) is known for encoding experiences and is one of the major inputs to the hippocampus (Wang *et al.*, 2018). Thus, projections to and from the LEC could enable associative odor experiences with contextual cues and lay the basis for associative memory.

These different pathways allow to reconstruct odor information and compare it with previously encoded experiences, with or without emotional values. The PC has even been suggested as a coincidence decoding region for spatial map reconstruction in complex odor environments (Poo *et al.*, 2022).

We have detailed here the main steps of the canonical olfactory pathway. As commonly seen for most biological processes, the straightforward way is not sufficient to explain the biological complexity of this system, where each step is carefully controlled and modulated by various means. Thus, we will now explore these diverse ways of tuning the olfactory message and their relevance for our study.

## Chapter II. Modulation of the canonical olfactory pathway

### 1. Centrifugal connections

The described pathway of the olfactory message has been, although in parallel ways for the cortical part, a one-way ride. From the MOE to cortical areas, the electrical information climbed its way up, leading us to a feed-forward, bottom-up understanding of olfaction. However, in olfaction, as in other sensory systems, a considerable number of cortical neurons project back to the first relays of the message, in what is called a top-down, or centrifugal, manner. These inputs carry information about the individual internal state as well as the expected outcome based on previous experiences. The presence of cortical top-down projections from the sensory cortex back onto earlier sensory relays (such as the thalamus for visual and auditory systems, or the OB in olfaction) is a standard feature of sensory systems (Gilbert and Li, 2013). Here, we will focus on the neurons innervating the main OB (Figure V, Right), allowing the consequent integration of the message before transmission to cortical regions. Although important advances have occurred in the anatomical and function characterization of these cortical top-down projections, their functional role in olfaction is still far from being deciphered.

For a better understanding, we can split the centrifugal connections into two main groups. On the first hand, neuromodulatory regions of the brain send axons to the OB where numerous neuromodulator receptors are expressed, radically changing the intrinsic properties of OB neurons in given contexts. On the other hand, several cortical regions directly or indirectly targeted by OB projection neurons send messages back to the OB.

#### a. Neuromodulatory regions and their influence on the olfactory bulb

The OB is one of the many targets of neuromodulatory brain regions. The main regions are the Locus Coeruleus (LC), the Raphe Nuclei (RN), namely the dorsal and median raphe, and the Horizontal limb of the Diagonal band of Broca (HDB) located in the basal forebrain (BF). These

three regions send projections to a large number of brain areas and release different neuromodulators, respectively NorEpinephrin (NE), Serotonin (5-HT) and AcetylCholine (ACh) (Matsutani and Yamamoto, 2008).

In the OB, these three molecules are released according to the activation of their source region and have various effects on the bulbar cells:

- The LC sends massive projections to the OB, compared to other regions, and its terminals secrete NE, a catecholamine known to play a role in attention, arousal, and various physiological and cognitive processes (Schwarz and Luo, 2015). It is estimated that 40% of the noradrenergic neurons emerging from this nucleus send collaterals to the OB, with axon terminals mainly located to the GCL and IPL and, to a lesser extent, in the GL (Shiple, Halloran and de la Torre, 1985; Gómez *et al.*, 2005) NE secretion enhances the firing activity of principal cells by a disinhibition mechanism, through inhibition of GCs (Jahr and Nicoll, 1982b), which is coherent with the NE role in arousal that could facilitate odorant perception. However, more recent studies showed that NE release in the OB participates in the suppression of odor responses in the glomeruli (Eckmeier and Shea, 2014), but is involved in the enhancement of the signal to noise ratio (Manella, Petersen and Linster, 2017). Thus, the role of noradrenergic modulation in the OB still needs to be clearly deciphered. The variety of adrenergic receptors and their differential expression in the OB cell types will not be discussed here.
- Serotonin is known to be involved in various cognitive processes, from visual perception to anxiety and depression (Berger, Gray and Roth, 2009). Most serotonin originates from the RN and spreads through the whole brain. These fibers modulate the treatment of olfactory information in the bulb by acting on multiple targets (Hardy *et al.*, 2005; Liu *et al.*, 2012; Schmidt and Strowbridge, 2014; Steinfeld *et al.*, 2015; Brunert *et al.*, 2016; Kapoor *et al.*, 2016). In the OB, fibers from the RN predominantly innervate the GL (McLean and Shipley, 1987). Previous work showed that serotonin release impairs odor-evoked glomeruli activation through OSNs GABA<sub>B</sub> receptors by increasing JGCs activity (Petzold, Hagiwara and Murthy, 2009). Indeed, inhibitory cells in the GL display higher responses to odors with RN activation (Brunert *et al.*, 2016). However, experimental evidence of excitation of M/T cells or eTCs by the same 5-HT-releasing

neurons makes it difficult to draw precise conclusions on serotonin effect on olfactory integration (Kapoor *et al.*, 2016). Consequently, the diversity of targeted cell types and 5-HT receptors (Brill *et al.*, 2016) suggests a fine-tuning ability for these fibers, although not precisely determined and probably state-dependent. Broad serotonin effects on olfactory perception have been reviewed by (Gaudry, 2018).

- Acetylcholine terminals also seem to preferentially target the GL of the OB, especially the juxtglomerular cells (sSACs, PGCs and eTCs), and come mainly from the HDB and the MagnoCellular PreOptic area (MCPO) (Carson, 1984a; Jeune and Jourdan, 1993). These afferents innervate the majority of the bulb's layers (Ichikawa and Hirata, 1986; Kasa *et al.*, 1995; Rothermel *et al.*, 2014) modulating several interneuron populations (Castillo *et al.*, 1999; Pressler, Inoue and Strowbridge, 2007; Ma and Luo, 2012; Rothermel *et al.*, 2014; Burton, 2017). ACh can activate both ionotropic (nicotinic) and metabotropic (muscarinic) receptors, although PGCs and M/TCs seem to express essentially nicotinic receptors, resulting in their excitation (Castillo *et al.*, 1999). On the other hand, GC activity seems decreased through muscarinic receptors, but this results in a paradoxical increase in GABA release onto MCs (Pressler, Inoue and Strowbridge, 2007). When triggered, ACh release in the bulb exhibits an increase in M/T cells spike firing, whether or not an odor is presented coincidentally (Rothermel *et al.*, 2014). Altogether, results indicate that ACh modulation of the olfactory message may filter the strongest message, laying the basis of attention in the OB (D'Souza and Vijayaraghavan, 2014).

These three systems add an intricate layer of neuronal tuning in the OB, with probable roles in attention and learning, in a state-dependent manner. A common feature of these centrifugal projections is the presence of varicosities along their axon terminals (Shipley, Halloran and de la Torre, 1985; Ichikawa and Hirata, 1986; McLean and Shipley, 1987), which allow the release of neuromodulators in a wide spatial range of the OB when activated. Thus, neuromodulatory centrifugal connections could have a broad effect on OB transmission, while glutamate and GABA projections from olfactory areas would target smaller zones and potentially precise glomeruli.

## b. Centrifugal projections from non-neuromodulatory areas

Various studies sought to draw a map of cortico-bulbar projections, starting as early as 1911 with the drawings of Cajal identifying these afferences. After the basics of feedforward transmission were deciphered, people started looking at centrifugal innervation of the OB (Carson, 1984b; Shipley and Adamek, 1984). Later, technical advancements in tracing methods helped determine and quantify the afferences to the bulb, especially thanks to the use of rabies viruses. Thus, the latest works were able to recreate whole brain maps of centrifugal projections and distinguish them according to their preferential targets in the OB (Padmanabhan *et al.*, 2019; Schneider *et al.*, 2020; Wang *et al.*, 2023). The authors confirmed that the AON, PC, CoA, nLOT, TT, LEC, BF, LC, RN, Hippocampus, among others, all send projections directly to the OB. These projections are actually found in a higher number than OB-to-cortex projections. Interestingly, of all structures receiving afferences from the OB, only the olfactory tubercle seems not to possess reciprocal connections. Here, we will try to give an overview of the role of these projections in olfaction, focusing on the two regions sending the most input, the AON, and PC.

### i. AON projections to the OB

The AON is the main cortical region projecting to the OB. Indeed, an estimate of roughly 54% of cortico-bulbar projections arise from this region, which receives extensive bulbar inputs from both types of projecting cells. The neurons that project to the OB are distributed throughout all layers of the AON (de Olmos, Hardy and Heimer, 1978; Haberly and Price, 1978; Davis and Macrides, 1981; Luskin and Price, 1983; Shipley and Adamek, 1984).

Moreover, the AON is one of the only regions, along with the nLOT, to project to both the ipsi and contralateral OB (in a ratio of approximately 10:1), with only the ipsilateral AON terminals reaching the glomerular layer, while the rest is mainly located in the superficial part of the GCL (Davis and Macrides, 1981; Rothermel and Wachowiak, 2014). Knowing the fact that this region already projects to its own contralateral counterpart, we can assume that the AON is crucial for interhemispheric synergy in olfactory processing. Lastly, AONpP is the

predominant subregion sending axons to the OB, where they mainly target the GCL, despite some contradictory data on this last feature (Wen *et al.*, 2019; Wang *et al.*, 2023). This difference in projections depending on the AON sub-regions from which they originate, suggests they may have different functions.

But how do these projections impact the OB circuit? In the first functional studies, the AON centrifugal fibers were identified as glutamatergic cells that target both bulbar interneurons and principal cells (Markopoulos *et al.*, 2012a). *In vivo* activation of these projections suppresses odor evoked responses, but also displays some temporally limited rise in principal cells activity, suggesting a role in selecting precisely timed activity in the OB. Interestingly, BF activation can trigger activity in OB-projecting AON cells, suggesting a complementary role in relaying descending information to the OB (Rothermel and Wachowiak, 2014).

*In vivo* inhibition of these neurons, especially those of AON pars Medialis (AONpM), shows an enhanced olfactory capacity in mice presented to very weak odorants, but also in social and novelty recognition tasks, with variable effects of their activation (Aqrabawi *et al.*, 2016). These neurons seem to receive strong inputs from the ventral Hippocampus (vHPC), where optogenetic activation is enough to elicit strong depolarization on most AONpM cells and recapitulated the effects seen with direct activation of AONpM.

Another particularity of these centrifugal connections is their innervation by oxytocin-expressing neurons from the ParaVentricular Nucleus (PVN), which were shown to activate AON neurons projecting to bulbar GCs, triggering the inhibition of OB principal cells. This resulted, as seen in ACh modulation, in a better signal-to-noise ratio when presented with an odor (Oettl *et al.*, 2016).

Finally, a very recent study showed that AON projections exhibit their effects mainly on TC odor-evoked responses, rather than through MCs, which are in turn more targeted by the APC (Chae *et al.*, 2022).

## *ii. Piriform cortex projections to the OB*

Projections from the PC to the OB mainly arise from layers IIb and III (Diodato *et al.*, 2016), with layer IIb preferentially targeting M/T cells and layer III terminals predominant in the GCL.

Numerous cells in layer IIb also project, sometimes collaterally, to medial Prefrontal Cortex (mPFC), while cells in layer IIa mainly project to CoA and LEC. Moreover, although these projections seem to innervate all cell types in the OB, the majority target the GCL rather than the MCL or EPL (Wang *et al.*, 2023).

Moreover, anterior PC cells do not send contralateral projections (Davis and Macrides, 1981; Luskin and Price, 1983; Boyd *et al.*, 2012a). Interestingly, centrifugal fibers are still somewhat organized: PC neurons that are in close proximity to each other are more likely to project their axons to similar regions of the OB (Padmanabhan *et al.*, 2016), which could reflect the existence of coding strategies for olfactory information.

PC-OB projections, when activated, result in disynaptic inhibition of M/T cells by directly exciting GCs (Boyd *et al.*, 2012a). However, they also elicit feed forward inhibition of these very GCs, indubitably by activation of dSACs, that receive more inputs from PC than the GCs. Thus, these connections allow precisely timed, short-latency inhibition of OB projecting cells, suggesting a predominant role in odor-response time resolution. Lastly, PC neurons also activate sSACs and PGCs in the glomerular layer, enhancing their ability to suppress odor-evoked-responses. Later findings showed that these PC feedbacks have enhanced activity during mouse wakefulness and show scattered activity across various glomeruli after odor presentation, which is not related to the odor-responding glomerulus (Boyd *et al.*, 2015).

Studies have shown that AON and PC centrifugal fibers may in fact diverge in their targets. Data suggest the PC neurons have a predominant effect on MC activity, while the AON would rather influence TC responses (Otazu *et al.*, 2015; Chae *et al.*, 2022). Thus, a region-specific centrifugal tuning might occur, with a differential effect, as a result of their individual inputs. This would correlate with the differential innervation of these regions by M/TCs and might be an argument in favor of a “feedback” mechanism, although this has not yet been proven.

Besides, most of the described non-neuromodulatory top-down projections are excitatory, but recent work shed more light on the existence and function of inhibitory centrifugal fibers.

### *iii. Inhibitory centrifugal projections*

The third most important region sending afferences to the OB is the BF. Although we already discussed its cholinergic activity in a previous section, several studies showed evidence that this region also projects GABAergic long-range fibers to the OB, targeting inhibitory neurons (Gracia-Llanes *et al.*, 2010). These fibers selectively innervate GCs (Nunez-Parra *et al.*, 2013), SACs (Case *et al.*, 2017) and some subtypes of PGCs (Sanz Diez, Najac and De Saint Jan, 2019). Co-release of GABA and ACh can occur and make the broad result of these axons activation unpredictable (De Saint Jan, 2022). Overall, these fibers seem to participate in disinhibition of M/TCs and are required for proper discrimination of similar odorants (Nunez-Parra *et al.*, 2013).

Furthermore, while most studies characterized glutamatergic AON projections to the OB, our lab recently discovered that a subregion of the posterior AON, namely AON *pars posterioralis* (AONpp), gave rise to GABAergic projections that resulted in direct inhibition of most cell types of the OB, except interneurons in the GL (PGCs and sSACs), in contrast with BF inputs (Mazo *et al.*, 2022). These cells, which display broad odor-evoked effects, seem to be involved in fine odor discrimination, but their precise role still needs to be explored. It is also interesting to note that these fibers, unlike their excitatory counterparts, project only to the ipsilateral OB. These insights provide a particular interest to the study of inhibitory top-down fibers projecting to the OB, which have in the AON been greatly overlooked. Moreover, GABAergic long-range projections are now found in a greater number of systems (Caputi *et al.*, 2013a), which gives relevance to attempts to understand their function.

### *iv. Other regions projecting to the olfactory bulb*

Another region sending consequent projections to the OB is the nLOT, which interestingly also projects contralaterally. However, only a few studies looked upon its function and the results do not give satisfying conclusions (Vaz *et al.*, 2017). Data suggest that the nLOT could encode the valence of the stimulus in a go/no-go task (Tanisumi *et al.*, 2021), but further investigation would help to understand the role of this nucleus.

Regions like the CoA, LEC and even hippocampus send direct projections to the OB, which are yet to be correctly characterized.

We can note that reciprocal connectivity is a feature that is encountered not only with respect to the OB, but throughout the olfactory path, creating a complex dynamical representation of odor dimensions (Courtiol and Wilson, 2017). This allows constant, spatio-temporal modulation of the olfactory message and results in the complex integration necessary for proper interpretation and behavioral responses.

Together, the feedforward message and the top-down tuning, whether excitatory, inhibitory or neuromodulatory, result in a complex message containing both external and internal information, passing through the bulb.

### c. Integrated roles of centrifugal fibers in the olfactory system

Several roles have been poised for centrifugal fibers, among which we find the generation of different oscillation rhythms and also for conveying internal information.

#### i. *Oscillations in the OB*

Brain oscillations are generated by rhythmic activity in neuronal circuits and can be studied with LFP recordings or EEG. Oscillations range in frequency, with defined bandwidths corresponding to various processes in the brain.

In the OB, sensory stimulation is initially paced by the rhythmic flow imposed by respiratory cycles, which give rise to an initial range of slow oscillations (Theta; 1-12Hz). The mechanism underlying their generation can be complex and, at least, partially centrifugally driven, given that a certain patterning of MC firing with respiration remains when the nasal epithelium is bypassed by tracheal breathing (Ravel and Pager, 1990).

However, the most studied rhythms in the OB are gamma oscillations (from 40 to 100 Hz). They are evoked by sensory stimulation and coupled to a specific phase of the theta rhythm, especially at the transition between inspiration and expiration (Manabe and Mori, 2013). They emerge from the synchronous firing of M/TCs and rely on the activity of reciprocal dendro-

dendritic synapses between M/TCs and GCs (Lagier *et al.*, 2007). Gamma oscillations are also found in the PC where they rely on the interaction between principal neurons and interneurons (Eeckman and Freeman, 1990; Litaudon, Garcia and Buonviso, 2008).

Most studies converge to the fact that gamma oscillations are necessary for the coding of information in the brain. They were first described in the olfactory system (Bressler and Freeman, 1980) and are now widely studied in various cognitive processes. Several models are hypothesized for the generation of gamma oscillations, all of which require GABA<sub>A</sub>-mediated inhibition (reviewed in Buzsáki and Wang, 2012). Findings indeed suggest the gamma rhythm in LFP recordings are allowed by IPSPs originating from local interneurons that synchronize the spiking of principal cells. In the OB, gamma oscillations are necessary for correct odor discrimination in a GABA<sub>A</sub>-dependent manner and rely on the activity of reciprocal dendrodendritic synapse between MCs and GCs (Lepousez and Lledo, 2013a), and their role in these processes was recently demonstrated in the human olfactory cortex (Yang *et al.*, 2022). There is also evidence that long-range modulation can trigger gamma-range synchrony across various brain regions (Buzsáki and Wang, 2012), especially involved in interhemispheric synchronization, which could answer questions about the role of contralateral connections emerging from the AON and nLOT.

Other ranges of oscillation have been found useful in olfactory processing, with Beta oscillations (15-40Hz) also playing a role in odor discrimination (Kay and Beshel, 2010; Kay, 2014). Different rhythms could act in synergy in odor sampling contexts, with succession of fast, slow gamma, and beta rhythms allowing different information processing to occur (Frederick *et al.*, 2016). It has been proposed that gamma rhythms result from local neuronal interactions in both the OB and PC, triggered by sensory stimulation, while beta oscillations are generated by distal relationships, especially centrifugal information from the PC, or even Hippocampus (Martin and Ravel, 2014), and are the ones modified after olfactory learning. Most remarkably, inhibitory centrifugal fibers from the AON have been demonstrated to increase beta synchronization in the OB (Mazo *et al.*, 2022).

Lastly, data showed that MCs and TCs differently synchronize along the gamma range (Burton and Urban, 2021), which could give another insight on the difference between these two projection cells.

Altogether, these data suggest a role for the complex OB circuitry and the various centrifugal projections, especially for long-range GABAergic fibers from the AON and HDB, in generating precisely timed activity patterns resulting in oscillation rhythms that promote activity and information coding. Hypotheses about their strong role in these processes are comforted by their conservation across species (Kay, 2015).

*ii. Centrifugal fibers and integration of internal state information*

The OB receives massive inputs from both neuromodulatory and various other non-neuromodulatory regions, such as various parts of the olfactory cortex, amygdala, and hippocampus. This suggests that a general role of these centrifugal connections could be the transmission of internal state information, such as hunger or fear, to the OB. It is quite easy to understand for neuromodulatory regions, since their function is directly linked to physiological state and its broad influence on brain function: Norepinephrine-secreting neurons are activated by arousal (Poe *et al.*, 2020), serotonin levels are tuned by anxiety or sleep (Berger, Gray and Roth, 2009), and acetylcholine is involved in attention processes, among others. Thus, these centrifugal controls are highly dependent on the individual's internal state, as has already been shown in the visual system (Polack, Friedman and Golshani, 2013).

For non-neuromodulatory fibers, what hints toward their ability to tune OB activity according to internal information is the fact that they all are contacted by various regions of the brain that are crucial in responding to this processing. For example, the hypothalamus is greatly modulated by various information coming from blood factors, and projects to the PC, the AON, and directly to the OB.

The first insights on the internal modulation of top-down projections were provided by recordings of AON and PC fibers in awake and anesthetized mice, which displayed enhanced activity in the OB during wakefulness (Rothermel and Wachowiak, 2014; Boyd *et al.*, 2015). As a result, the activity of GCs is increased in awake mice, resulting in sparser, more time-specific responses of M/TCs (Kato *et al.*, 2012). In social contexts, data show that oxytocin-secreting hypothalamic projections to the AON enhance the signal-to-noise ratio through top-down projections and allow social recognition (Oettl *et al.*, 2016).

Finally, these dop-down fibers are also controlled by neuromodulators as a result of internal states such as hunger. Indeed, fasting induces an increase in the production of endocannabinoids, which are known to bind to presynaptic CB1 receptors to dampen neurotransmitter release (Iremonger, Wamsteeker Cusulin and Bains, 2013). It was shown that deletion of CB1 receptors at cortico-bulbar glutamatergic axons is sufficient to suppress fasting-induced hyperphagia in mice by lowering olfactory detection thresholds, thereby enhancing odor detection (Soria-Gómez *et al.*, 2014). Thus, hunger appears to influence olfactory processing in the OB through modulation of centrifugal transmission. At the local network level, CB1Rs are present at both GC- and dSAC-contacting synapses and have been demonstrated to be capable of bidirectional modulation of M/TC activity through inhibition or disinhibition (Pouille and Schoppa, 2018).

A last kind of internal information that is hypothesized to be transmitted through top-down projections is the prediction of the ascending message. Depending on contextual cues and previous experiences, top-down inputs could modulate the entering message by probabilistic predictions (Rauss and Pourtois, 2013; Nave *et al.*, 2020). These mechanisms help explain visual illusions (O'Callaghan *et al.*, 2016) and speech prediction (Cope *et al.*, 2017; Asilador and Llano, 2021) in humans. In olfaction, only *in silico* models have for now deciphered a top-down role in predictive coding, emphasizing the role of cortico-bulbar projections in context-dependent olfactory processing (Adams *et al.*, 2019).

Centrifugal projections are a key component of odor processing by modulating various steps of the ascending olfactory message. They allow the OB to act as a coincidence detector between external stimulation coming from the MOE and the internal state, encoding previous experiences or through other sensory inputs coming from cortical areas. However, a last, but not least, features of the OB give rise to even more complex processing, namely the integration of new neurons throughout the whole life of individuals as a result of neurogenesis.

## 2. Bringing new cells in the circuit: neurogenesis in the olfactory bulb

As with most of the central nervous system, the olfactory regions, and particularly the OB, originate from the differentiation of ectodermic cells during embryonic development. As a result of genetically encoded developmental programs, the OB emerges at the most anterior part of the telencephalon due to the carefully balanced expression of various signaling molecules, such as FGF, BMP, Wnt, SHH, and differentiation is triggered by the arrival of pioneering OSN axons, limiting cell proliferation (Hébert *et al.*, 2003; Hirata *et al.*, 2006). Early development of OB circuits, as well as neuronal and axonal maturation, have been extensively reviewed and will not be further discussed here (Sakano, 2020; Tufo *et al.*, 2022).

Focusing on interneurons (PGCs, SACs and GCs, mainly), we now know that most of them originate from ventral telencephalon, particularly the SubVentricular Zone (SVZ) during embryonic development and the first neonatal weeks (Batista-Brito *et al.*, 2008).

However, for most of the 20<sup>th</sup> century, neuroscientists thought that once the brain was fully developed, no new neurons were produced. Cajal stated, in 1928, that *“In the adult centers, the neural paths are something fixed and immutable: everything may die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree”*. Despite early studies showing incorporation of tritiated thymidine in brain cells, first in hippocampus, then in the OB (Altman, 1962, 1969; Altman and Das, 1965), this phenomenon was only confirmed later thanks to development of new techniques such as bromodeoxyuridine (BrdU) staining and electron microscopy (Kuhn, Dickinson-Anson and Gage, 1996).

Further evidence accumulated confirming that a reduced number of brain regions were capable of proliferation and neuronal differentiation during adulthood. Today, proliferation of new neurons has been proven in rodents to occur at least in the dentate gyrus of the hippocampus, the SVZ, and more recently the hypothalamus (Bartkowska *et al.*, 2023), with hypotheses about possible adult neurogenesis in prefrontal cortex, striatum and amygdala (Jurkowski *et al.*, 2020). This generation of new neurons however decreases with age, due partially to the exhaustion of stem cells (Kuhn, Dickinson-Anson and Gage, 1996; Enwere *et al.*, 2004; Katsimparidi and Lledo, 2018). For this study, we will mainly focus on neurogenesis in the olfactory system (Figure VIA).

#### a. Generating new cells in the adult life

The production of new neurons during adult life happens, in rodents, in neurogenic niches present in the SVZ and the dentate gyrus, although some other brain regions display putative neurogenic activity (Feliciano, Bordey and Bonfanti, 2015; Jurkowski *et al.*, 2020). A common characteristic of these niches is the presence of Neural Stem Cells (NSCs) in a particular environment allowing quiescence and proliferation of these cells.

In the SVZ, located on the border of the lateral ventricles, ependymal cells are in direct contact with the cerebrospinal fluid (CSF). Lining these ventricles, the actual stem cells, called the type-B cells, are radial glia-derived cells presenting astrocytic characteristics (Figure VIII B), such as the expression of the marker GFAP (Doetsch *et al.*, 1999), which was also shown to be expressed in the SubGranular Zone (SGZ) of the DG (Garcia *et al.*, 2004).

B-cells can either remain quiescent or become activated (Codega *et al.*, 2014). Once activated, they slowly divide symmetrically but via two different ways: roughly 20% of the divisions result in self-renewal of the B cells, while 80% give rise to C-type cells, which are fast-dividing neural progenitors that provide amplification of the progenitor pool (Ponti *et al.*, 2013; Obernier *et al.*, 2018). After a few divisions, these cells will themselves become A-type cells, which are migrating neuronal progenitors that continuously travel through the SVZ, at the center of B/C-cells bordered channels (Thomas, Gates and Steindler, 1996; Doetsch, García-Verdugo and Alvarez-Buylla, 1997).

B-cells interestingly show characteristics of ependymal cells, with a single cilium extending through the ventricle wall to contact the CSF and are also in contact with blood vessels. Thus, their neurogenic activity can be controlled by humoral factors coming from the blood or the CSF, as well as various neuronal afferences originating from the VTA, striatum or hypothalamus. Precise regulation of adult neurogenesis in the SVZ has been extensively studied and reviewed (Lim and Alvarez-Buylla, 2016; Katsimpardi and Lledo, 2018; Obernier and Alvarez-Buylla, 2019). It is also important to note that regional heterogeneity in the SVZ

recapitulates stem-cell diversity, which is linked to the subtypes of interneurons generated in the OB (Merkle, Mirzadeh and Alvarez-Buylla, 2007; Young *et al.*, 2007; Merkle *et al.*, 2014).

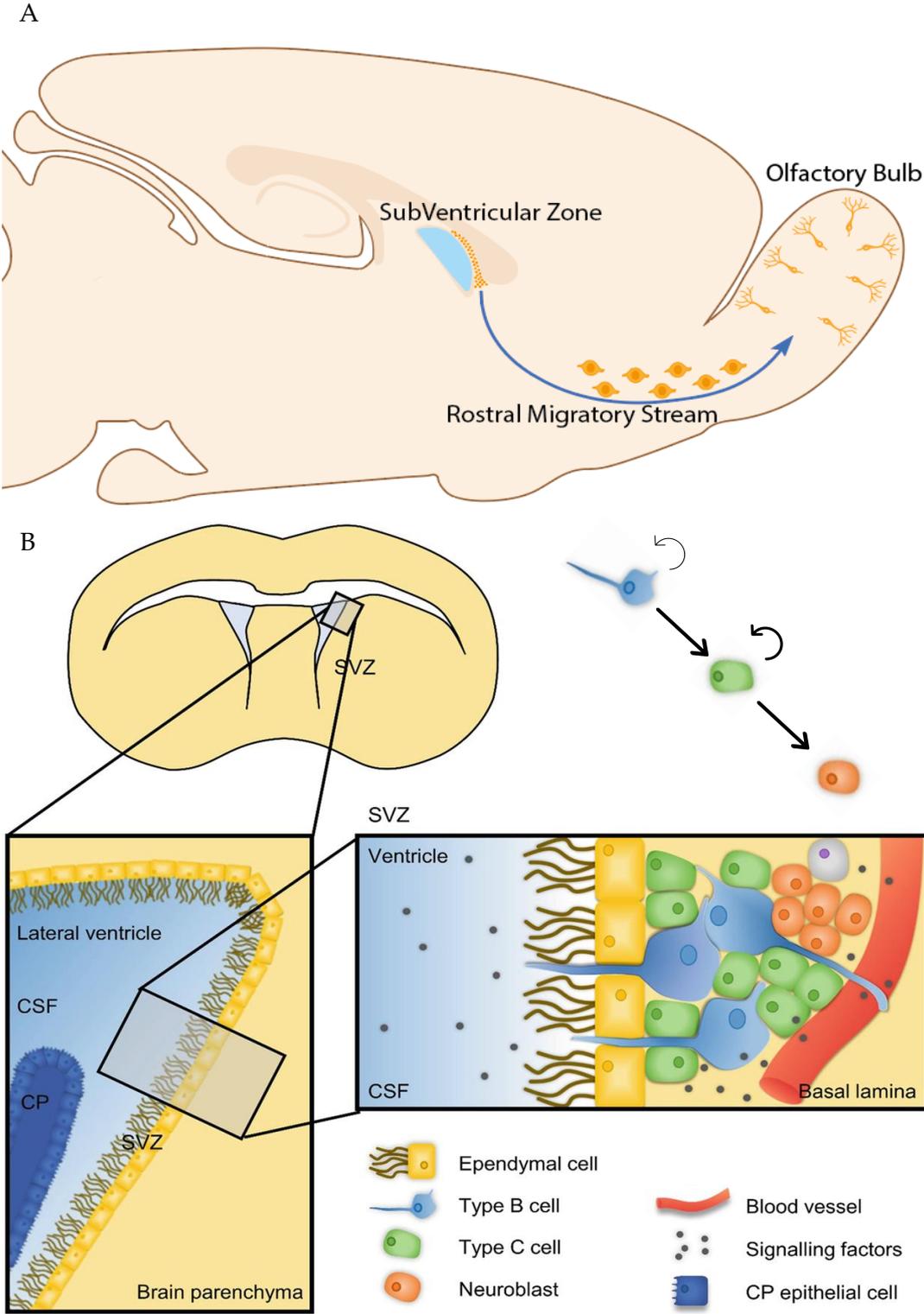


Figure VII. Generation of new neurons in the Subventricular zone. A. General overview of neurogenesis in the olfactory system. Neural progenitors are generated in the Subventricular Zone, proliferate and produce neuroblasts migrating through the Rostral Migratory Stream, to finally reach the Olfactory bulb where they differentiate into Granule and PeriGlomerular Cells and integrate the bulbar network. B. Schematic representation of the adult subventricular zone (SVZ) neurogenic niche. SVZ lines the lateral walls of the lateral ventricles and is comprised of three main cell types: the multipotent type B NSCs that give rise to type C cells (fast dividing transient amplifying cells) that, in turn, generate type A neuroblasts. Type B cells interact basally with blood vessels and apically with the cerebrospinal fluid (CSF). The composition of the CSF is modified by the choroid plexus, a thin vascularized membrane mainly composed by epithelial cells, which secretes several cytokines and trophic factors to the CSF. Adapted from Filipa F. Ribeiro & Sara Xapelli, 2021

Thus, this zone of proliferation and generation of neural progenitors shows complex functioning and strong regulation, giving birth to most of the cells that will then migrate towards the OB.

#### b. Migration of the neural progenitors in the rostral migratory stream

Once the neural progenitors are generated in the SVZ, they need to migrate to the OB, which is a distance greater than 5 mm anterior in mice, before differentiating into interneurons and integrating the network. This phenomenon has been demonstrated in early neurogenesis work (Lois and Alvarez-Buylla, 1994) and has been later elegantly confirmed with time-lapse recordings of migrating neuroblasts in acute slices (Bakhshetyan and Saghatelyan, 2015).

Two kinds of migration occur successively. First, neuroblasts migrate tangentially (parallel to the surface of the brain) to reach the OB via the Rostral Migratory Stream (RMS). Then, once at the center of the bulb, they migrate radially to integrate at the proper spot required to accomplish their function.

##### *i. Tangential migration to the olfactory bulb*

Along the RMS, neuroblasts present a bipolar morphology and travel at a rate between 40 and 80  $\mu\text{m}/\text{h}$  (Davenne *et al.*, 2005). Thus, it takes them 2 to 7 days to cover the 5 mm distance from the SVZ to the OB, passing by several brain regions such as the striatum and even through the

AON. Neuroblasts are linked together by N-CAM adhesion protein and form small chains of migrating cells (Rousselot, Lois and Alvarez-Buylla, 1995).

As seen in the SVZ, neuroblasts migrate while surrounded by an astrocytic sheath that seem to act as a migration-inducing indicator and provides guidance cues (Mason, Ito and Corfas, 2001). The control of migration speed is dependent on the ambient GABA concentration, which is balanced by both GABA release by the neuroblasts and GABA uptake by ensheathing astrocytes (Bolteus and Bordey, 2004).

As with all cell migration processes, this migration is controlled by numerous factors such as cytoskeleton regulating proteins, extracellular matrix, and guidance molecules, whether attractive or repulsive (Lalli, 2014; Bressan and Saghatelian, 2021). Thus, tangential migration is a carefully controlled process that results in the arrival of neuroblasts at the center of the MOB.

*ii. Radial migration: centripetal travel into the olfactory bulb network*

The end of tangential migration is controlled by both extrinsic and intrinsic factors, first inducing the dissociation of the migration chains. For example, the matrix protein Tenascin-R initiates the detachment of migrating cells, thus stopping their movement and triggering radial migration (Saghatelian *et al.*, 2004). In the meantime, a switch in neuroblast genetic expression occurs, and molecules involved in cell-cell attachment like Sphingosine 1-Phosphate Receptor 1 are down-regulated, easing the transition to a more solitary journey (Alfonso *et al.*, 2015). Several other molecular cues such as Reelin expression regulate the transition between the two forms of migrations (Hack *et al.*, 2002), which in the end lead to the positioning of neuroblasts into their final place.

Most neuroblasts stop their radial migration in the GCL (95%), while a some GCs continue migrating to reach the GL and differentiate into PG cells (5%) (Luskin, 1993). Other subtypes of interneurons can be generated through these mechanisms, but their number remains anecdotic (Brill *et al.*, 2009). However, they need to become functional neurons to be fully integrated into the OB network.

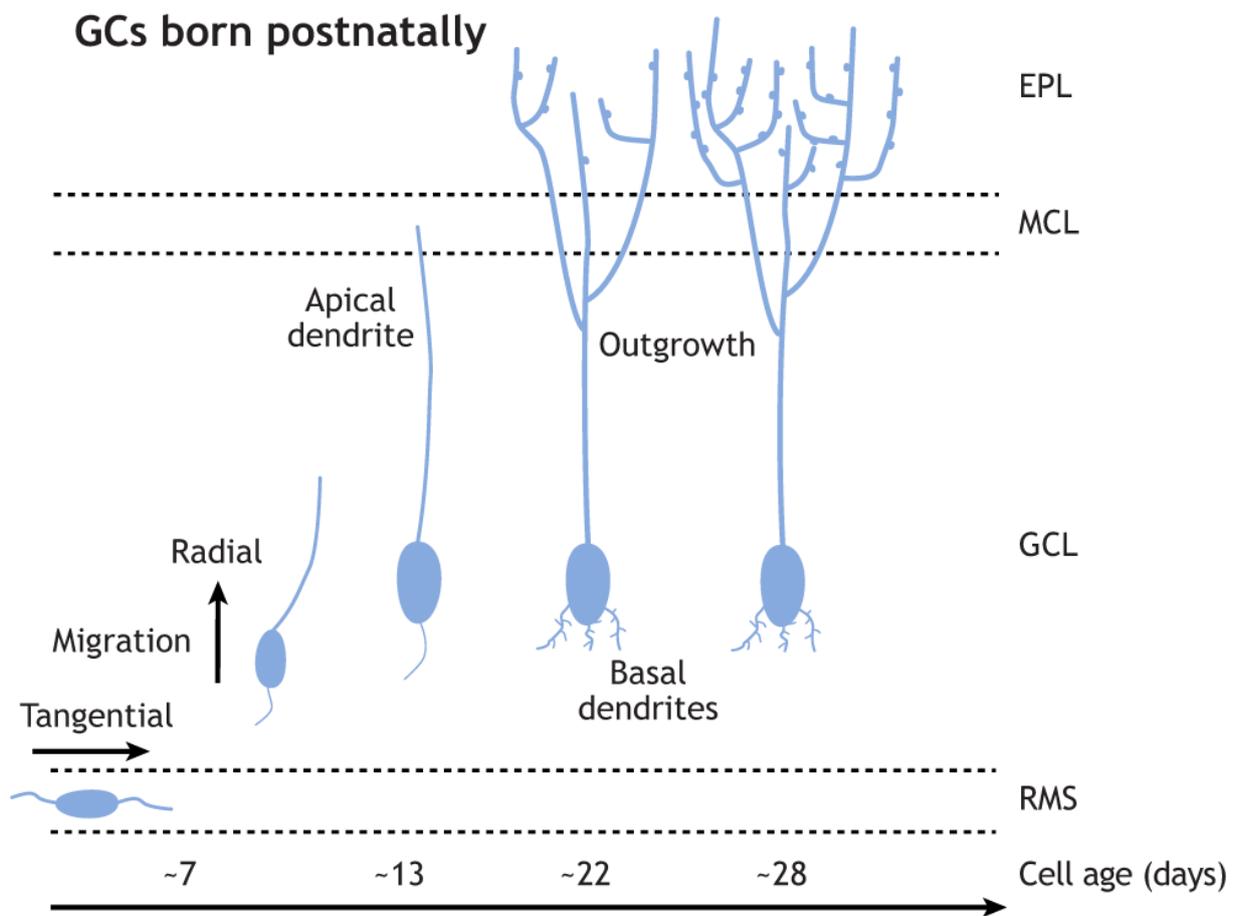


Figure VIII. Morphological maturation of granule cells (GCs) generated postnatally. After migrating tangentially in the RMS, immature neurons migrate radially into the GCL, before extending an apical dendrite to the mitral cell layer (MCL). Over the next ~2 weeks, the apical dendrites extend further into the external plexiform layer (EPL), branch extensively and gain spines. Smaller basal dendrites also develop over this time period. From Tufo *et al.*, 2022.

### c. Differentiation and integration of the adult-born neurons

After migration, neuroblasts that will become GCs settle earlier than those differentiating into PGCs because of the shorter distance they need to travel to their final location. Moreover, future PGCs present a second switch from radial to lateral migration in the GL (Liang *et al.*, 2016). We will mainly focus on the development of newborn GCs.

Several stages of maturing GCs have been morphologically described by early studies (Figure IX). Starting at around day 7, during radial migration, determined progenitors start to develop an unbranched apical process toward the external layers of the OB, while a small basal one faces the opposite direction. From day 9 to 13, the apical process elongates into a prominent dendrite through the MCL, which will keep growing and start branching to reach the EPL between days 11 and 22 (Petreanu and Alvarez-Buylla, 2002).

Dendritic spines in the EPL develop from day 14 (Whitman and Greer, 2007) and their stability was demonstrated to be activity dependent, and this activity also controls the production of new neurons and the complexity of their apical dendrite branching (Saghatelian *et al.*, 2005). The location of mature abGCs is also regulated by the activity of projection neurons in the OB through the activity-dependent release of glutamate and BDNF and has consequences on MC response synchronization (Breton-Provencher *et al.*, 2016).

Along this morphological maturation, new GCs also display functional, electrophysiological changes that are necessary for them to become fully integrated into the OB network (Figure X). As we already know, during the tangential migration, GCs start to express GABA<sub>A</sub> receptors that are crucial for the control of their migration. Then, GCs start to express AMPARs, and only during the radial migration do they express NMDARs (Carleton *et al.*, 2003). In their late development, when their dendrite arborization is fully extended, newly generated GCs display voltage-dependent Na<sup>+</sup> currents sufficient to trigger action potentials. At this point, they can respond to olfactory nerve stimulation (Belluzzi *et al.*, 2003) and are well activated by physiological odor presentation (Carlén *et al.*, 2002; Dietz, Markopoulos and Murthy, 2011) Moreover, abGCs display the ability to develop glutamatergic long-term potentiation during a short period of time, which is not observed on pre-existing ones (Nissant *et al.*, 2009) and could hint at a unique role of these abGCs in olfaction (Figure XB).

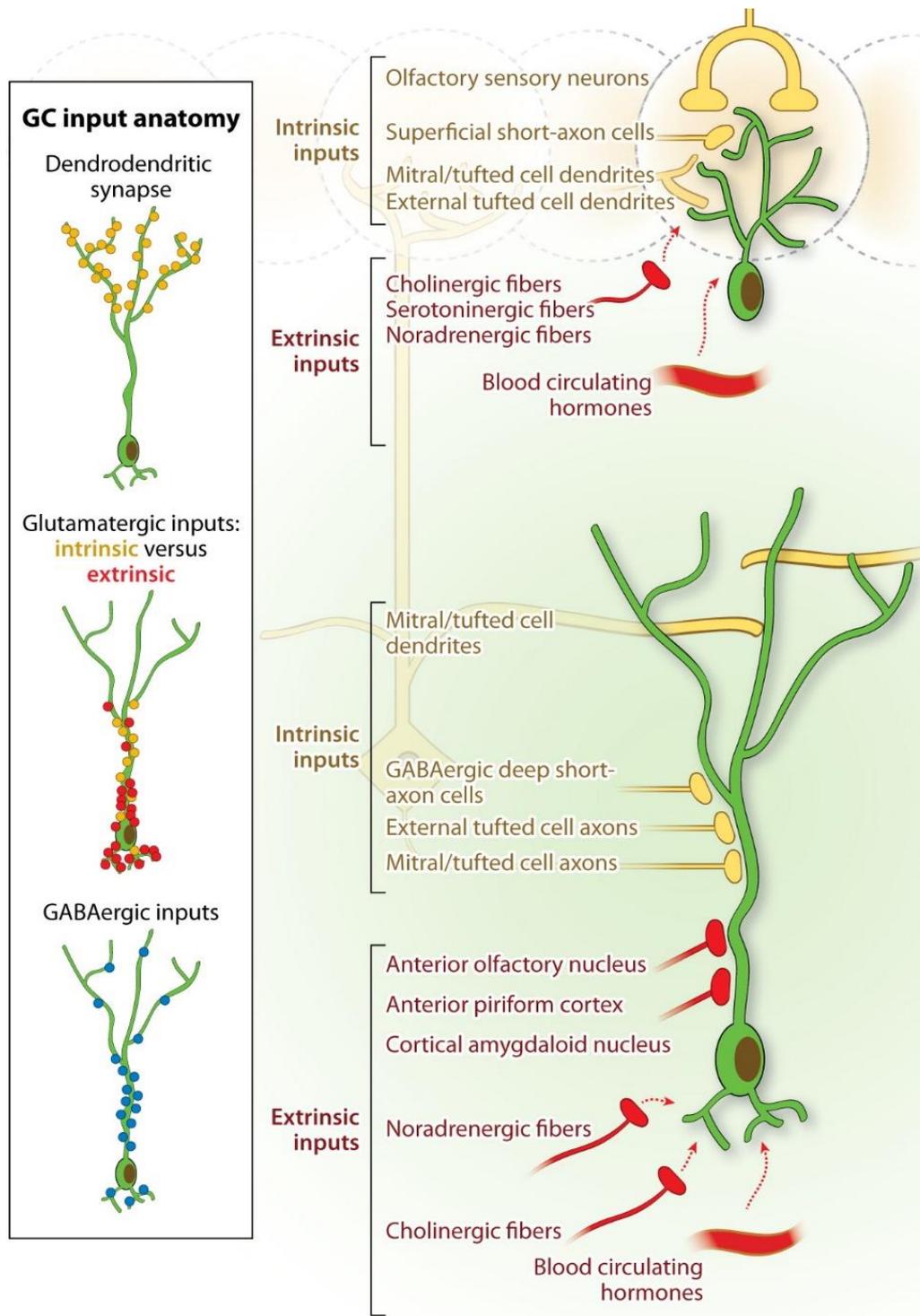


Figure IX. Connectivity of adult-born Granule Cells (abGCs). (Left) abGC input anatomy. Dendrodendritic inputs are restricted to the apical part of GC dendritic tree. Glutamatergic and GABAergic axodendritic inputs are restricted mainly to the proximal and basal parts. Most GABAergic inputs onto GCs are derived from deep short-axon cells. (Right) Adult-born periglomerular cells (PGCs) and GCs are targeted and influenced by different intrinsic neurons of the olfactory bulb network (intrinsic inputs; yellow), by top-down fibers originating from different distant brain regions, and by blood-circulating hormones (extrinsic inputs; red). Adapted from Lepousez, 2013.

Interestingly, before the apical arborescence is functional, new GCs already receive inputs from centrifugal projections that are necessary for their survival and maintenance in the OB (Hanson, Swanson and Arenkiel, 2020). This gives insight on the crucial role of cortico-bulbar inputs on OB neurogenesis. Once the GC dendrites mature, synapses start to form onto them, with relative regional differences: in abGCs post-synaptic excitatory loci first emerge on the proximal segment of the apical dendrite, and later onto both the basal dendrite and the distal part of the apical dendrite. Reciprocal synapses, located on the distal segment, start to develop quickly after dendrites reach the EPL (Kelsch, Lin and Lois, 2008) (Panzanelli *et al.*, 2009) and seem to reach full functional capacity to inhibit MCs 5 to 7 weeks after generation (Bardy *et al.*, 2010). Centrifugal inputs from the AON and PC, on the other hand, develop barely more than 2 weeks after the birth of the new cells (Deshpande *et al.*, 2013).

Functional inhibitory synapses are exhibited by abGCs as soon as their apical dendrite starts to extend in the GCL and they outnumber glutamatergic ones at early stages (Panzanelli *et al.*, 2009). This may be explained by the fact that GABAergic signaling is necessary for the correct development of adult-born neurons and drive their dendrite and spine formation (Pallotto *et al.*, 2012; Deprez *et al.*, 2015). Later, excitatory synapses progressively outnumber inhibitory ones.

Despite a great number of neurons being integrated in the OB every day, their population quickly decreases by 30 to 50% at between 15 and 50 days after their generation in the SVZ (Petreanu and Alvarez-Buylla, 2002; Winner *et al.*, 2002), although some contradictory data has been recently published (Platel *et al.*, 2019), suggesting that the observed reduction may be due to BrdU toxicity. Nonetheless, abGC survival is strongly reduced by sensory deprivation, meaning that odor-evoked activity leads to maintenance of at least part of the abGCs (Figure XC) (Saghatelian *et al.*, 2005; Yamaguchi and Mori, 2005; Sawada *et al.*, 2011). Conversely, increased odor stimulation seems to favor generation of adult-born progenitors (Rochefort *et al.*, 2002; Alonso *et al.*, 2008; Bonzano *et al.*, 2014), leading to a general conclusion that the whole process of OB neurogenesis, from proliferation of stem cells to the survival of newly integrated cells, is strongly linked to olfactory activity.

As we will discuss in chapter 3, a strong link between survival and different forms of olfactory learning have been demonstrated.

Overall, the survival of neo-neurons in adult rodents is therefore a highly regulated process (Petreanu and Alvarez-Buylla, 2002; Saghatelian *et al.*, 2005; Lledo, Alonso and Grubb, 2006; Sawada *et al.*, 2011; Lepousez, Nissant and Lledo, 2015).

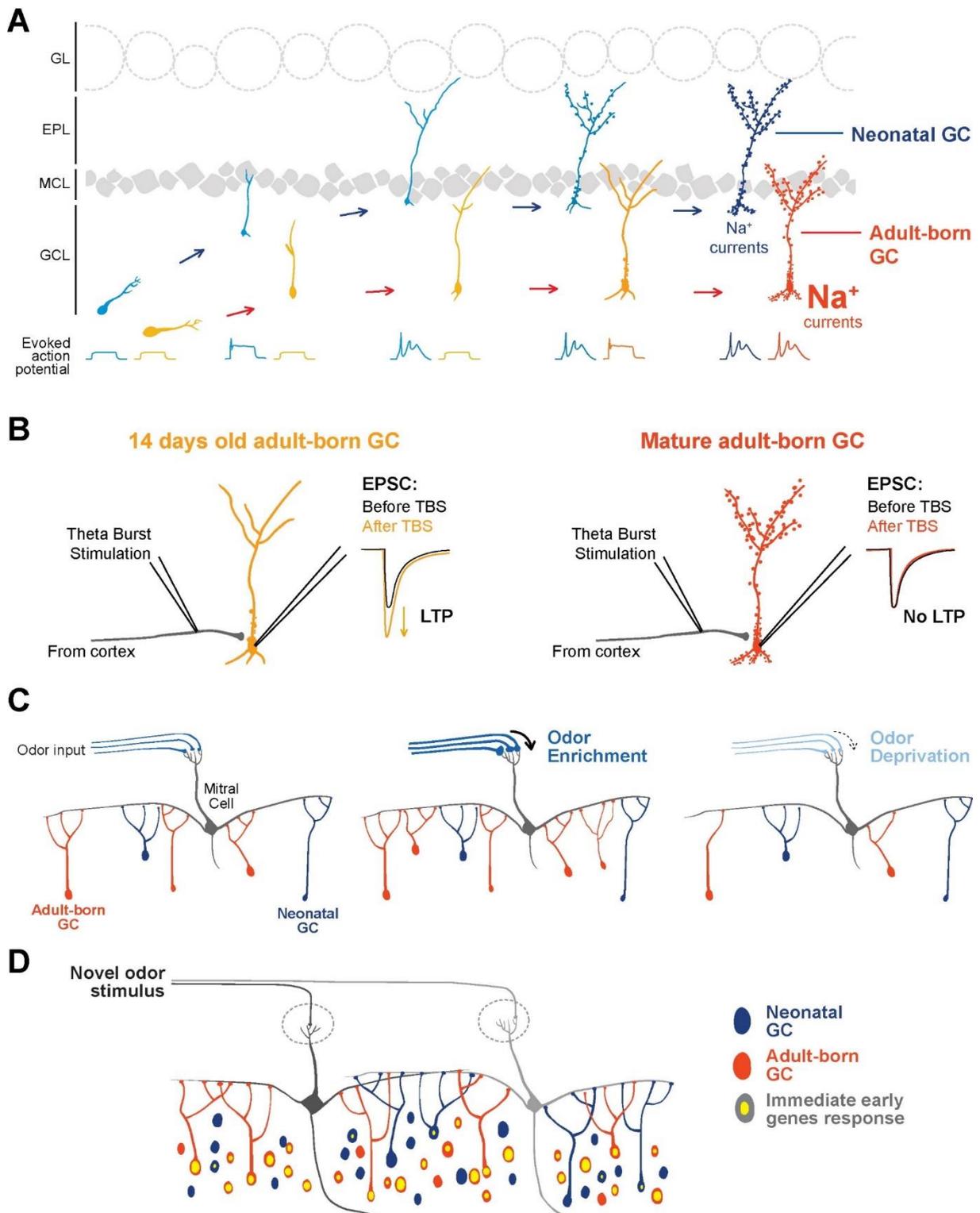


Figure X. Distinct maturation and function of postnatal and adult-born granule cells in the OB. A. Model of the maturation of neonatal versus adult-born granule cells. The different shading of red (neonatal) and blue (adult-born) represent the granule cells at their different stage of maturation. Adult-born cells exhibit differences in location, timing of synaptic input formation and sodium channel properties. Adult-born cells tend to have their soma located in the deeper layer of the OB whereas the neonatal cells are placed in the superficial layer (closer to the mitral cell layer). As a result, their dendritic arborization is reaching different part of the external plexiform layer (EPL). In contrast to neonatal granule cells, interneurons generated during adulthood receive synaptic input before forming synaptic output with mitral cell dendrites. In addition, adult-born granule cells fire action potentials at later maturational stages and tend to have stronger sodium currents when they are fully mature. B. Shortly after arriving at the adult OB (14 days), theta burst stimulation (TBS) induces long-term potentiation (LTP) at the glutamatergic synapses between cortical projections and adult-born GCs. This feature is lost with maturation of adult-born GCs. C. Effect of odor enrichment and deprivation on adult-born cells survival and morphology. After odor enrichment there is an increase in the number of adult-born cells surviving in the OB. Odor deprivation on the other hand decreases the survival and number of spines of the adult-born GCs. The pre-existing population of GCs remains intact. D. Greater number of adult-born cells respond to novel odors, as assessed by the expression of immediate early genes, compared to the pre-existing population of interneurons. GL: glomeruli; EPL: external plexiform layer; MCL: mitral cell layer; GCL: granule cell layer. From Breton-Provencher et al, 2012

We now know that functional GCs are continuously integrated to the OB circuits, where they contact a wide variety of both projecting cells and interneurons (Bardy *et al.*, 2010), with a much higher proportion of M/TCs displaying IPSCs after abGCs stimulation compared to interneurons. However, this does not yet explain the fundamental role they play in olfaction.

Intriguingly, neurogenesis also gives rise to glutamatergic sSACs in the GL, which project to neighboring glomeruli (Brill *et al.*, 2009).

#### d. Functional role of adult-born granule cells in the olfactory bulb

To understand the function of abGCs, we must first determine whether they display different morphological or physiological properties compared to neonatal GCs (nnGCs). It is worth noting that abGCs represent 40 to 60% of the total GC population a year after labeling SVZ progenitors (Imayoshi *et al.*, 2008). This study also showed that ablation of the abGC population caused loss of integrity of the OB network. Thus, adult neurogenesis is predominant in the OB and is hypothesized to help the replacement of old GCs, contributing to homeostasis of the network throughout life. However, this idea is now challenged as neurogenesis in the OB could be seen as a simple addition mechanism, supported by the observation that OB size increase throughout life (Platel *et al.*, 2019), or a balance of both hypotheses. It has also been

shown that abGCs mainly occupy a deeper part of the GCL compared to nnGCs (Figure XA) (Lemasson *et al.*, 2005), which may influence their effect on sensory message tuning since the length and branching of the apical dendrite does not differ between deep and superficial GCs. This could underly the distinct processing differences of TCs (superficial) and MCs (deep) in the EPL (Orona, Scott and Rainer, 1983).

Furthermore, electrophysiological differences exist between abGCs and nnGCs. abGCs acquire their ability to generate action potentials later than nnGCs but display greater excitability once they are fully mature (Carleton *et al.*, 2003). They also show an increase in voltage-dependent GABA release upon sensory deprivation-induced synaptic loss, which was not observed on nnGCs (Saghatelian *et al.*, 2005). This reliably shows adaptation abilities specific to newborn GCs. Finally, selective depletion of abGCs impaired the generation of oscillations in the OB network, which has been proven crucial for correct odor perception (Breton-Provencher *et al.*, 2009).

All these morphological and functional distinctions have been reviewed in (Breton-Provencher and Saghatelian, 2012). Finally, our team demonstrated that mature adult-born neurons express unique features in their synaptic output that allow them to avoid GABA<sub>B</sub> mediated auto-inhibition (Valley *et al.*, 2013a). It has also recently been demonstrated that abGCs display high flexibility of their apical dendrites synapses, however the dynamics were not different from nnGCs (Sailor *et al.*, 2016).

Interestingly, fully mature abGCs are less reactive to odors than their neonatal counterparts, suggesting that sensitivity depends on GC maturation level and the context of the odor stimulation (Magavi *et al.*, 2005; Grelat *et al.*, 2018).

Moreover, immature abGCs exhibit higher activation when animals are presented with new odors, accompanied with greater immediate early gene expression, suggesting that these cells undergo different experience-dependent modifications than nnGCs (Figure XD) (Magavi *et al.*, 2005). This indicates a dialogue between olfactory perception and abGCs that are a putative substrate for creating new olfactory information channels directly into the OB. Most behavioral effects that have been put in light so far involve learning mechanisms, which will be the main focus of our next chapter.

How can these functional differences between the two GC populations be interpreted? Differential functional roles of adult and neonatal-born GCs might be shaped by distinct top-down feedback from higher order brain areas. Moreover, this connectivity may change because of odor experience. We also need to keep in mind that although nnGCs and abGCs have different positioning, odor responses and electrophysiological properties, a third population is constituted by immature abGCs who exhibit their own properties and are more prone to display plasticity (Gao and Strowbridge, 2009; Nissant *et al.*, 2009). Thus, the heterogeneity of this population raises questions on their differential implication in olfactory behaviors and learning mechanisms.

In this regard, we need to emphasize the fact that neurogenesis, taken alone, can be considered as an extreme form of network plasticity. Adult-born neurons integrate various high-order attributes and behavioral states into the olfactory representation and thus contribute to olfactory-driven behaviors. Thus, it seems only logical that this subpopulation of GCs is the best candidate for experience-induced long-term changes in the OB. We will consequently focus on learning mechanisms at various scales and their close interactions with all the olfactory system features that we have already discussed.

## Chapter III. At the crossroads: Olfactory learning

### 1. Behavioral responses to odors

In the last two chapters, we have determined that an odorant molecule, recognized by various specific receptors in the main olfactory epithelium, triggers a GPCR-mediated transduction generating an electric message transmitted to the main OB glomeruli. Then, after consequent integration both at the bulb microcircuit level (mainly in the GL and the GCL) and by cortical afferents, M/TCs transmit this odor-evoked message to cortical areas allowing various interpretation such as odor identification, discrimination in the case of multi-odor stimulus, and of course behavioral response, the inevitable conclusion to most sensory perception. This response to odors and how it could be shaped by experience will be the focus of this chapter.

#### a. Spontaneous responses to odor stimuli

Most of the behavioral responses to odors, like other sensory clues, are the consequence of previous experiences dictating how to react to a given stimulus. Nonetheless, in olfaction, we can observe the existence of innate, spontaneous responses to odor detection.

Indeed, it is easy to demonstrate that domestic mice, bred in a perfectly controlled environment, display strong behavioral responses to natural predator-associated odors such as TriMethylThiazoline (TMT) which is found in fox urine. Detection of TMT by a laboratory-bred mouse will result in fast immobilization, known as freezing behavior, a response that the mouse has never learnt in the stimulus-poor environment it has always wandered (Fendt, Endres and Apfelbach, 2003; Fendt *et al.*, 2005).

This type of innate response can mostly be observed for value-associated odors, with both innate attractiveness for food-related (peanut butter) or opposite-sex conspecific urine and innate aversiveness for predator- (TMT, TMA) and death-related odors (various volatile amines like putrescine and cadaverine). Interestingly, a class of OR seems to be specialized in

detecting amine-containing odorants and is activated in innate avoidance behavior in zebrafish (Hussain *et al.*, 2013) and mice (Pacifico *et al.*, 2012).

The odors associated with innate values present a specific representation in the OB: innately aversive odors activate glomeruli predominantly in the dorsal OB, which have been shown to be necessary for triggering avoidance behavior (Kobayakawa *et al.*, 2007; Cho *et al.*, 2011). Moreover, a single receptor is sufficient to induce freezing when its related glomerulus is activated, while its ablation robustly dampens the innate response to TMT (Saito *et al.*, 2017).

In the ventral OB, aversive odors evoked activity in the posteroventral regions, while appetitive odors exhibit a more anterior glomerular activation map, thus suggesting the existence of innate value topography directly in the OB (Kermen *et al.*, 2016). This gives rise to another role of the OB, the partial encoding of innate associations, which is supported by the direct connections to the cortical amygdala, a region known for encoding emotions.

Direct MC inputs to various amygdala nuclei enable the innate behavior responses without a need for identification of the odor or even recall of previous experiences through cortical areas. These connections are a specialization of the olfactory system, making it unique in its ability to bypass cortical processing to directly target emotion centers for appropriate response. Particularly, MCs directly projecting to the CoA mediate innate aversive responses (Root *et al.*, 2014; Saito *et al.*, 2017) while projections to the Medial Amygdala (MeA) appear to be involved in appetitive social cues (Inokuchi *et al.*, 2017; Li *et al.*, 2017). Another nucleus of the amygdala, the Amygdala Piriform transition area (AmPir), has been shown to mediate a hormonal response subsequent to predator-related odors, linking olfactory perception to systemic effects for fight-or-flight behaviors (Kondoh *et al.*, 2016).

These innate odor responses, although flexible and wired during embryonic and early post-natal development (Qiu *et al.*, 2021), can be seen as evolutionary-encoded memories that are necessary for both the survival of the individual through predator avoidance, and that of the species with food-associated and social odor appetitive responses required for mating or breeding. However, they are not sufficient to adapt to novel olfactory environments and thus demand other mechanisms enabling the acquisition of new odor-meaning associations for each individual.

## b. Olfactory learning: emergence of new odor-induced responses

In contrast to spontaneous responses to odor stimuli, acquired or experience-dependent responses mostly rely on learning processes that will change the behavioral output subsequently to odor perception.

As defined in the dictionary, learning is “the ensemble of memorization processes implemented by an individual in order to elaborate or modify specific behavioral schemes under the influence of environment and experiences” (Larousse). This definition yields a striking idea: the final result of learning processes is to create new behavioral patterns, or to change those already existing, according to external cues. Thus, stable change of behavior in any animal in a given context is an indicator of learning processes. Of course, this assertion is not new and has been the foundation of a considerable number of studies aiming to decipher learning – and by extension memory – mechanisms in the brain (Thorndike, 1898; Kupfermann, 1975; Pavlov (1927), 2010).

For our study, we will keep the definition of learning as “the ensemble of molecular, cellular and network processes leading to stable changes in the behavioral response to a given set of stimuli”.

Although we will not give a full historic view of discoveries related to behavioral assessment of learning and memory, it is worth noting that the first successful attempts to decipher molecular substrates of learning were looking at habituation on the gill-retraction reflex in *Aplysia californica*, which showed that repetitive tactile stimuli inhibited an innate protection process in the aquatic mollusk, laying the basics of behavioral studies and understanding of neural mechanisms underlying these observations (Castellucci *et al.*, 1970). From there, numerous studies have discovered fundamental rules of learning at the behavioral, cellular, and molecular scales.

Refocusing our demonstration on olfaction, most work in this topic was performed on associative learning. Indeed, as odor detection gives insights about our environment components, it is mostly used to understand what constitutes this environment. Thus,

previously encountered odorants are often associated with either other sensory stimuli, emotions, or contextual memories. However, other types of learning have been demonstrated, which we will give a quick overview before looking deeper into mechanisms underlying these behaviors.

### *i. Habituation*

The simplest learning behavior that can be easily observed is the ability to repress a behavioral response such as a reflex after repetitive or prolonged stimulations in a short time window, which do not result in some type of benefit or detriment as was first described in the spinal cat (Spencer, Thompson and Neilson, 1966). It can be seen as a way for animals to get rid of non-relevant inputs and thus leave room for other, more salient, or relevant cues to be analyzed. This habituation process is usually only seen as a short-term type of learning since the end of the stimulus or a change in its intensity rapidly triggers back the repressed behavior (Wilson and Linster, 2008).

In olfaction, habituation can be intuitively understood, seeing as we easily stop perceiving the perfume we wear or even become quickly used to a bad ambient smell. In rodent, habituation behavior is assessed by measuring novelty-induced bradycardia (Best *et al.*, 2005) or the decrease in time spent exploring a new odor (Cleland *et al.*, 2002). Interestingly, the TMT-induced fear response does not decline after repeated exposure in rats, underlying a hard-wired mechanism of predator avoidance (Wallace and Rosen, 2000).

Earlier work showed that olfactory habituation was allowed by short- and long-term depression of MCs projecting to the APC in a mGluR dependent manner, resulting in a reduced representation of the odor in the cortex (Best and Wilson, 2004). Moreover, this habituation, according to the duration of the stimulus, can last between a few minutes and several hours, and even repress responses to odors similar to the habituated one in a phenomenon known as cross-habituation (McNamara *et al.*, 2008). Olfactory habituation has been reviewed in (Wilson, 2009).

## ii. *Association learning*

Most stimuli are initially neutral for animals, meaning they do not have either hedonic value (appetitive or aversive), nor evoke contextual-related experiences. In everyday life, associative learning plays a key role in several relevant animal behaviors including food detection, painful stimuli avoidance, etc. It simply consists of linking the perception of a stimulus, here an odorant, with an output that is – in experimental conditions – usually a reward (sucrose for example) or a punishment (a mild electric shock) but can simply be a particular place or another sensory stimulus. When associated to a precise behavior, this can result in conditioning, with a conditioned behavior occurring whenever a certain initially unrelated stimulus is presented, as demonstrated by Pavlov at the end of the 18<sup>th</sup> century (Domjan, 2005).

Numerous studies have been performed on various animals, from *Aplysia* (Walters, Carew and Kandel, 1981) to rodents, indicating a mechanism present in a great diversity of *taxa*. Indeed, associating sensory stimuli is crucial for the survival of an individual and its correct interaction with its environment.

Association learning, or conditioning, can be divided in two categories:

- Classical conditioning was described by Pavlov in a famous experiment with dogs where an unconditioned stimulus (US, food) was presented along a neutral conditioned stimulus (CS, a bell sound), which became sufficient to evoke salivation in the absence of food, in what was termed a “conditioned response” (Pavlov (1927), 2010). This Pavlovian conditioning primarily relies on reflex responses that are conditioned by environmental stimulation, independent of the individual.
- Theorized even before Pavlov’s work, the second type of conditioning describes association learning where the individual learns how its own behavior induces an environmental response. The first demonstration of this behavior was performed by Thorndike, who allowed cats to access food by rubbing against their cage, and was termed “instrumental conditioning” (Thorndike, 1898). Later, Skinner used pigeons and rats in positive and negative reinforcement conditioning and renamed the paradigm as “operant conditioning” (Epstein, Lanza and Skinner, 1980).

In olfaction, neutral odors can easily be associated with pleasant or unpleasant stimuli, triggering behaviors such as food-seeking and freezing even when the associated reward or

shock is not present. Furthermore, associating a meaning to an odor can modify its representation in the brain and ease discrimination between an ethologically-relevant odor and a neutral one, even if the odors have very close initial representations in the olfactory areas (Linster *et al.*, 2001). Associative learning is also a means to create new odor values and thus modulate preferences for certain molecules over others, with changing signals in the brain.

A common task used to assess associative behavior is the operant go/no-go task, where an individual is presented with various olfactory stimuli, only one stimulus or some that are rewarded (*i.e.*, sugar water). Individuals learn to respond with a “go” for the reward only when presented with the associated odors, which indicates that they correctly learned the association (Friedrich, 2006).

In the present study, olfactory learning was performed in a Go/No-go paradigm using automated olfactometers. These devices were used for olfactory learning for more than 20 years (Bodyak and Slotnick, 1999; Slotnick and Restrepo, 2005) for various association learning protocols. They allow automated, computer-controlled presentation of odors, with or without subsequent reward presentation. After mice have learnt that poking their snout into the odor port allows them to obtain a water-reward on the left waterspout, 2 odors are presented in a pseudo-random manner with only one of which rewarded. These devices enable the study of various aspect of olfactory perception and learning:

- Discrimination learning, which is the most intuitive aspect of this task. The difficulty of the task can be modulated by using more or less similar odor pairs, allowing the study of “easy” versus “difficult” discrimination. For finer discrimination, odor can be mixed in various proportions to increase the similarity of presented stimuli.
- Reversal learning, *i.e.*, the ability to reassign previously learnt values gives insights on the behavioral flexibility of the individual. Once the success criterion is reached, the existing rule is reversed (S+ becomes unrewarded, S- triggers the reward) and the number of trials necessary to overcome the old rule (from 0 to 50% performance) or to learn the new one (from 50 to 100%) can be determined.
- Detection threshold, in tasks where learnt odor concentrations are progressively lowered. This task allows determining detection thresholds and is a good readout of odor perception.

- Memory, where performance of a previously acquired association is assessed after a delay of 24 hours (short-term memory) to 30 days (long-term memory), usually without any reward.

Other psychometric parameters are also measurable thanks to these devices (Abraham *et al.*, 2004), such as the delay time mice take between odor presentation and odor port head removal (detection time), which could be interpreted as the time the animal needed to sample the stimuli before taking the behavioral decision. In addition, this device allows measuring the time between head removal and the first lick of the waterspout as an indicator of the motor skills required to perform the task or the confidence in the identified odor.

It is interesting to note that once mice have repeatedly performed this type of task, new associative learning of the same difficulty becomes easier, which gives insight into the type of learning, called rule learning (Barkai, 2014). Thus, these protocols, although relevant and adapted to study associative learning, involve other types of learning that can modulate the behavioral output and that consequently must be considered when interpreting the results.

### *iii. Perceptual learning*

Repeated passive exposure to perceptually close odors increases the ability to discriminate between them (Mandairon *et al.*, 2006; Fleming, Wright and Wilson, 2019). This process, called perceptual learning, has also been shown in other sensory modalities and in humans (Beste and Dinse, 2013).

We can consider that perceptual learning is an important part of most behavioral tasks aimed at associative learning, since these often require several hundreds of odor presentations (for operant conditioning) to reach a performance criterion. This makes it difficult to distinguish between the two processes in such behavioral paradigms. However, it seems that reward-associated learning and perceptual learning have the same effect on discrimination, both in discrimination improvement (Escanilla, Mandairon and Linster, 2008) and electrophysiological responses in the OB (Buonviso and Chaput, 1999).

Consequently, we need to keep in mind for further experiments that discrimination of odors is a combination of various types of learning rather than a specific transcription of associative learning.

As we stated in our own definition of learning, these changes in behavior are enabled by short- and long-term changes at the network, cellular, and molecular level, of which we only scratched the surface for now. The next section will allow us to apply the olfactory features studied in our two first chapters to learning and memory processes to better grasp the mechanisms underlying profound changes in olfaction and will focus on the OB circuit.

## 2. The dialogue between learning and olfactory bulb features

### a. Plastic changes in the olfactory network

To understand the plastic changes occurring in the olfactory system, and more specifically in the OB, we must have a quick look at present evidence on how the exchange of information between 2 neurons could change with time.

#### *i. Long-term changes underlying learning and memory*

In the brain, long-term modifications can occur at the synaptic level, giving rise to stable changes in electrical transmission and underlying network modifications with potential behavioral implications. These long-term changes can either decrease the activity of the concerned neuron, known as long-term depression (LTD), or increase it in long-term potentiation (LTP).

The first hypothesis described to explain changes in neuronal transmission came from Donald Hebb's work, stipulating that repetitive activation of a connection between two cells would result in an increase of this connection's strength (Hebb, 1949). This Hebbian plasticity suggests

that synapses undergo modifications according to their activity, resulting in increased or decreased strength, due to either presynaptic or post-synaptic changes, or both. However, other forms of plasticity such as homeostatic and intrinsic plasticity have been demonstrated. Here, we will briefly describe the various neuronal properties that can change and subsequently modify network responses.

Most plasticity-induced changes occur at the synaptic connections between neurons:

- Pre-synaptic changes usually occur through changes in the probability of neurotransmitter release in the synaptic cleft, and can be triggered by several mechanisms such as coincidence of retrograde messenger and presynaptic activity, binding of released glutamate on presynaptic mGluR (Pelkey *et al.*, 2005), activation of neuromodulation receptors such as CB1R (Heifets and Castillo, 2009; Cui *et al.*, 2016), or structural changes allowing tuning of the vesicle pool at the synapse (Bourne, Chirillo and Harris, 2013). Presynaptic plasticity, although initially overlooked, has been extensively reviewed (Yang and Calakos, 2013; Monday and Castillo, 2017). Given the variety of mechanisms and their sometimes-contradictory effects, the integrated effect of network activation on a synapse remains difficult to predict. However, dysregulation of these mechanisms can contribute to brain disorders such as schizophrenia or Alzheimer's disease (Siegert *et al.*, 2015; Maingret *et al.*, 2017).

Interestingly, this form of plasticity could share similar mechanisms in excitatory and inhibitory neurons, which is not the case for post-synaptic plasticity since activation-induced mechanisms are fundamentally different.

- In post-synaptic long-term plasticity, the observed changes mainly involve the number and types of receptors available at the post-synaptic membrane. For instance, the most commonly known mechanism relies on coincidental depolarization and synaptic release of glutamate allowing the activation of NMDA receptors, which induces the externalization of more AMPA receptors, thus causing the increase of inward current when glutamate is further released (Lüscher and Malenka, 2012). NMDA receptors have been identified early as necessary for spatial learning in the hippocampus (Morris, 1989). Moreover, NMDA activation activates the CaMKII protein which, once autophosphorylated, promotes AMPAR synaptic trapping (Opazo *et al.*, 2010) and

activates plasticity transcription (Giese, 2021). In some cases, NMDAR activation can also induce AMPAR endocytosis, resulting in LTD (Hrabetova *et al.*, 2000).

For inhibitory synapses, post-synaptic plasticity relies logically on the modulation of GABA<sub>A</sub>R number at the synapse. Just like in excitatory synapses, activation of NMDAR can promote the exocytosis of receptors, inducing LTP in an CaMKII-dependent manner (Marsden *et al.*, 2007). However, this does not explain how plasticity occurs with repetitive release of GABA by the presynaptic element. GABA<sub>A</sub>R trafficking is highly dependent on scaffold proteins such as gephyrin, which phosphorylation by the GSK3 signaling pathway results in synaptic density modulation (Tyagarajan *et al.*, 2011). Moreover, it seems that the overall number of available GABA<sub>A</sub>Rs is controlled by the level of degradation in the endoplasmic reticulum before reaching the plasmic membrane, which appears to be activity-dependent (Saliba *et al.*, 2009). Thus, both the number and location of GABA receptors seem to be regulated by neuronal activity, underlying postsynaptic plasticity in inhibitory synapses (Luscher, Fuchs and Kilpatrick, 2011; Barberis, 2020).

Moreover, apart from unique synapse strengthening or weakening, long-term changes can occur by creation (Leggio *et al.*, 2005), maintenance or pruning of synapses, which will result in an increase or decrease of the overall message generated by the output neuron after integration and support experience-dependent remodeling of microcircuits. This structural plasticity and the maintenance of new dendritic spines has been proven crucial in long-term memory formation (Yang, Pan and Gan, 2009).

A last mechanism that changes how a neuron will react in a given context is the modification of intrinsic properties of the cell, called intrinsic plasticity, as has been shown in zebra finches during song learning (Ross *et al.*, 2019). This type of plasticity relies on the modulation of cell excitability through changes in voltage- and Ca<sup>2+</sup>-gated ion channels that modulate how postsynaptic currents are transmitted along the dendrites, the action potential-generation threshold, or the firing mode, among others (Beck and Yaari, 2008). This type of plasticity has been demonstrated in the olfactory system (Cansler, Maksimova and Meeks, 2017).

All these mechanisms are present in most of the brain and lead to long-term modifications underlying learning and memory. They have been the topic of several reviews (Kotaleski and Blackwell, 2010; Baltaci, Mogulkoc and Baltaci, 2019).

*ii. Long-term modifications in the OB*

In the OB, various studies have looked upon learning traces at each step of message integration. As the first actors of olfactory transmission, OSNs were shown to undergo long-term plasticity after learning. Indeed, a recent study showed that turnover of OSN synapses in the OB is dependent on olfactory input (Cheetham, Park and Belluscio, 2016). Moreover, the output of these neurons is enhanced after appetitive (Abraham *et al.*, 2014) and fear learning (Kass *et al.*, 2013; Bhattarai *et al.*, 2020) and can be returned to its original strength by extinction of the fear association (Morrison, Dias and Ressler, 2015).

It is commonly accepted that learning causes changes in odor representation in the OB by modulating the glomerular activation map (Salcedo *et al.*, 2005), as seen in both perceptual and associative learning. M/T cells have sparse activation and show a long-lasting activity decrease in an odor-specific manner in awake animals, suggesting that bulbar representation of odors is shaped by previous experience (Kato *et al.*, 2012). Furthermore, repetitive odor discrimination modifies the activation patterns in M/T cells, leading to better performance in distinguishing similar stimuli through pattern separation (Doucette and Restrepo, 2008; Gschwend *et al.*, 2015). Interestingly, only MCs seem to change their pattern enough to discriminate odors (represented in Figure XI), specifically in active learning contexts (Yamada *et al.*, 2017). This leads to a probable distinct role of mitral and tufted cells in olfactory learning, which could be linked to their different targets in cortical regions as well as the cortico-bulbar loop they integrate (Chae *et al.*, 2022).

Intriguingly, activation patterns of MCs do not undergo the same changes in discrimination involving dissimilar or similar odorants (Chu, Li and Komiyama, 2016). In easy discrimination tasks, MCs actually displayed broader activation, which is poised to improve efficiency, while close odorants increased pattern separation in an attempt to favor robustness of discrimination. Thus, a trade-off between efficiency and accuracy seems to exist in bulbar learning signatures, which is also observed in perceptual learning.

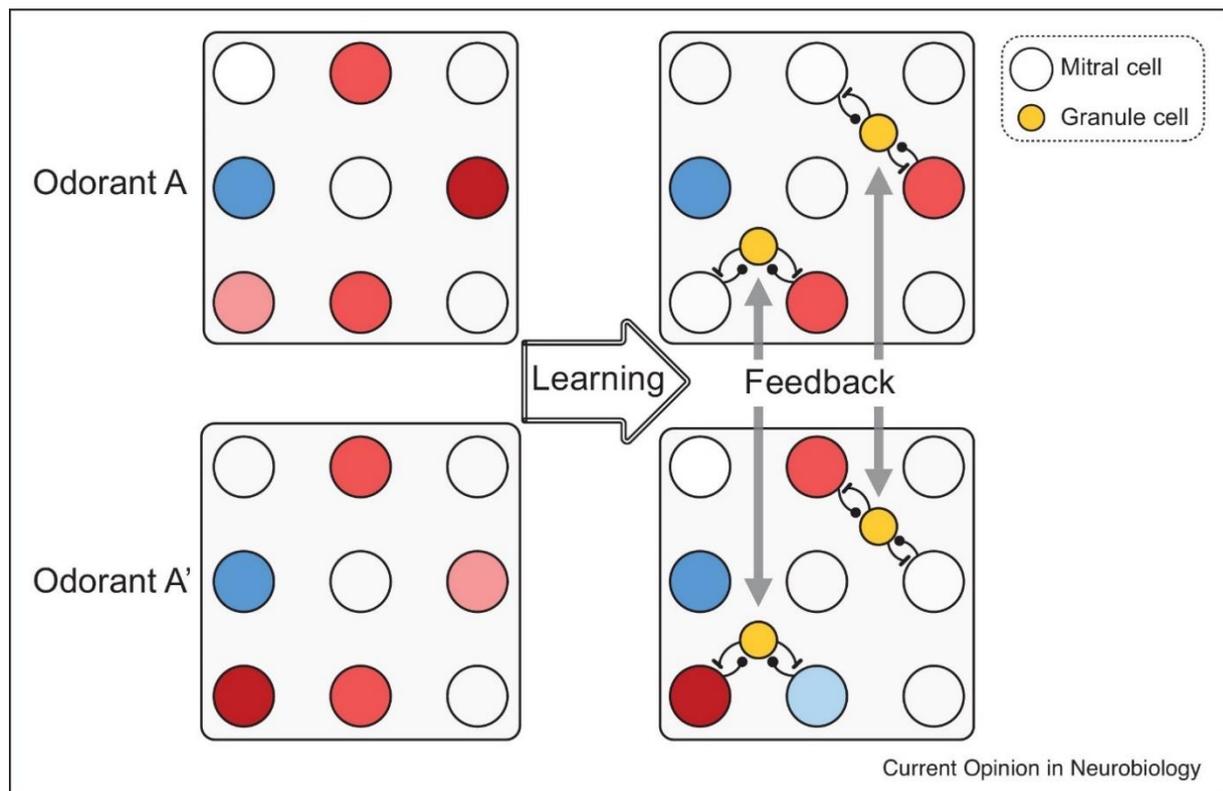


Figure XI. A model of perceptual learning-related plasticity in the olfactory bulb. Similar odorants (A and A') evoke similar response patterns in mitral cells before learning (left). Perceptual learning induces pattern decorrelation of mitral cell representations of the learned odorants (right). The enhanced pattern separation is mediated by adaptive and selective lateral inhibition from granule cells, particularly adult-born granule cells. In addition to mitral cell inputs, granule cells also receive feedback innervation from higher brain centers, which enables context-dependent odor processing. Red circles: mitral cells with excitatory odor responses; blue circles: mitral cells with suppressive odor responses. The intensity of colors represents the strength of the responses. Yellow circles: granule cells. From Wu, Yu and Komiyama, 2022

The changes in activation patterns of OB projecting cells are enabled by the interneurons, which are thought to provide lateral inhibition allowing sparser activity (Yokoi, Mori and Nakanishi, 1995; Mori, Nagao and Yoshihara, 1999). In learning, artificial tuning of GC activity was proved effective to modulate discrimination ability in mice, with increased GC activity leading to quicker learning (Abraham *et al.*, 2010; Nunes and Kuner, 2015), while their inactivation strongly impairs discrimination ability (Gschwend *et al.*, 2015).

It was also demonstrated that olfactory learning leads to increased activation of GCs by activated MCs, showing that strongly activated MCs could influence lateral inhibition of adjacent bulbar columns (Huang *et al.*, 2016). Finally, the GC/MC synapse displays LTP during social learning (Liu *et al.*, 2017), reinforcing the idea that olfactory memory is partially encoded directly in the OB.

Functionally, several studies looked at the effect of learning on oscillations in the OB. LFP recordings converged towards a decrease in gamma-band concurrent with an increase in beta rhythms (Ravel *et al.*, 2003; Martin *et al.*, 2004), which highly relies on centrifugal projections (Martin *et al.*, 2006). However, other studies showed that gamma oscillations increased with task difficulty, translating engagement in the task (Beshel, Kopell and Kay, 2007) and are dependent on local inhibition during odor discrimination (Lepousez and Lledo, 2013b).

Effects of olfactory learning has also been studied and demonstrated in most of the regions involved in the processing and interpretation of the olfactory message, notably in the AON (Aqrabawi and Kim, 2020), PC (Meissner-Bernard *et al.*, 2019; Terral *et al.*, 2019), LEC (Boisselier, Ferry and Gervais, 2014), CoA (Iurilli and Datta, 2017) and PFC (P. Y. Wang *et al.*, 2020).

Overall, plastic changes occur at each step of the olfactory pathway in the bulb, leading to tuning of the message in an experience-dependent manner directly in the first relay of olfactory processing (Wu, Yu and Komiyama, 2020). However, as seen, this message is greatly complicated by both centrifugal projections and adult neurogenesis in basic odor perception, it would be naïve to think that these mechanisms do not play a predominant role in odor learning and memory encoding.

#### b. Olfactory learning and centrifugal projections

We saw in chapter 2 that most cells in the OB are targeted by both neuromodulatory and non-modulatory fibers coming from various regions of the brain. These projections are involved in attention processes, reflect internal states of the individual, and participate in the generation of oscillatory rhythms in the OB, among other roles.

Several neuromodulatory afferences have been shown to be involved in olfactory learning in the brain, from cholinergic activity being modulated by reward-associated olfactory learning (Hanson, Brandel-Ankrapp and Arenkiel, 2021) to noradrenergic tonus enhancing the stability of olfactory memories (Linster *et al.*, 2020). However, for our study, we will focus on the role of non-neuromodulatory top-down projections in olfactory learning.

*i. Role of glutamatergic centrifugal fibers in olfactory learning*

Glutamatergic inputs from the AON and APC were the first non-modulatory centrifugal projections to be described (Boyd *et al.*, 2012a; Markopoulos *et al.*, 2012a). Early work looking at broad effect of centrifugal projections through lesions of the olfactory peduncle tried to describe their plasticity and/or their involvement in olfactory learning and memory (Martin *et al.*, 2004; Kiselycznyk, Zhang and Linster, 2006). These studies showed that cortical feedback is crucial for proper olfactory learning. Moreover, *in vitro* studies showed that proximal excitatory inputs on GCs, likely to originate from the cortex, were able to display LTP (Gao and Strowbridge, 2009; Nissant *et al.*, 2009).

The fact that AON activity is sensitive to brain states and receives afferences from various brain regions such as the BF of the ventral hippocampus (involved in episodic memory) (Aqrabawi and Kim, 2018a, 2020) suggests that the role of its top-down projections would be of great influence. First, we know that, through the activation of presynaptic GABA<sub>B</sub> receptors, AON and APC excitatory feedback projections activity selectively diminishes on OB interneurons, but not on principal cells, and participate in the generation of beta oscillations (Mazo *et al.*, 2016).

Their behavioral effect has been looked at in specific cases, concluding that these fibers are involved in odor processing (Quintela *et al.*, 2020), social recognition (Oettl *et al.*, 2016) and food preference transmission (C. Y. Wang *et al.*, 2020). Thus, it seems that glutamatergic cortical projections tune the olfactory message in a brain state-dependent manner. In other sensory systems, corticofugal projections have been shown to increase learning rate in visual tasks (Ruediger and Scanziani, 2020) for instance.

These findings primarily focused on excitatory cortical feedback onto the OB. However, we saw that inhibitory fibers also emerge from both the AON and the BF and display prominent roles in OB computation.

*ii. Role of GABAergic centrifugal fibers in olfactory learning*

GABAergic fibers from the AON were recently discovered and, although their effect on behavior remains unclear, seem to influence fine discrimination in go/no-go associative tasks

and help in generating beta oscillatory rhythms in the OB, usually related to learning processes (Mazo *et al.*, 2022). Moreover, artificial stimulation of these fibers results in general inhibition of M/TCs, which suggests they could be relevant for pattern separation in the context of olfactory learning.

In the BF, GABAergic projections to the bulb show increased activity when mice are presented with a reward-associated odor, and a strong suppression when receiving the reward (Hanson, Brandel-Ankrapp and Arenkiel, 2021). This suggests BF GABAergic neurons might play a role in identifying the rewarded odor in the OB, but this still needs to be confirmed.

Furthermore, we already saw that artificial activation of these fibers inhibits the GCs and impairs odor discrimination (Nunez-Parra *et al.*, 2013). In a more recent study, the same authors showed that the learning of new odor associations shows an increase in GABAergic BF neuron responsiveness at odor arrival, with most of these neurons responding even before the odor in what seems to be an anticipatory activation (Nunez-Parra *et al.*, 2020). Hypothetically, this could induce inhibition of the OB circuitry to favor the detection of the relevant message at odor presentation.

Taken together, these results show that centrifugal inputs to the OB are all part of the integration allowing the deciphering of odor identity, concentration, and value for the individual, in accordance with internal state, attention and motivation. However, we see that inhibitory cortico-bulbar afferences, despite anatomical and electrophysiological descriptions, still lack full functional characterization, which may unravel roles in perception and/or learning processes.

Nonetheless, centrifugal projections are not the only feature of the OB that are intertwined with learning processes. The arrival of new neurons during adult life is as dependent and crucial for these mechanisms than top-down projections.

### c. Olfactory learning and adult neurogenesis

We determined that, as well as in the dentate gyrus, new neurons constantly integrate into the OB circuit, mainly becoming GCs (95%) and PGCs (5%). It appears most logical that providing

new, naïve neurons in a network throughout life would serve as substrate for novel processes such as learning.

The first evidence of new neuron implications in learning and memory mechanisms was found in the hippocampus, where the deletion of neurogenesis impaired short-term memory associative task (Shors *et al.*, 2001). Moreover, it was shown shortly after that learning increased survival of the newly generated neurons both in the dentate gyrus (Gould *et al.*, 1999; Leuner *et al.*, 2004) and the OB (Alonso *et al.*, 2006; Lledo, Alonso and Grubb, 2006), although other studies showed that survival could be decreased in easy learning tasks (Mandairon *et al.*, 2006; Mouret *et al.*, 2008). These early works made a strong case for the implication of this process in learning and memory but were not sufficient to decipher their actual importance on the matter. Evidence later on proliferated and a real dialogue was shown between adult neurogenesis and learning.

#### *i. Effects of learning on adult neurogenesis*

We have already seen that learning increases both survival and plasticity of adult-born GCs in the OB. However, we must note that the effect of learning on survival is time dependent. Indeed, a critical period exists that dictates the fate of the new neurons according to learning, which is between 18 and 30 days after cell birth (Mouret *et al.*, 2008). Interestingly, in the days following this critical period, learning seemed to promote elimination of the adult-born neurons. This could be a way for the OB to turnover and dispose of cells that did not take part in relevant information processing. This effect was mainly observed deep in the GCL, which correlates with the fact that adult born neurons usually integrate deeper, rather than superficially in the GCL.

Once integrated and mature, new neurons are less subject to elimination. However, odor enrichment triggers an increase in synaptic stability on adult-born GCs and PGCs, suggesting a plastic substrate allowing generation and maintenance of new connections (Livneh and Mizrahi, 2012). Intriguingly, recruitment of these newborn neurons in various learning tasks also depends on their age. While simple stimulation appears to induce activation of immature abGCs, associative learning stimulates the more mature neurons (Belnoue *et al.*, 2011). Furthermore, passive learning causes an increase in MC inhibition originating from abGCs,

while association reduces this inhibition, causing a respectively lower and higher odor-evoked response in the OB (Mandairon *et al.*, 2018).

Finally, a recent study found that complex enrichment, and consequently perceptual learning, induces an increase in the number of dendritic spines on all domains of abGC dendrites (Forest *et al.*, 2020), while only few changes were observed in nnGCs. This supports a specific role of abGCs in olfactory learning.

#### *ii. Cortical projections and adult-born neurons plasticity in olfactory learning*

One particularly interesting aspect of abGCs is the growing evidence that learning-induced modifications are dependent on centrifugal activity. First, data show that piriform inputs to the OB promote abGC apoptosis, specifically in postprandial sleep (Yokoyama *et al.*, 2011; Komano-Inoue *et al.*, 2014), and also after noxious stimulation (Komano-Inoue *et al.*, 2015). Although these are not specific to olfactory learning situations, they give hints on top-down effects on the abGC population according to specific context and experience.

Previous work in our lab showed that discrimination learning increased synapses on abGCs specifically on the basal dendrites and the proximal and distal parts of the apical dendrites, but not on the apical part which is usually in contact with M/T cells (Lepousez *et al.*, 2014a). These segments are the location where GCs receive inputs from top-down projections, especially glutamatergic inputs from the AON and APC, and are strengthened by learning. Furthermore, this paper demonstrated the abGCs received higher excitatory cortical input following olfactory learning, although this effect was not assessed in nnGCs. Thus, abGCs are also connected by cortical regions and these connections are modulated by experience.

A recent study also demonstrated that excitatory piriform inputs are necessary for abGCs plasticity during learning (Wu *et al.*, 2020). More precisely, blocking of piriform excitatory feedback abolished learning induced plasticity in abGCs, while artificial stimulation of piriform feedback proved sufficient to mimic plasticity in the absence of odor learning. Finally, the authors demonstrated that plasticity was not only specific to abGCs, but also limited to those activated by odor presentation during learning, suggesting a new substrate of learning-induced pattern separation.

Thus, olfactory exposure and learning are critical for abGC survival, elimination, recruitment, and plasticity in the OB, which pushes even further their potential implication in precise learning processes. Cortical projections are also implicated in learning process, with specific roles for abGCs opening new questions on the effect of memory on ulterior learning in relation to adult neurogenesis.

### *iii. Effect of adult neurogenesis on learning and memory*

Adult-born neurons in the OB are not only modulated by learning. We know they have an important part in olfactory processing, so if they undergo specific modifications with learning, they must be involved in learning processes. Evidence of their role came from perceptual learning studies that showed not only this learning also favored the survival of abGCs, which was accompanied by an increase in bulbar inhibition, but also that blocking cellular proliferation in the SVZ completely abolished improvement in discrimination (Moreno *et al.*, 2009).

Using genetic or irradiation tools, adult neurogenesis in the OB has been deemed necessary for odor discrimination but not memory (Gheusi *et al.*, 2000). While confusing results were obtained to assess the involvement of abGCs in associative learning and memory, with some studies claiming their participation ((Lazarini *et al.*, 2009; Valley *et al.*, 2009; Sultan *et al.*, 2010; Arruda-Carvalho *et al.*, 2014) and other refuting it (Imayoshi *et al.*, 2008; Breton-Provencher *et al.*, 2009). These differences seem to originate from the behavioral paradigm used in the studies, with abGCs involved mostly in operant conditioning, but not passive association (Mandairon *et al.*, 2011). Moreover, impairment of adult neurogenesis in the OB also resulted in deficits in innate responses to odors (Sakamoto *et al.*, 2014) and social discrimination (Feierstein *et al.*, 2010; Garrett *et al.*, 2015).

Using finer manipulation, specific optogenetic activation of abGCs strongly increased the performance of animals in a difficult associative learning task when light was concomitant with odor presentation (Alonso, Lepousez, Wagner, *et al.*, 2012). Moreover, this performance increase was only observed for 40 Hz frequency stimulation, but not with 10 Hz stimulation, which showed to induce substantially higher GABA-induced current on MCs.

This confirms that general increase of inhibitory activity upon M/TCs is crucial for learning in the OB, with the abGCs being a good candidate to generate this inhibition. It is also worthy to note that selective inhibition of adult-born GCs abolishes odor discrimination faculties after prior perceptual learning, a type of learning that showed increased activation of abGCs (Forest *et al.*, 2020).

In an elegant study, abGCs, but not neonatal ones, were shown to be specifically activated by presentation of an already associated rewarded odor (S+). Optogenetic stimulation of abGCs only for the S+ odor was sufficient to improve the performance of animals and also to generate an association without odor, suggesting an encoding of value directly in abGCs or their inputs (Grelat *et al.*, 2018). Stimulation of these cells, but not of pre-existing ones, finally increased the ability of animals to switch the association rule, showing a role in early learning of positive odor association. Evidence also showed that adult neurogenesis is involved in olfactory memory maintenance (Sultan *et al.*, 2010), thus confirming that these cells are key actors in learning and memory in the OB.

At the network level, adult-born neurons were shown to shape the MC activation pattern during associative learning, which could explain their role in fine discrimination tasks. Indeed, specific ablation of abGCs, but not random ablation of GCs, showed a decrease in difficult discrimination performance associated with a decrease in suppressive responses to odors in MCs (Li *et al.*, 2018). Consequently, these results reinforce the hypothesis that abGCs improve the odor discrimination tasks by inhibiting projection neurons in the OB. However, this study did not look at tufted cells responses and thus did not decipher the distinct role of adult-born neurons on different populations of OB principal cells.

Finally, several studies have shed light on the role of neuromodulatory inputs. Survival of abGCs is indeed dependent on neuromodulatory activity, such as cholinergic input, since activation of B2-containing nicotinic receptors drastically impairs the number of abGCs in the OB, inducing deficits in short-term memory (Mechawar *et al.*, 2004). In the same line, noradrenergic inputs are thought to be activated by novelty in olfactory processing (Veyrac *et al.*, 2009) and are able to regulate the survival of abGCs, with A2-receptor agonist shown to improve discrimination after perceptual learning (Moreno *et al.*, 2012). Thus, abGC population

numbers are strongly regulated by neuromodulatory inputs and are in return necessary for proper olfactory learning.

In summary, we have seen that both perceptual and associative learning increase the number and synaptic density of abGCs in the OB, and that these cells are required for proper associative learning and discrimination following perceptual learning. Moreover, these cells appear to be more responsive to reward-associated odors and, although their effect on tufted cells remains unclear, display a strong inhibitory drive on MCs during learning tasks and recall. More importantly, in the context of the present study, the survival, maturation, and functional role of these cells is highly linked to centrifugal fibers arriving to the OB, especially during learning processes.

## Problematic

In our demonstration, we showed that detection of odorants at the olfactory epithelium triggers the generation of electrical messages transmitted to the OB by sensory neurons, which are then integrated through complex circuitry to create olfactory representations in the bulb. This information travels to cortical regions that ideally decipher, among other parameters, the identity of the perceived molecules, their respective concentration, but also their significance for the individual, either innate or acquired by learning.

Furthermore, the message integration is highly shaped by projections coming from said cortical regions, namely the anterior olfactory nucleus, piriform cortex, basal forebrain, amygdala, and neuromodulatory nuclei, directly in the OB, adding a layer of complexity that translates internal states, attention processes, and is also dependent on previous olfactory experiences. This results in a constantly changing representation of the same odors in the brain through plastic modulation of odor representations, the creation of olfactory memory through learning processes and the influences of changing internal states, and multisensory integration.

Finally, new GCs are constantly brought to the OB during the life of an individual cohabitating with pre-existing ones generated at embryonic and post-natal ages, which have been proven crucial for correct olfactory processing, learning and memory.

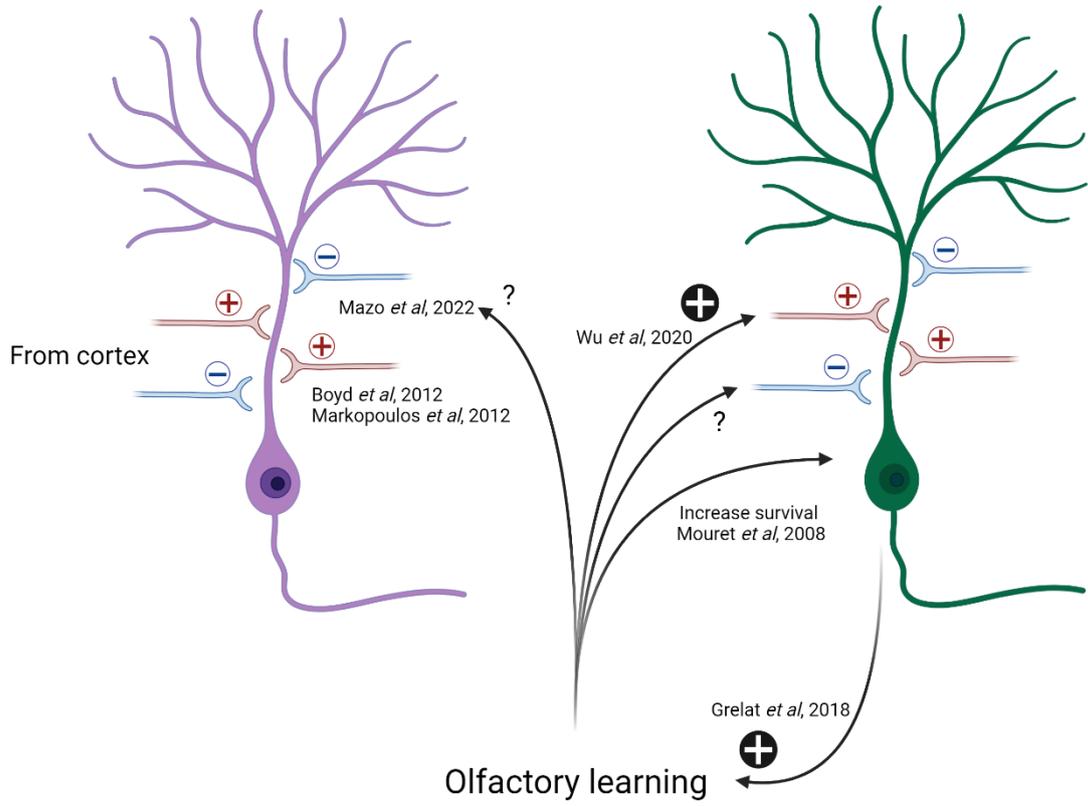
Despite proficient work dedicated to understanding the complex system allowing animals to transform volatile chemical clues into interpretable representations, stable memories, and allowing to trigger adapted behavior, many questions remain.

In particular, among all centrifugal fibers that arrive to the OB, recent results highlight the existence of long-range GABAergic fibers, which role is far from being understood. In the OB of rodents, these centrifugal inputs impinge on multiple partners, notably GC interneurons, in an extremely dynamic circuit.

In this work, I will address the following questions:

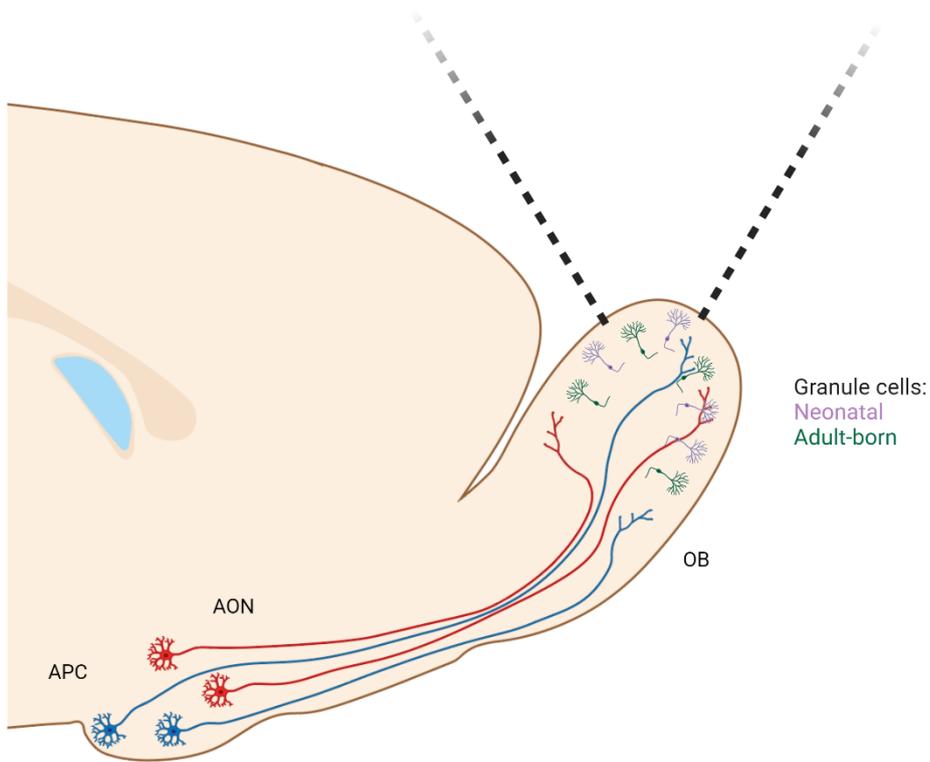
- ➔ Do cortico-bulbar GABAergic connections to GC populations change with odor experience and olfactory learning? If so, what are the synaptic changes induced by learning?
- ➔ How do GABAergic neurons projecting to the OB become activated during odor presentation? Does this activation change with olfactory learning?
- ➔ What is the functional role of AON GABAergic top-down inputs on olfactory perception, learning, and memory?

Although my work could not bring an answer to all these complex questions, I tried to bring different pieces of this system together to improve our understanding of a beautiful system that leads us not only to understand our immediate environment and detect danger and food, but to enjoy the smell of freshly cut grass in the spring rain and take great delight over the complex aromas of the finest wines.



Neonatal granule cells

Adult-born granule cells



# Materials and Methods

## Mice

Adult Wild-Type (WT, n=42) C57BL/6J and vGAT::Cre (*Slc32a1<sup>tm(cre)Low1</sup>*, MGI ID: 5141270, maintained on a C57BL/6J background, n=120) mice were used in this study. Mice were housed (2-5 per cage), under standard housing conditions ( $23 \pm 1$  °C; humidity 40%) in a 14/10h light/dark cycle with dry food and water available ad libitum except during behavioural experiments. All behavioral tests were conducted during the light period (10am – 7pm). All procedures were performed in compliance with the French application of the European Communities Council Directive of 22 September 2010 (2010/63/EEC), approved by the local ethics committee (CETEA 89, project dap220049) and were reviewed by the Animal Welfare Committee of the Institut Pasteur. We used the minimum number of animals, estimated from our previous knowledge in performing the same type of experiments. Both female and male mice were used in all experiment in similar numbers (except histology in figure 4), with no observed differences.

## Viral injections

Various viral vectors were used in this study, all presented in table 1. For adult mice injections, P60 mice (postnatal day 60) were anesthetized intraperitoneally with a ketamine (35 mg/kg) and xylazine (4 mg/kg) mixture and placed in a stereotaxic apparatus. A small craniotomy was performed over the targeted region on right and left hemispheres and viral solutions were injected (See Table 2 for stereotaxic coordinates) through a glass micropipette connected to a Nanoject III microinjector (Drummond Scientific). For neonatal mice, P6 pups were anesthetized with isofluorane (3.5%; 372 mL/min; Iso-Vet, Piramal Healthcare) and positioned in a stereotaxic frame using a homemade cast. Small craniotomies were drilled above the injection sites with a needle, and bilateral viral injections (350 nL per site) were made into the

RMS (stereotaxic coordinates: +2.4 mm anteroposterior and  $\pm 0.6$  mm mediolateral from bregma and -2.7 mm dorsoventral from skull surface). When a second injection was needed (Results figure 1), one month after lentiviral injection, AAV9 Syn-ChR2-GFP was injected in the APC as previously described. After all stereotaxic procedures, cranial skin was sutured, and animals were left to recover on a heated pad until complete wakefulness.

Paxinos brain atlas was used as reference to verify injection site in each animal.

Table 1. Viral Vectors

Virus	Provider	Titration	Figure
LV-Ubc-TdTomato (pFUGW-tdtomato or FUtdTW)	LV IRNEM Necker	$7 \times 10^8$ TU/mL	1, 2, 3, 4
pAAV-hSyn-hChR2(H134R)-EYFP (AAV5)	Addgene (26973-AAV5)	$7 \times 10^{12}$ vg/mL	1, 2, 3, 4
pGP-AAV-syn-FLEX-jGCaMP8f-WPRE (AAV1)	Addgene (162379-AAV1)	$5 \times 10^{12}$ vg/mL	5
pAAV-hSyn-DIO-EGFP (AAV5)	Addgene (50457-AAV5)	$\geq 7 \times 10^{12}$ vg/mL	6, 7
pAAV-CAG-FLEX-rc [Jaws-KGC-GFP-ER2] (AAV5)	Addgene (84445-AAV5)	$7 \times 10^{12}$ vg/mL	6
pAAV-EF1a-double floxed-hChR2(H134R)-EYFP-WPRE-HGHpA (AAV5)	Addgene (20298-AAV5)	$1 \times 10^{13}$ vg/mL	7

Table 2. Sterotaxic coordinates

Region	Coordinates	Volume injected	Figure
RMS	AP: +3.3 ML: +/-0.82 DV: -2.7 from brain surface	200nL	1, 2, 3, 4
AOC	AP: +1.9 ML: +/-2.1	200nL	1, 2, 3, 4

	DV: -3.8/4.0 from brain surface		
AONp	AP: +1.9 ML: +/-1.7 DV: -4.6 from Bregma	200nL per site	5, 6, 7
Olfactory Peduncle (Fibers implantation)	AP: +2.9 ML: +/-1.4 DV: -2.5 from brain surface	NA	5, 6, 7

## Fibers implantation

During stereotaxic procedures, right after viral injections, optic fibers (multimode, 430 $\mu$ m diameter, NA 0.5, LC zirconia ferrule) were bilaterally implanted above the olfactory peduncle (See table 2 for stereotaxic coordinates) and fixed to the skull with a liquid bonding resin (Superbond, Sun Medical) and dental acrylic cement (Unifast).

## Go/No-go task

Go/No-go operant conditioning task was performed using custom-built olfactometers as described previously (Alonso, Lepousez, Sebastien, *et al.*, 2012; Mazo *et al.*, 2022). At least 3 weeks after the last stereotaxic injection, mice were water-deprived (maintained at ~85% of their initial weight) and progressively trained to receive odors in a sampling port and then get a water reward from a waterspout 5 cm left from the odor port. Mice weight was closely monitored, and task training was performed without odor presentation. Once mice were able to stay at least 1.2 seconds in the odor port (presence detected by an infrared laser beam), odor association was allowed to proceed. For all trials, mice needed to enter the odor port and stay for at least 1 second to receive an odor.

Mice received a pair of odors in a pseudo-random fashion (10 presentations each, no more than 3 times the same odor in a row), and only one (S+) was accompanied by the obtention of

a water-drop, triggered by licking the spout, while the other one (S-) did not receive reinforcement of any kind. Odor was presented in the port for a maximum of 2 seconds, or stopped when mice removed their snout from the sampling port, which then left them 2 seconds to get the reward. If mice licked the spout after receiving S+, the result is a Hit, and a False Alarm (FA) after S-. If they did not lick after S+, result is a Miss, and a Correct Rejection (CR) after S-. The inter-trial interval was 5 seconds. Mice were tested on discrimination for 10 blocks of 20 trials, containing each 10 rewarded (S+) and 10 unrewarded odours (S-), with a 4 to 6  $\mu$ L water drop as a reward.

A pseudorandom protocol was used during behavioral experiments to assign animals to the different olfactometers (six olfactometers in total). A given animal was never trained more than two consecutive days in the same device.

For each 20-trial block, performance is calculated as a percentage according to the following formula:

$$Performance = \frac{n(Hit) + n(CR)}{20} \times 100$$

Performance scores  $\geq 85\%$  implied that mice had correctly learnt to assign the reward value to the S+ and the non-reward value to the S-. The number of blocks needed to reach the criterion level was counted as the number of blocks employed before reaching a block with 85% correct responses.

### Catch trial protocol

For the catch trial experiment, in addition to the 2 vials of odors used for discrimination learning, two other vials are used, containing respectively the highly diluted S+ odor and mineral oil. Each of the 4 vials are presented 5 times in a block of 20 trials, and only the non-diluted S+ odor is rewarded. Catch number is the number of times the mouse licks the water port when the diluted S+ odor is presented, across 100 trials (25 presentations of each odor).

## Reversal protocol

For reversal, mice undergo 2 blocks of the initial rule, which are followed by 5 blocks where initial S+ becomes unrewarded while S- is now rewarded. If a given mouse does not reach the 85% criterium in the first two blocks, the results of the reversal task are discarded.

## Memory protocol

24h or 2 weeks after last reinforced learning, memory is assessed by two blocks of 20 trials where previously learnt odors are presented but not rewarded. Memory score is then calculated according to:

$$\text{Memory} = \frac{\text{Mean score of the 2 test blocks}}{\text{Mean score of the last 3 reinforced learning blocks}}$$

## Reaction time analysis and definition

Automated olfactometer allow precise measurement of the time of events during the task. The odor-sampling time, called Reaction Time (RT), is measured between the arrival of the odor and the moment the mouse removes its head from the odor port, while First Lick (FL) is calculated between the end of RT and the first detected lick on the waterspout.

Odors were presented using glass vials containing the following couples: Hexanol/Octanal, Amylacetate/Ethylbutyrate, (+)-Limonene/(-)-Limonene. All odours were pure monomolecular odorants (Sigma-Aldrich), diluted in mineral oil at 1/100 except for catch trials. Odors were generated by passing a 120mL/min stream of air over the surface of diluted odorants in disposable 50mL glass tubes. The odorant vapor was mixed with 3.20 L/min clean air before its introduction into the sampling port. Thus, the odor concentration delivered was 4.3% of the head space above the liquid odorant.

To provide photostimulation for optogenetics excitation and inhibition experiments, mice were tethered to the optic fibers before being placed in the olfactometer. Laser onset was synchronized with the closing of the diversion valve, allowing bilateral stimulation during odor presentation with 473nm (5mW) and 658nm (10mW) laser at 33Hz (5ms pulses) for 1

second or continuously for 2 seconds, respectively. The absolute light intensity was calibrated before each experiment.

### *Ex vivo* electrophysiological recordings

Mice were deeply anesthetized with intraperitoneal injection of Ketamine (100mg/Kg) and Xylazine (10mg/Kg) and swiftly decapitated. The OB and frontal cortices were rapidly dissected and placed in ice-cold ACSF containing in mM: 124 NaCl, 3 KCl, 2.8 MgSO<sub>4</sub>, 26 NaHCO<sub>3</sub>, 1.25 NaHPO<sub>4</sub>, 20 glucose, 0.5 CaCl<sub>2</sub> (~310 mOsm, pH 7.3 when bubbled with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>; all chemicals from Sigma France). They were then glued to a block of 4% agarose and placed, submerged in ice-cold ACSF, in the cutting chamber of a vibrating microtome (Leica VT 1200S). Horizontal slices (300  $\mu$ m thick) of the OBs were placed in bubbled ACSF in a warming bath at 35°C for 30 min and then at room temperature (*i.e.*, 22  $\pm$  1°C).

For whole-cell recordings, individual slices were placed in a chamber mounted on a Zeiss Axioskop upright microscope, and continuously perfused (1.5 mL/min) with 30°C ACSF containing in mM: 124 NaCl, 3 KCl, 1.3 MgSO<sub>4</sub>, 26 NaHCO<sub>3</sub>, 1.25 NaHPO<sub>4</sub>, 20 glucose, 2 CaCl<sub>2</sub> (~310 mOsm, pH 7.3 when bubbled with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>; all chemicals from Sigma France) (Warner Instrument inline heater). Slices were visualized using a 40x water-immersion objective. We obtained whole-cell patch-clamp recordings from visually targeted TdTomato-labeled GCs (Fig.3E). Patch pipettes, pulled from borosilicate glass (OD 1.5mm, ID 0.86mm, Sutter Instrument, UK; P-97 Flaming/Brown micropipette puller, Sutter Instrument Co, UK), had resistances of 6–10 M $\Omega$  and were filled with a Cesium-Methane sulfonate based solution (in mM: 126 Cs-MeSO<sub>3</sub>, 6 CsCl, 2 NaCl, 10 HEPES, 0.2 Cs-EGTA, 0.3 GTP, 2 Mg-ATP, 280–290 mOsm, pH 7.3). All membrane potentials indicated in the text are corrected for a measured liquid junction potential of +10mV. Labelled cells were identified by the presence of TdTomato in the tip of the patch pipette after membrane rupture. Recordings were obtained via an Axon Multiclamp 700B. Signals were filtered at 2 kHz and sampled at intervals of 20–450  $\mu$ s (2.2–50 kHz) according to the individual protocols. Series resistance (R<sub>s</sub>),

and membrane resistance ( $R_m$ ) were estimated using peak and steady-state currents, respectively, observed in response to a 5mV membrane step. Currents mediated by  $Na^+$  voltage-gated channels were measured under voltage-clamp conditions. Depolarizing pulses (100 ms) from  $-70$  mV to incremental steps (5 mV), up to  $+10$  mV, were given at a rate of 1 Hz.  $Na^+$  currents were measured after subtraction of scaled passive current responses to the appropriate voltage steps. IPSCs were recorded at  $V_c = 0$  mV and EPSCs at  $V_c = -70$  mV.

Synaptic events were elicited by photo-activation of  $ChR2^+$  axon terminals stimulation using a 470 nm light-emitting diode (LED; Xcite by Lumen Dynamics) illuminating the sample through the objective. Duration of the light pulses was adjusted (from 0.1 to 6,4ms) for each cell to evoke minimal to maximal PSCs. Data were acquired using Elphy software (Gerard Sadoc CNRS; Gif sur Yvette, France) and analysed with Elphy and IgorPro (Neuromatic by Jason Rothman).

## Calcium imaging using fiber photometry

During behavioral tasks, neurons infected with  $GCamp8f$  vector were continuously excited with a 473-nm solid-state laser (Crystal Lasers) via a 430- $\mu$ m multimode optical fibre (output intensity  $< 0.1$  mW). The emitted fluorescence was collected by the same fibre, filtered through a dichroic mirror and a GFP-emission filter (452–490 nm/505–800 nm; MDF-GFP, Thorlabs), filtered ( $525 \pm 19$  nm) and then focused on a NewFocus 2151 Femtowatt photodetector (Newport). Blue light reflected in the light path was also filtered and measured with a second amplifying photodetector (PDA36A; Thorlabs). The signals from the two photodetectors were digitized by a digital-to-analogue converter (Micro1401-3 A/D interface, CED) at 5000 Hz and then recorded using Spike2 software (CED, UK). Paxinos Brain atlas was used as reference to check viral vector injection and fiber optic implantation site in each animal. Brain hemispheres where injection or the fiber optic were outside were discarded for the analysis (X Hemispheres,  $n = X/X$ , Mice,  $n = X/X$ ).

## Immunolabelling

Mice were deeply anesthetized with intraperitoneal injection of ketamine (100mg/Kg) and Xylazine (10mg/Kg) and were intracardially perfused first with Saline (0.9% NaCl), then with 4% paraformaldehyde in 0.1M phosphate buffer. Brains were removed and cryoprotected in PBS-Azide 0.02 %-sucrose 30% overnight. Sagittal slices (60µm thick) were obtained with a microtome and were stored with PBS-Azide 0.02%. Immunostaining was performed on free-floating sections. Non-specific staining was blocked in PBS-Triton (PBST) 0.5% with 10% Normal Goat Serum for 1h30 at room temperature followed by incubation in primary antibody solution (see Table 3) in PBST 0.5% over 1 or 2 nights at 4 °C. Sections were subsequently washed 3 times in PBST 0,5% (for 15 min each) and then transferred to secondary antibody solution containing a DNA-specific fluorescent probe (Hoechst; 1:5000) in PBST 0.5% for 2 hours at room temperature. Sections were again washed 3 times in PBST (for 15 min each) before being mounted onto glass slides and coverslipped using polyvinyl alcohol mounting medium.

Table 3. Antibodies

Antibody	Provider	Dilution	Figure
Chicken anti-GFP	Abcam (ab13970)	1/1000	3, 6, 7
Mouse anti-RFP	Rockland (200-301-379)	1/1000	3
Rabbit anti-vGAT	Synaptic System (131002)	1/1000	3
Guinea pig anti-cFos	Synaptic System (226308)	1/1000	6, 7
Rabbit anti-pS6	Cell Signalling (4858)	1/500	6, 7
Goat anti-Chicken 488	Invitrogen (A11039)	1/1000	3, 6, 7
Goat anti-Mouse 568	Invitrogen (A21134)	1/1000	3,
Goat anti-Guinea Pig 594	Invitrogen (A11076)	1/1000	6, 7
Goat anti-Rabbit 647	Invitrogen (A21244)	1/1000	3, 6, 7

## Imaging and quantification

### Inhibitory synapses in abCGS

For VGAT puncta analysis, images of slices were obtained using a confocal laser-scanning microscope (LSM 980, 63x oil objective; Zeiss®) in stack of optical slices (0.36  $\mu\text{m}$  thick along the Z axis). Images acquisition and quantification were performed blindly to the brain slice condition.

Image analyses were performed using the software Imaris®. Surfaces were created for both eYFP (GABAergic fibres) and TdTomato (adult-born GCs) with a minimum number of voxels of 2500. Spots with a diameter of 1  $\mu\text{m}$  were created for VGAT staining. Then spots were filtered for the proximity to both surfaces. Spots were selected if they were 0.5  $\mu\text{m}$  close to the eYFP and 0.5  $\mu\text{m}$  close to the TdTomato. Spots were divided in four groups depending on their location: either on the soma, on the proximal dendrite (<30  $\mu\text{m}$  from soma), on the distal dendrite (>30  $\mu\text{m}$ ) or on the apical dendrite (located in the EPL).

### Statistical Analyses

Statistical analyses were performed with GraphPad Prism 9.0. Most of the tests used in this study are non-parametric tests (Mann-Whitney test, Kruskal-Wallis test with Uncorrected Dunn's test for multiple comparisons, Friedman test) due to the low sampling and non-normal distribution of data. When a parametric test was performed (Unpaired t-test, One-way ANOVA), normality was verified using the Kolmogorov-Smirnov test. Differences were considered significant for  $P < 0.05$ . Outliers were identified with ROUT column analyses and were removed for  $Q \geq 0.5\%$ .



# Results

## Chapter I. Study of cortical inputs to various populations of granule cells

### Adult born granule cells receive less top-down excitation and inhibition from Anterior Olfactory Cortex (AOC) than neonatal ones

In this study, we sought to decipher the functional connectivity of corticofugal inputs with specific populations of local interneurons in the OB circuits. More precisely, we want to untangle the specific impact on adult-born vs neonatally generated granule cells and their potential changes with olfactory learning. To do so, we labelled GCs progenitors generated at specific ages of mice thanks to stereotaxic injections of TdTomato-expressing lentiviral vectors in the RMS of pups at postnatal day 6 (P6) or adult 2-month-old mice (P60) (Figure 1A-B). This allowed us to observe a cohort of red-labelled cells, that were generated either postnatally (neonatal group) or at an adult stage (adult-born group). Importantly, in the present work, we restricted our study to mature postnatal-born interneurons (either formed at early postnatal ages or throughout life) by starting experiments at 12 weeks post injection (wpi), when these neurons are already integrated in the bulbar circuit and their density in the GCL reached a plateau (Bardy *et al.*, 2010).

Anatomical and physiological studies have shown that the olfactory cortex (OC), notably the APC and the AON, send dense projections back to the OB where they impinge notably onto GCs (Boyd *et al.*, 2012b; Markopoulos *et al.*, 2012b; Mazo *et al.*, 2022). To assess the functional impact of these projections on different GC populations, we injected in the same animal an adeno-associated virus (AAV) expressing channelrhodopsin-2 (ChR2) fused to the green fluorescent protein YFP into the APC and AON (Figure 1C). One month later, ChR2-YFP was present in axonal fibers with most of the labeling in the GCL, and a lesser expression in the internal plexiform layer (IPL) and mitral cell layer (MCL).

By using this approach, we were able to perform *ex vivo* patch clamp electrophysiological recordings in acute slices (Figure 1D), to study the synaptic currents elicited by cortical stimulation onto GCs depending on their age of generation as previously performed (Lepousez *et al.*, 2014b; Mazo *et al.*, 2022). Importantly, ChR2-YFP cells were exclusively located in the AOC, and we did not detect any viral diffusion from the injection site directly to the bulb. In addition, injection in the APC and AON did not significantly label M/TCs in a retrograde manner ( $2.2 \pm 1.5$  M/T-cells per section,  $n=6$  animals) nor did it significantly label migrating neuroblasts en route to the OB ( $0.32 \pm 0.18$  cell/mm<sup>2</sup> in GCL,  $n=6$ ), as measured in (Lepousez *et al.*, 2014b).

This protocol enabled the study of AMPA-, GABA<sub>A</sub>- and NMDA-mediated currents by changing the membrane potential and using antagonists to isolate specific responses (Figure 1E). Importantly, we did not find any difference in membrane resistance when comparing cells generated at neonatal and adult age (neonatal =  $1001 \text{M}\Omega \pm 188.5$  vs Adult-born =  $969.5 \text{M}\Omega \pm 94.5$ ; Mean  $\pm$ SEM, Mann-Whitney test,  $p=0.2627$ ), although a higher amplitude of voltage-gated Na<sup>+</sup> current was observed in adult-born neurons, in agreement with previous results (Carleton *et al.*, 2003) (Figure 1F).

Interestingly, we observed that after addition of the AMPA receptor antagonist NBQX, virtually no change was noticeable in the amplitude of inhibitory currents (Figure 1E, +0mV, blue trace). This confirms that the observed inhibitory responses are directly coming from the AOC rather than occurring in a feed-forward fashion through dSACs. As expected, SR9551 (antagonists of GABA<sub>A</sub> receptors) abolished inhibitory synaptic responses.

We observed that nnGCs displayed stronger excitation (Figure 1G) and inhibition (Figure 1H) than adult-born ones when cortical fibers were stimulated. Finally, postnatally generated cells also showed higher AMPA/NMDA ratio than abGCs (Figure 1I).

Together, these results show that neonatal GCs receive either stronger connections or more inputs from the OC than adult-born ones, suggesting a differential control of top-down fibers on both GC populations.

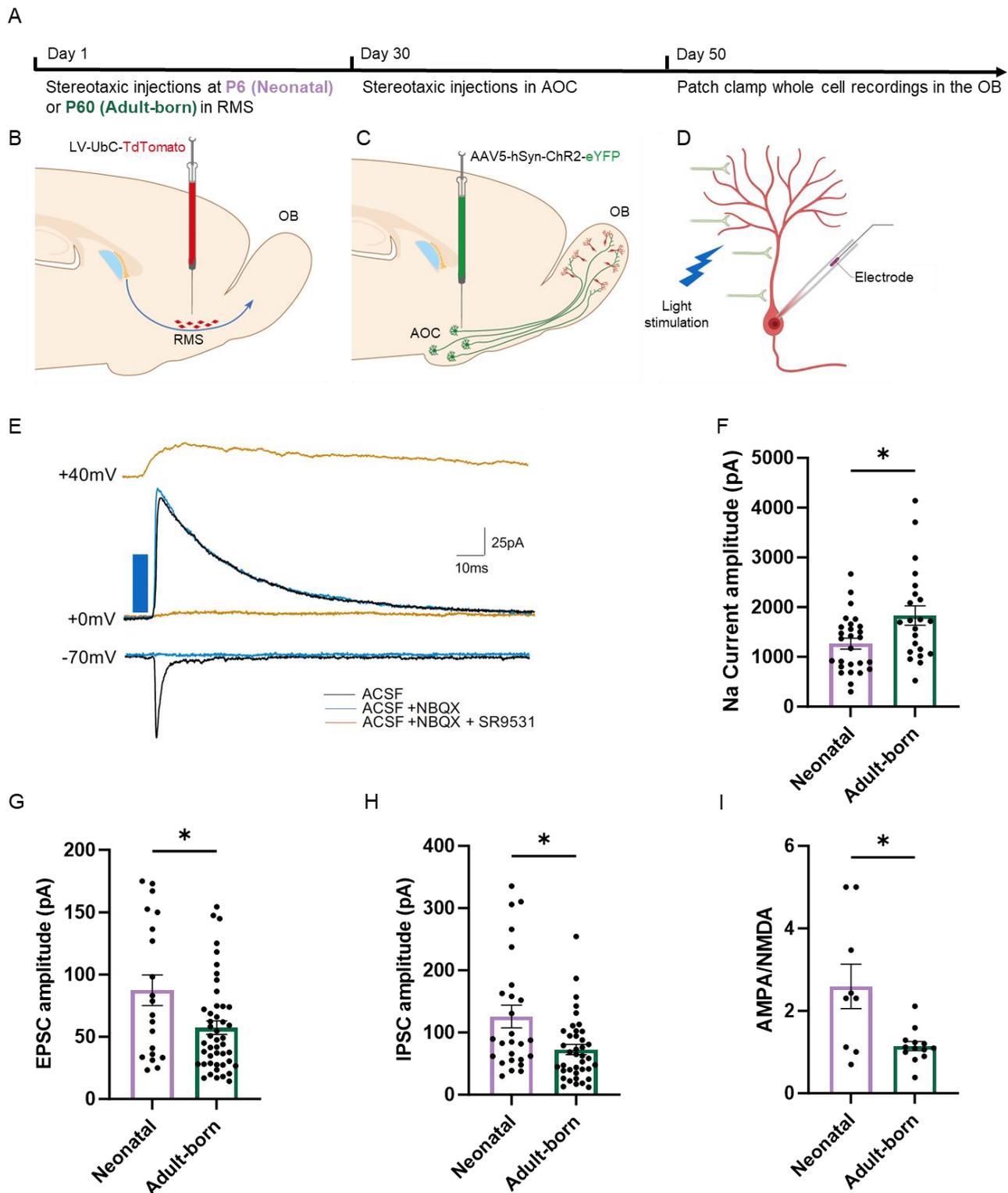


Figure 1. Adult born granule cells receive less top-down excitation and inhibition from Anterior Olfactory Cortex (AOC) than neonatal ones. A. Chronology of stereotaxic injections and electrophysiology recordings. B. Stereotaxic injections of TdTomato-expressing lentiviral vector in the Rostral Migratory Stream (RMS) at neonatal (P6) or adult (P60) age. C. Injections of ChR2-expressing virus in the AOC. D. Schematic of ex vivo electrophysiological recordings of labeled granule cells in the olfactory bulb (OB). E. Representative mean traces of electrophysiological voltage-clamp recordings at various voltages. F. Voltage-gated sodium current amplitude (Welch's t test,  $p=0.0167$ ,  $n=27-22$ ). G. Light-evoked EPSCs amplitude (Mann-Whitney test,  $p=0.0446$ ,  $n=20-46$ ). H. Light-evoked IPSCs amplitude (Mann-Whitney test,  $p=0.0134$ ,  $n=26-39$ ). I. AMPA/NMDA ratio (Welch's t test,  $p=0.0279$ ,  $n=9-13$ ). Data are shown as mean  $\pm$  SEM and individual data points.

## Associative learning tunes cortical inputs only on adult-born granule cells

We next sought to determine whether these responses could be modified by prior olfactory learning. For that, after stereotaxic injections as previously performed (Figure 2A, B), we used an operant conditioning task in custom built olfactometers where mice learnt to discriminate a reinforced odor, associated with a water reward (positive stimulus: S+), against a non-rewarded one (negative stimulus: S-; Figure 2C). This go/no-go task was also performed in one group with a randomly given reward, which prevented the association with a specific odorant but exposed mice to the same odorants and reward (called pseudo-learning group). Animals were exposed daily to a pair of odorants (200 trial per day for 21 days). Learning group mice showed a rapid learning of discrimination, gradually reaching the performance criterium of 85% for 3 different pairs of odors (Example in Figure 2D, Supplementary figure 1A-B), while pseudo-learning ones remained at the 50% chance level, as expected. For both neonatal and adult-born groups, performances were similar in terms of the learning rate (Figure S1A-B). Meanwhile, control mice were not exposed to odors neither to reward and were kept in their home cage throughout the experiment.

Once all learning tasks were completed and 24 hrs after the last training session, patch clamp recordings were performed. Overall, we recorded 311 cells from 42 mice. When recording nnGCs, no changes were observed in excitatory and inhibitory responses from the AOC in both learning and pseudo learning groups respect to control mice (Figure 2E, F). Amplitude of voltage-gated sodium currents (Figure 2G) and AMPA/NMDA ratios (Figure S1C) were not modified by learning or pseudo-learning. When recording abGCs, we found no difference in the amplitude of EPSCs (Figure 2H) or in AMPA/NMDA ratios between groups (Figure S1C). However, we showed that amplitude of IPSCs greatly increased on P60-generated cells after olfactory learning in respect to both pseudo-learning and control animals (Figure 2I). Finally, the amplitude of voltage-gated sodium currents decreased in the learning and pseudo-learning groups (Figure 2J) in respect to control mice.

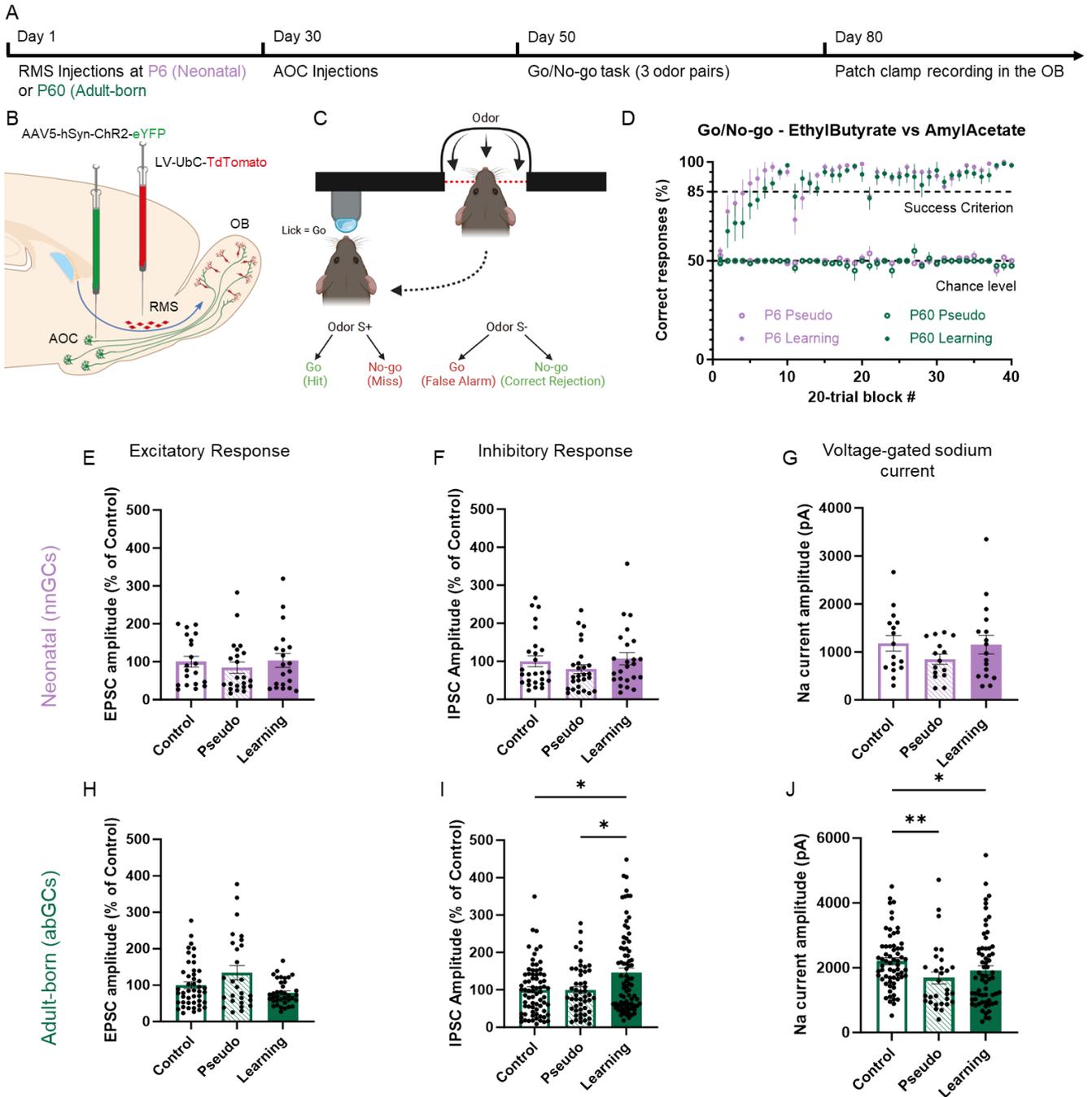


Figure 2. Associative learning tune cortical inputs only on adult-born granule cells. A. Timeline of the experiments. B. Stereotaxic injections of TdTomato-expressing virus in the Rostral Migratory Stream (RMS) at neonatal (P6) or adult (P60) age and ChR2-expressing virus in the Anterior Olfactory Cortex (AOC). C. Schematic description of the Go/No-go olfactory task. D. Example of discrimination learning performance across days. E-G. Responses elicited in neonatal granule cells. E. Light-evoked EPSCs amplitude (Kruskal-Wallis test,  $p=0.5387$ ,  $n=20-22-20$ ). F. Light-evoked IPSCs amplitude (Kruskal-Wallis test,  $p=0.2993$ ,  $n=26-27-24$ ). G. Sodium current amplitude (Brown-Forsythe analysis,  $p=0.2854$ ,  $n=16-15-17$ ). H-J. Responses elicited in adult-born granule cells. H. Light-evoked EPSCs amplitude (Kruskal-Wallis test,  $p=0.2235$ ,  $n=46-27-37$ ). I. Light-evoked IPSCs amplitude (Kruskal-Wallis test,  $p=0.0196$ ; Dunn's test, Control vs Pseudo,  $p=0.9615$ ; Control vs Learning,  $p=0.0137$ ; Pseudo vs Learning,  $p=0.0237$ ,  $n=73-50-80$ ). J. Sodium current amplitude (Kruskal-Wallis test,  $p=0.0077$ ; Dunn's test, Control vs Pseudo,  $p=0.0040$ ; Control vs Learning,  $p=0.0240$ ; Pseudo vs Learning,  $p=0.2808$ ,  $n=63-30-64$ ). Electrophysiology data is expressed as % of control mean value. Data is shown as mean  $\pm$  SEM and individual data points. OB: Olfactory Bulb.

## Adult born granule cells display more inhibitory synapses with cortical terminals after learning

We then decided to focus on the increase of inhibition observed after odor-reward association in adult-born neurons. To specifically label and manipulate GABAergic feedback, for the rest of this work, we will employ a conditional labelling approach. For that, we used the same injection protocol as previously described, but this time using a conditional AAV-Cre-dependent-ChR2-YFP in *vGAT::Cre* mice in order to specifically label GABAergic AOC cells (Figure 3A-B).

Using this approach, we first checked whether the observed effect of learning on inhibition was also present in *vGAT::Cre* mice. To refine our results, we isolated minimal and maximal responses in the recorded GCs by using incremental light pulse duration (from 0.1 to 6.4ms) (Figure 3H). Minimal responses were collected at the shortest time duration eliciting synaptic currents for both inhibitory. Most of the time, some failures are still occurring at that stage. Maximal responses were collected when the amplitudes of the responses reached a plateau. We found that both minimal (Figure 3E) and maximal (Figure 3F) light-evoked responses were increased by olfactory learning, confirming and extending our previous data on WT mice (see Figure 2I). Moreover, the Max/Min ratio was also higher in these cells, hinting that a greater number of synapses could be involved in the cortical feedback after learning (Figure 3G).

To probe the morphological correlates of functional changes, we performed histology on fixed slices to count the number of synapses according to their location on the abGCs cellular domains (Figure 3C-D). By using the same injection protocol, we observed that GABAergic cortical top-down axonal boutons (in green) made putative synapses on TdTomato+ abGCs (in red; Figure 3C). In addition, to validate the presumptive inhibitory inputs on abGCs, we labelled and quantified the presence of vesicular GABA transporter (VGAT, in grey), a presynaptic protein essential for GABA accumulation into synaptic vesicles.

First, we analyzed the distribution of presumptive inhibitory inputs throughout the dendritic tree and soma of GCs (Figure 3I). For that, GC dendritic trees were subdivided into five compartments: basal, somatic, proximal, distal, and apical domains (Figure 3D). The proximal compartment was defined as the first 30 $\mu$ m of the main dendrite starting from the soma, the

distal compartment as the adjacent segment starting 30 $\mu$ m away but still within the GCL, and the apical compartment as the dendritic arbor located in the EPL beginning at the first dendritic branch point, as previously performed (Lepousez *et al.*, 2014b). In our samples, basal dendrites were difficult to visualize – probably due to a reduced expression of TdTomato in this compartment – and were not included in our analysis.

Our results showed that putative GABAergic inputs (vGAT<sup>+</sup>/TdTomato<sup>+</sup>) are located throughout all cell compartments of abGCs although they are significantly more abundant on dendritic domains in respect to the soma. Moreover, no differences were found between groups suggesting that learning does not change the total amount of GABAergic synapses impinging on mature abGCs. However, when we analyzed the number of VGAT<sup>+</sup> puncta colocalizing with corticobulbar GABAergic fibers (vGAT<sup>+</sup>/TdTomato<sup>+</sup>/eYFP<sup>+</sup>), we found a significant global increase in learning group in respect to control group, mostly due to tendencies observed in both distal and proximal domains.

Our data validate the observed functional changes and suggested that odor-reward association increases the number of GABAergic synapses arriving from cortical long-range inputs onto abGC.

**A**  
 Day 1: RMS and AOC Injections at P60 | Day 30 to 60: Go/No-go task | Day 60: Patch clamp recording or histology

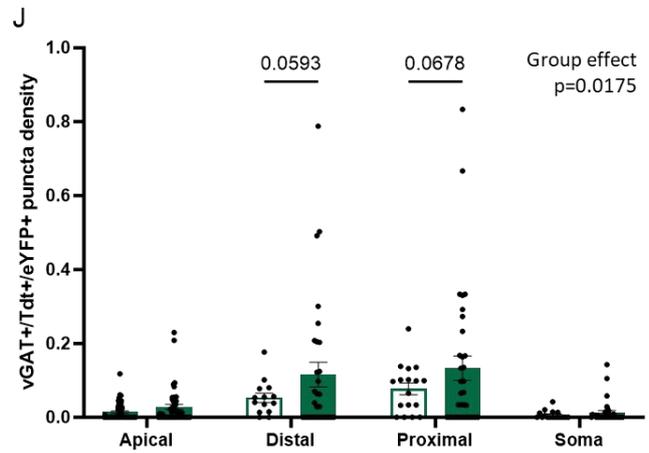
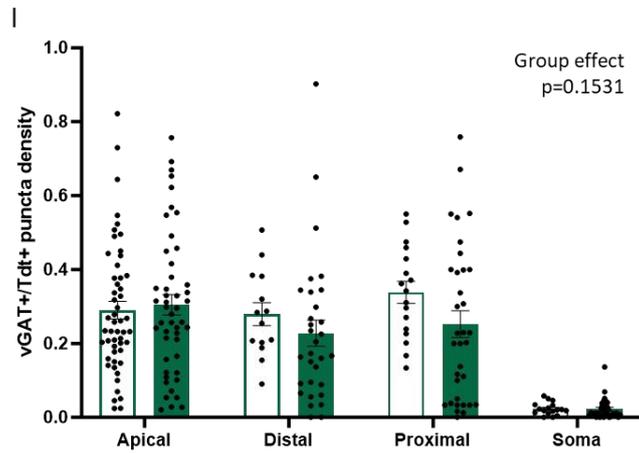
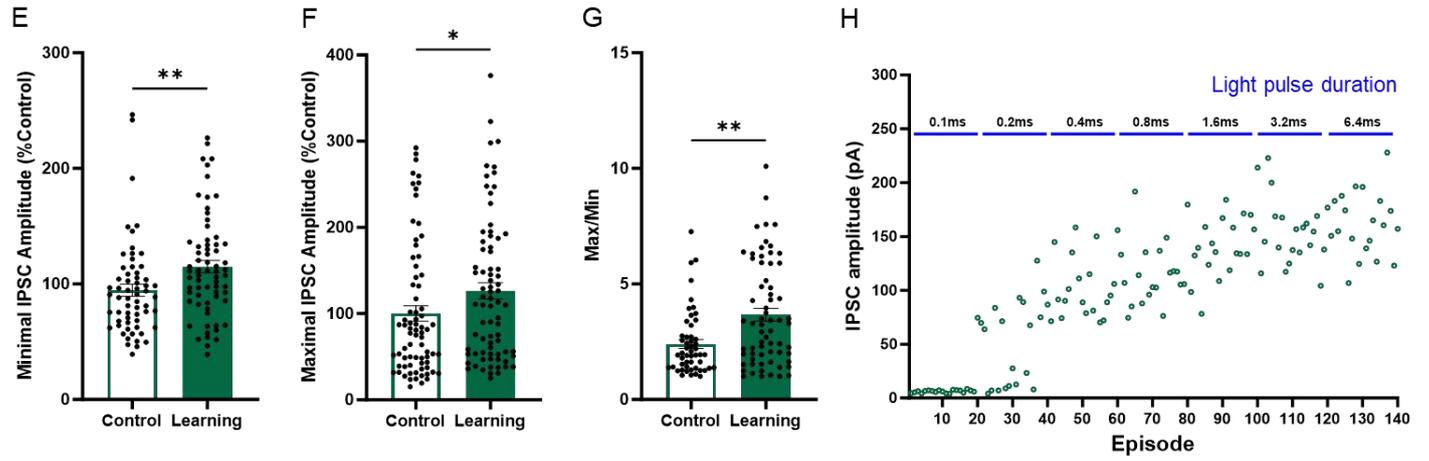
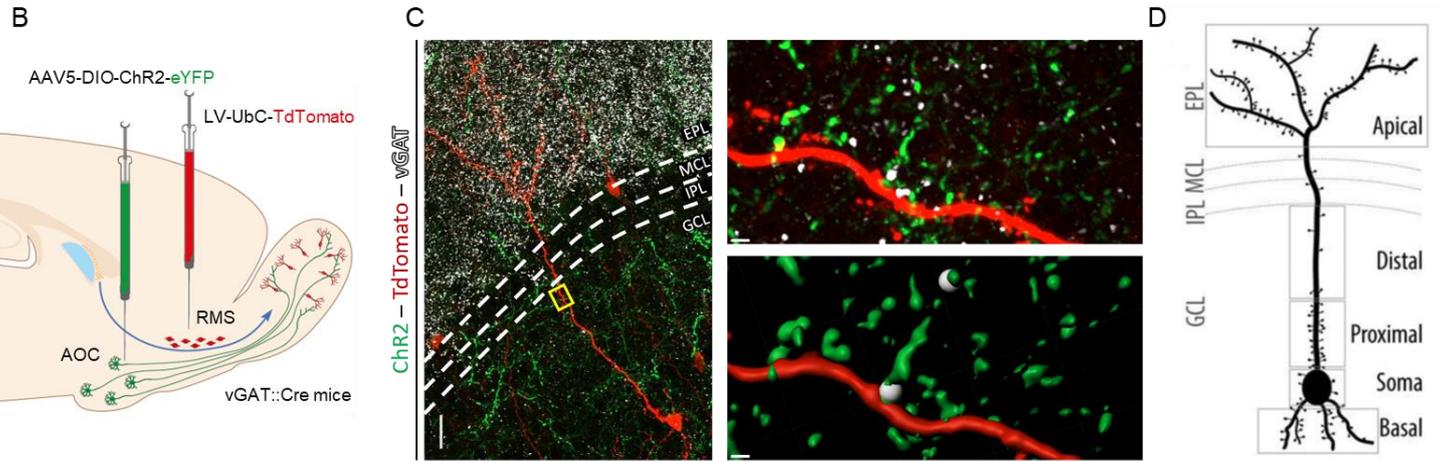


Figure 3. Adult born granule cells display more inhibitory synapses with cortical terminals after learning. A. Timeline of the experiments. B. Stereotaxic injections of TdT-expressing virus in the Rostral Migratory Stream (RMS) at adult age and ChR2-expressing virus in the Anterior Olfactory Cortex (AOC). C. Left, Example image of confocal images obtained after immunolabeling of adult-born GCs (Tdtomato, red), Cortical inhibitory afferences (eYFP, green) and VGAT synaptic protein (grey). Right, Zoomed in view of the yellow rectangle of the left panel (Top) and Imaris® 3D reconstruction of this same view (Bottom). (Scale bar 10 $\mu$ m (Left) and 2 $\mu$ m (Right)). D. Schematic representation of GCs anatomical segmentation. E. Minimal light-evoked IPSCs amplitude (Mann-Whitney test,  $p=0.0017$ ,  $n=61-67$ ). F. Maximal light-evoked IPSCs amplitude (Mann-Whitney test,  $p=0.0202$ ,  $n=75-77$ ). G. Maximal/minimal ratio (Mann-Whitney test,  $p=0.0019$ ,  $n=52-66$ ). H. Representative example of progressive stimulation intensity and associated inhibitory response amplitudes. I. Density of VGAT+ puncta (grey) in the different dendritic domains of abGC (red) ( $n=100-147$  dendritic segments per condition,  $n=3-7$  mice). The somatic density is per 10 $\mu$ m<sup>2</sup>, all other domains are per  $\mu$ m (Mixed-effect analysis, group effect  $p=0.1531$ ; domain effect  $p<0.0001$ ). J. VGAT+ puncta (grey) colocalized with GABAergic projecting axons (green) in different dendritic domains of abGC (red). The somatic density is per 10 $\mu$ m<sup>2</sup>, all other domains are per  $\mu$ m. (Mixed-effect analysis, group effect  $p=0.0175$ ; domain effect  $p<0.0001$ ; Apical,  $p=0.5025$ ; Distal,  $p=0.0593$ ; Proximal,  $p=0.0678$ ; Soma,  $p=0.8281$ ). Data are shown as mean  $\pm$  SEM and individual data points.

## Water deprivation promotes short-term depression at cortical GABAergic synapses

To complete our understanding of learning-induced synaptic changes between inhibitory cortical fibers and abGCs, we then looked at presynaptic properties by analysing short-term dynamics of the responses during light stimulation at various frequencies. We used 10Hz (Figure 4B-D) and 33Hz (Figure 4E-G) frequency stimulations as a representation of sniffing-related theta oscillations (4-12Hz) and learning-related beta frequencies (15-40Hz), respectively.

First, we observed that 10Hz stimulations exhibited a slight decrease of response amplitude directly after the 1<sup>st</sup> stimulation, which stabilized after the second one (Figure 4B). However, this short-term depression was accentuated in both pseudo-learning and learning conditions, suggesting an increased probability of GABA release in these conditions (Figure 4C-D). However, the exact same result was observed in abGCs of mice that were simply water-deprived for the same amount of time as learning ones. Thus, changes in presynaptic properties of cortical inhibitory afferences to abGCs seem to be caused at least by water-deprivation of the individual, but probably not odor exposure or learning mechanisms.

Looking at 33Hz stimulations, the same effect was observed with a quick short-term depression, more prominent in water-deprived, pseudo-learning and learning conditions compared to control (Figure 4F-G).

In this context, we also controlled that water-deprivation is not the reason for the previous observed differences in amplitude (see Figure 2I and Figure 3E, F), since control mice did not undergo deprivation. Although we confirmed that chronic water deprivation caused a significant weight loss across a long period of time (up to 35 days, Supplementary Figure 2B), similar to the one necessary for the Go/No-go task, it did not affect minimal, nor maximal inhibitory responses received by abGCs (Supplementary Figure 2C-D).

Taken together, our data show that olfactory learning specifically tunes inhibition arriving from the AOC specifically on the abGCs, but not in neonatal ones. Moreover, changes in inhibition might be mediated partly by an increase in the number of synapses with long-range

corticobulbar GABAergic inputs originated in the recently described neurons located in the AONp (Mazo *et al.*, 2022).

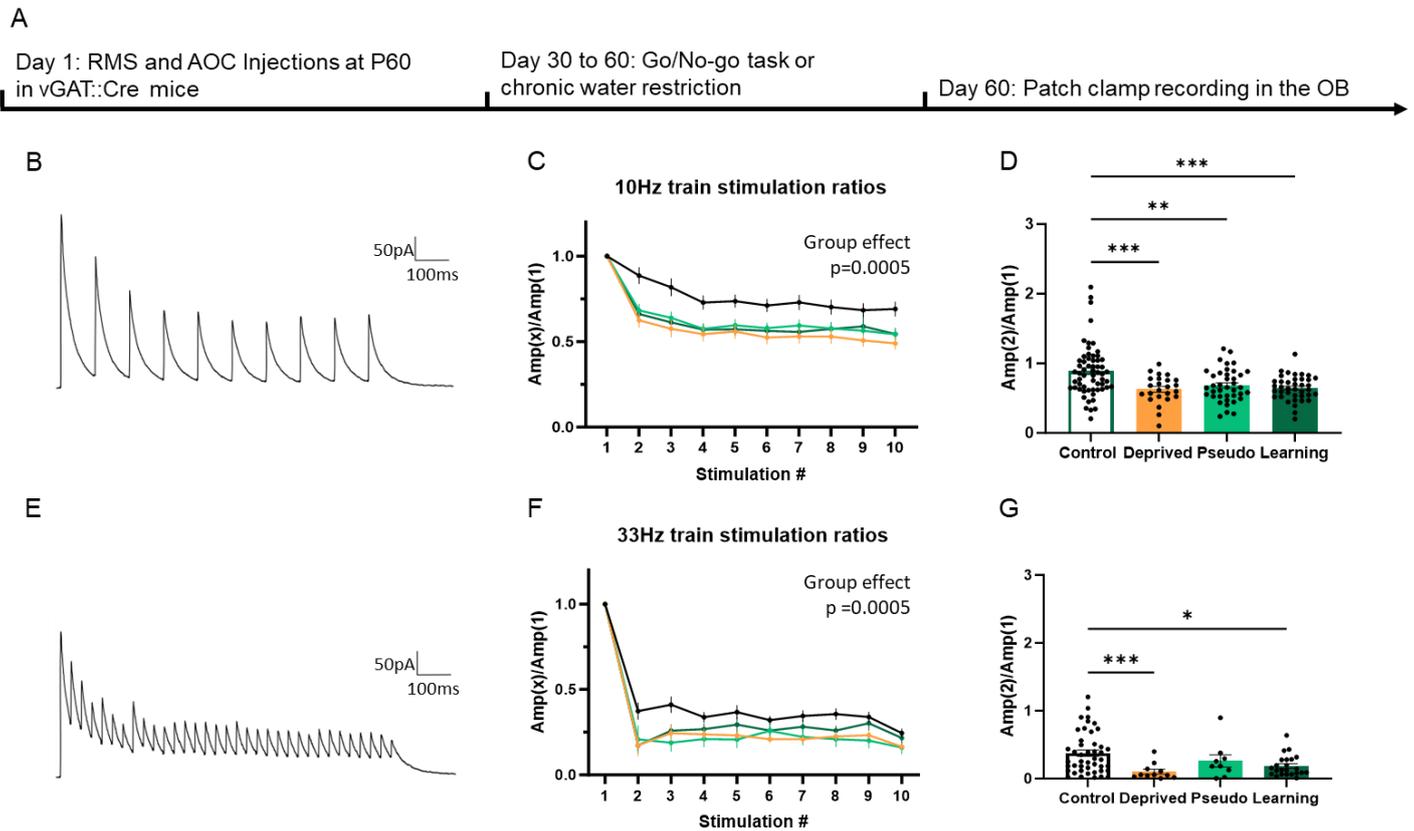


Figure 4. Water deprivation promotes short-term depression at cortical GABAergic synapses. A. Timeline of the experiments. B-D. 10Hz train stimulations of inhibitory inputs. B. Example trace of 10Hz train-stimulation of adult-born granule cells (5ms pulse). C. Amplitude ratios for each stimulation of a 10Hz train, normalized by first response amplitude (Two-way ANOVA,  $p=0.0005$  for group factor). D. Amplitude ratio of the second stimulation of 10Hz trains (Kruskal-Wallis test,  $p=0.0001$ ; Dunn's test, Control vs Deprived,  $p=0.0008$ ; Control vs Pseudo,  $p=0.0023$ ; Control vs Learning,  $p=0.0001$ ,  $n=60-24-39-39$ ). E-G. 33Hz train stimulations of inhibitory inputs. E. Example trace of 33Hz train-stimulation of adult-born granule cells. F. Amplitude ratios for each of the first 10 stimulations of a 33Hz train, normalized by first response amplitude (Two-way ANOVA,  $p=0.0005$  for group factor). G. Amplitude ratio of the second stimulation of 33Hz trains (Kruskal-Wallis test,  $p=0.0023$ ; Dunn's test, Control vs Deprived,  $p=0.0004$ ; Control vs Pseudo,  $p=0.2805$ ; Control vs Learning,  $p=0.0199$ ,  $n=45-11-9-23$ ). Data are shown as mean  $\pm$  SEM and individual data points.

## GABA<sub>B</sub> and CB1 receptors modulate GABAergic cortical synapses to adult-born granule cells

We know that cortical glutamatergic inputs onto GCs are modulated by GABA<sub>B</sub> (Mazo *et al.*, 2016) and CB1 receptors (Soria-Gómez *et al.*, 2014). We sought to determine their respective activity at cortical GABAergic synapses. Using the same protocol, without prior learning, we applied agonists and antagonists for GABA<sub>B</sub> and CB1 receptors and measured the IPSCs amplitude. We observed that GABA<sub>B</sub> (Figure 5A, B) and CB1 (Figure 5D, E) receptors agonists (100µM Baclofen and 10µM WIN 55212-2, respectively) induced strong decrease in light evoked IPSCs amplitude. Conversely, antagonists of GABA<sub>B</sub> (5µM CGP52432) and CB1 (10µM AM251) restored the amplitude measured in plain ACSF. Thus, Both GABA<sub>B</sub> and CB1 receptor are present at the synapse between cortical GABAergic terminals and abGCs in the OB. However, a direct application of antagonists (5µM CGP52432 or 10µM AM251) did not alter IPSCs amplitude, ruling out any basal activation of CB1 or GABA<sub>B</sub> receptors at this synapse under control conditions (Figure 5C, F, respectively).

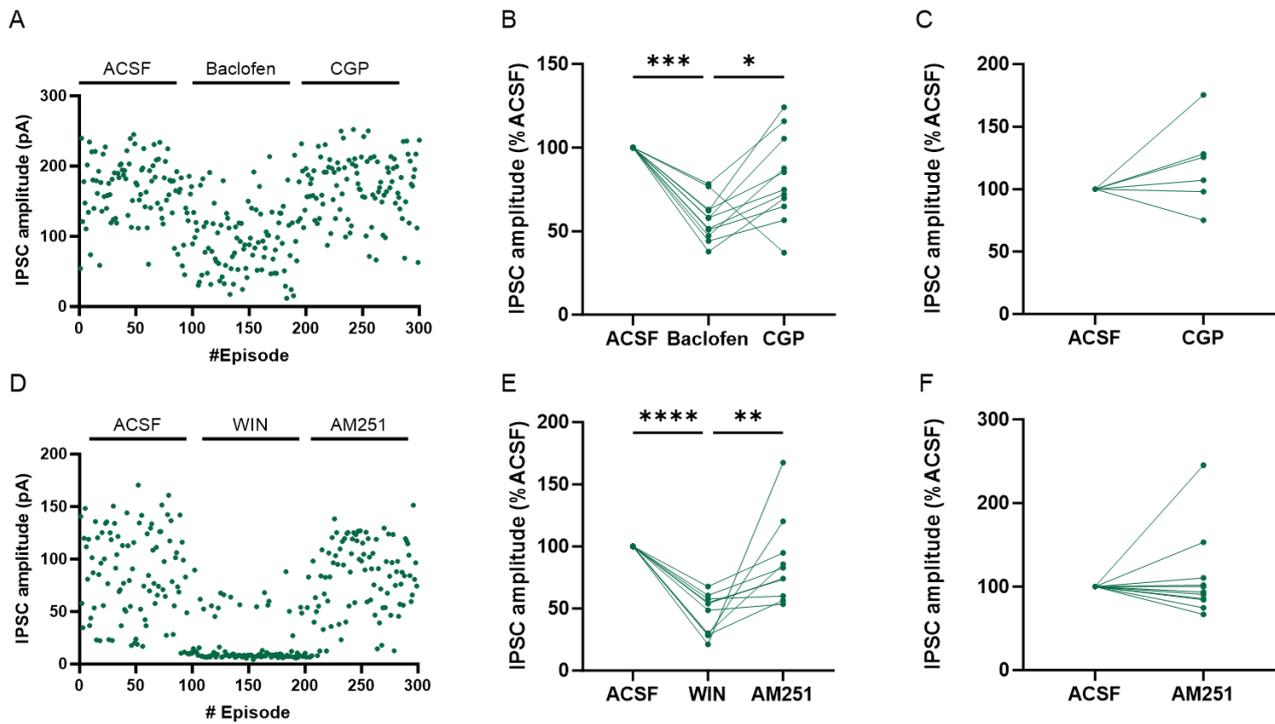


Figure 5. GABA<sub>B</sub> and CB1 receptors modulate GABAergic cortical synapses to adult-born granule cells. A-C. GABA<sub>B</sub>R modulation of IPSCs. A. Example amplitudes measured in voltage clamp recordings. B. Effect of GABA<sub>B</sub>R receptor agonist (Baclofen) and antagonist (CGP) on IPSCs amplitude (Friedman test,  $p < 0.0001$ ; ACSF vs Baclofen,  $p = 0.0001$ ; ACSF vs CGP,  $p = 0.2008$ ; Baclofen vs CGP,  $p = 0.0105$ ,  $n = 11$ ). C. Effect of GABA<sub>B</sub>R receptor antagonist (CGP) on inhibitory response amplitude (Wilcoxon test,  $p = 0.2188$ ,  $n = 6$ ). D-F. Effect of CB1R modulation on IPSCs amplitude. D. Example amplitudes measured in voltage clamp recordings. E. Effect of CB1 receptor agonist (WIN) and antagonist (AM251) on inhibitory response amplitude (Friedman test,  $p < 0.0001$ ; ACSF vs WIN,  $p < 0.0001$ ; ACSF vs AM251,  $p = 0.1797$ ; WIN vs AM251,  $p = 0.0073$ ,  $n = 10$ ). F. Effect of CB1 receptor antagonist (AM251) on inhibitory response amplitude (Wilcoxon test,  $p = 0.7002$ ,  $n = 11$ ).

## Chapter II. Physiological and behavioral relevance of GABAergic centrifugal fibers in the posterior AON

In the next part of this study, we intended to understand the role of these GABAergic afferences in behaving mice, especially during olfactory learning. We recently uncovered a corticofugal inhibitory feedback to OB, originating from a subpopulation of GABAergic neurons in the AON (Mazo et al, 2022). In vivo imaging and network modeling showed that optogenetic activation of cortical GABAergic projections drives a net subtractive inhibition of both spontaneous and odor-evoked activity in local as well as output neurons.

### GABAergic top-down projections from the Anterior Olfactory Nucleus are activated during Go/No-go task

First, we aimed to decipher the activity pattern of centrifugal fibers originating in the AONp during odor encounter and associative learning. For this, we injected a Cre-dependent GCaMP8f-expressing viral vector in AONp of vGAT::Cre mice and implanted optic fibers above the olfactory peduncle (Figure 6 A-B), thus allowing us to perform fiber photometry calcium activity recordings specifically of passing cortico-bulbar inhibitory projections while mice performed a Go/No-go task (Figure 6C).

Although further analysis and quantifications are need, in these preliminary results we observed that AONp fibers were activated in naïve mice already when entering the odor port (Figure 6D and E, -1 to 0s) and throughout odor presentation (Figure 6D and E, 0 to 1s), with no clear difference between S+ correct response (Hit, Figure 6D) and S- errors (False Alarm; Figure 6E). Intriguingly, after a week of training, overall activation seems to be higher when in the odor port, and still decreasing after head removal for both S+ (Figure 6F) and S- (Figure 6G).

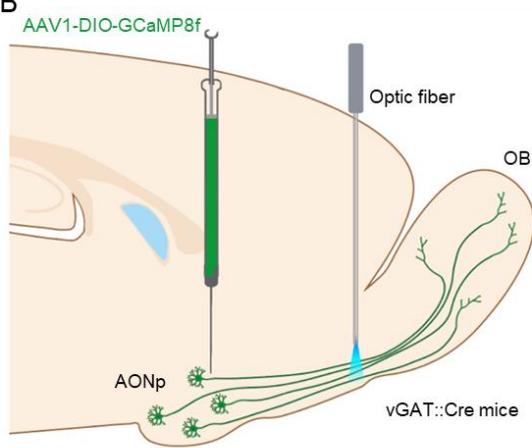
However, recording in an expert mouse (more than a month of training prior recordings) showed an intense plateau of activation for the whole time the head was in the odor port, with apparent hypoactivity afterwards, again similar for S+ (Figure 6H) and S- (Figure 6I).

A

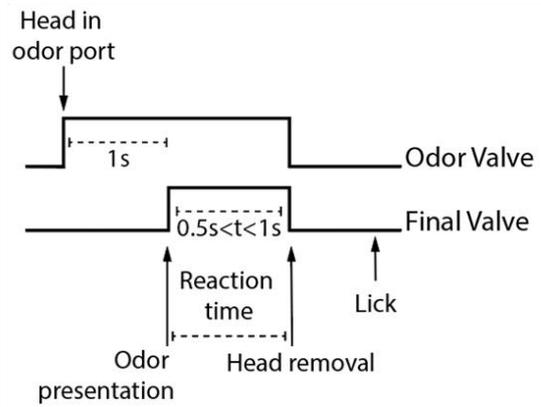
Day 1: AONp injections and fibers implantation

Day 30 to 50: Go/No-go task

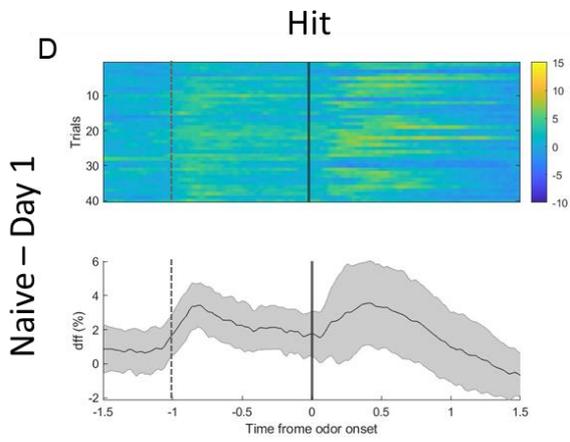
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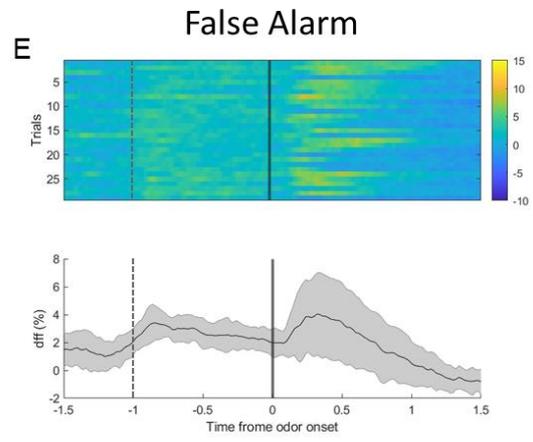
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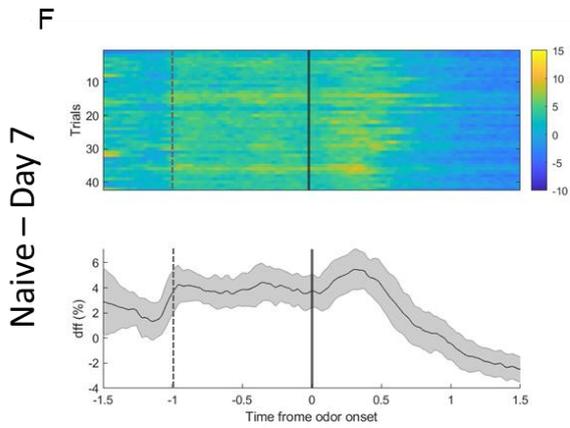
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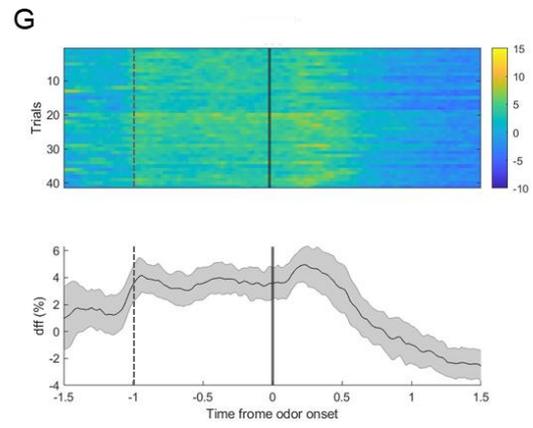
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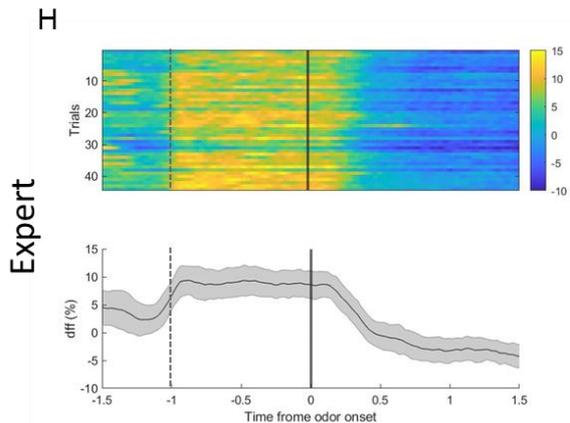
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H



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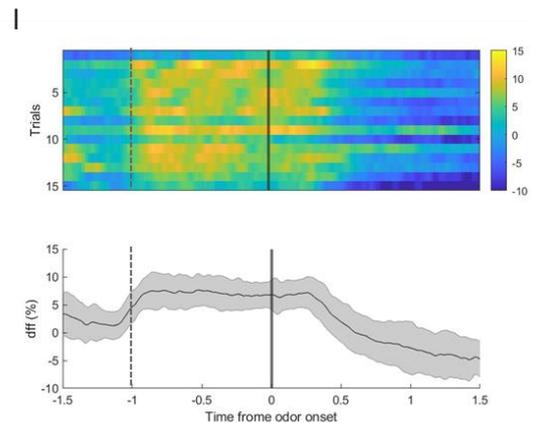


Figure 6. AONp GABAergic projections are activated during Go/No-go task. A. Timeline of the experiments. B. Stereotaxic injection of GCaMP8f vector in AONp and optic fiber implantation above the olfactory peduncle to allow recording of fibers projecting to the olfactory bulb. C. Timeline of olfactory learning trials. D-E. Signal recording in a representative naive mouse on the first day of discrimination learning (Hexanol vs Octanal, 1/100) during "Go" response after S+ (Hit, D) or S- (False Alarm, E) trials. F-G. Signal recording in a representative naive mouse on the seventh day of discrimination learning (Hexanol vs Octanal, 1/100) during Hit (F) and False Alarm (G) trials. H-I. Signal recording in a representative expert mouse during discrimination learning (Hexanol vs Octanal, 1/100) during Hit (H) and False Alarm (I) trials. Data are shown as individual trails  $dF/F$  (Upper panels) and mean  $\pm$  SD (Lower panels).

## Optogenetic inhibition of AONp GABAergic neurons impairs fine odor detection and learning but not memory

We next sought to manipulate GABAergic corticobulbar fibers to probe whether changes in the activity of these inputs are sufficient to alter animal behavior. We tried to understand their contribution to olfactory learning by inhibiting them during odor presentation. To do so, we used optogenetic inhibition through the expression of JAWS-YFP protein (Jaws group) exclusively in GABAergic neurons of the AONp by using vGAT::CRE mice. This approach results in a cohort of light-sensitive neurons projecting to the OB. To stimulate light-sensitive neurons *in vivo*, all the mice were implanted bilaterally with optic fibers on top of the olfactory peduncles, as we previously described, to allow yellow light stimulation (Figure 7A, B). Control mice were injected with AAV-expressing GFP only (control group).

In a first initiation task without light stimulation, using the operant conditioning task previously described, both control and Jaws groups performed similarly, which allowed us to discard potential off-target effects of viral vector expression (Figure 7C). Once animals reached the criterion, they were trained in the same task while receiving a continuous light stimulation beginning at the odor onset and lasting for 2 seconds (Figure 7C; see timeline in Figure 7E). This duration was set taking into account the mean reaction time measured for this task (Figure 7H) and to prevent an effect of rebound activation in Go/No-go decision (Chuong *et al.*, 2014).

Light stimulation did not modify performance of already learned task (Figure 7C), indicating that inhibition of GABAergic fibers did not alter odor discrimination acuity. However, Jaws mice displayed significantly reduced number of Hit when suprathreshold low concentrations of the rewarded odorant were presented as catch trials (Figure 7E), supporting the role of long-range GABA inputs in fine odor detection.

Next, we presented a new, more difficult couple of odors ((+)-Limonene vs (-)-Limonene, 1/200) while stimulating during the whole learning phase. We found that JAWS-expressing mice displayed a significant impairment in learning this task (Figure 7F). Although all the mice learned the task to criterion, mice spent more effort to learn the discrimination when JAWS-positive corticobulbar fibers were inhibited, as reflected in the number of blocks required to

reach criterion (Figure 7G). No difference was apparent in reaction time or first lick between groups (Figure 7H).

This result suggests a predominant role of GABAergic inputs in the early phases of the odor–reward association. To challenge this idea, animals were trained in a reversal version of the Go/No-go task. Mice had to reverse the values associated with an odor pair that they had already learnt. For this, the odorant previously associated with a reward was unreinforced and vice versa (Figure 7I, J). As expected, during the first few blocks of the reversal task, all animals performed below the chance level, as they kept following the previous odor–reward association rule. However, the adjustment to the new odor–reward association was not affected by GABAergic fibers inhibition (Figure 7I, J). Finally, we analysed whether light inhibition also altered olfactory memory. For these experiments, animals were trained until reaching success criterion while GABAergic fibers were activated as previously described. Then, mice were tested for odor memory recall 24hs (Figure 7K) and 2 weeks (Figure 7L) after the end of the training session without light stimulation. The data was normalized to last blocks performance during training to rule out differences due to learning acquisition. Once again, memory was not altered in the Jaws group respect to control mice.

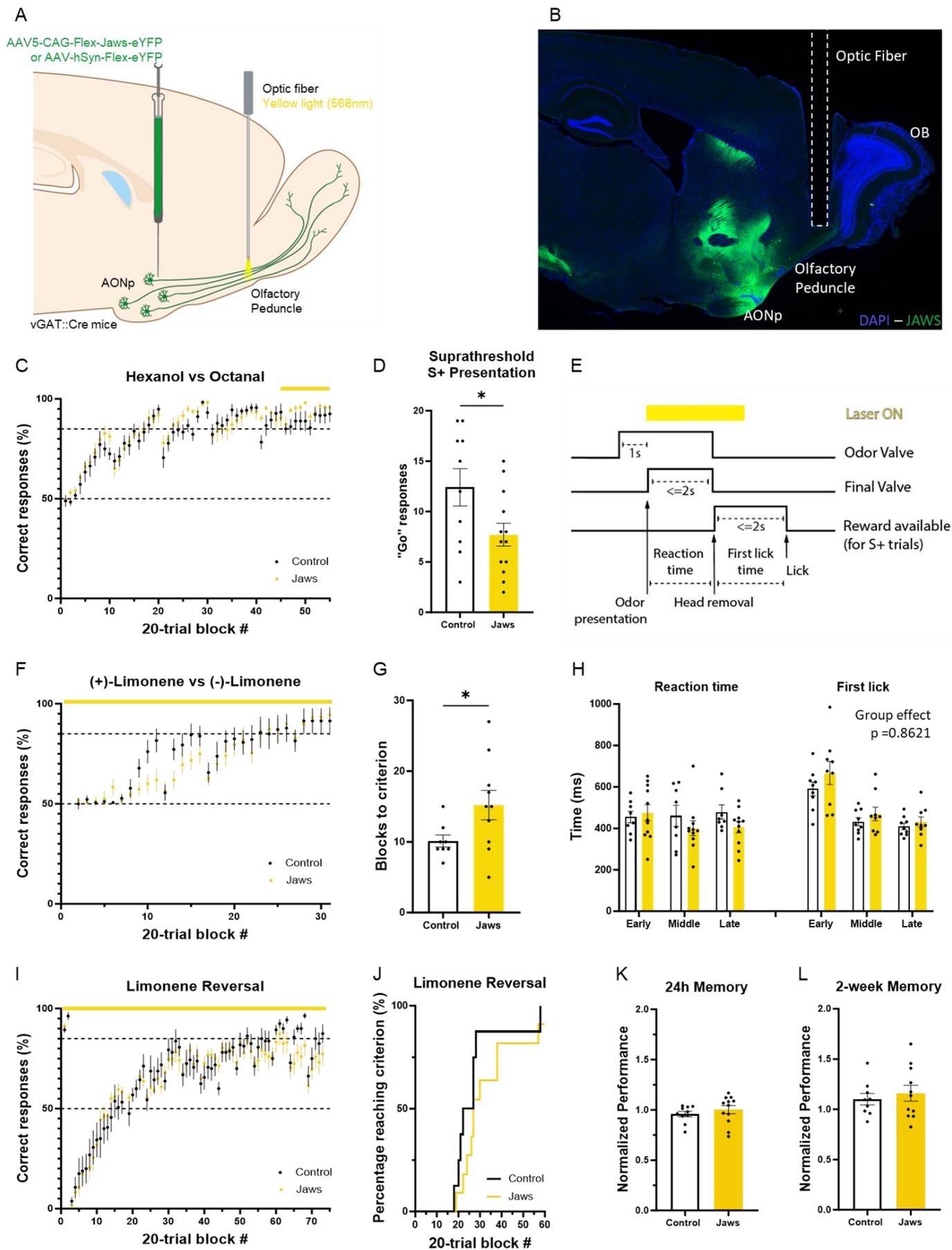


Figure 7. Optogenetic inhibition of AONp GABAergic projections impairs fine odor detection and learning but not memory. A. Stereotaxic injection of JAWS-expressing or control vector in AONp and optic fiber implantation above the olfactory peduncle to allow stimulation of fibers projecting to the olfactory bulb. B. Histological verification of viral vector expression and optic fiber implantation. C. Learning performance of a discrimination task (Hexanol vs Octanal, 1/100) (Mixed effect analysis,  $p=0.8107$ ,  $n=10-13$ ). Yellow band represents trials when continuous light was ON. D. Number of “go” responses when a suprathreshold ( $10^{-4}$ ) S+ odor was presented (Unpaired t-test,  $p=0.0333$ ). E. Timeline of light stimulation during odor presentation in each trial. F. Learning performance of a second discrimination task ((+)-Limonene vs (-)-Limonene, 1/200) (Mixed-effect analysis,  $p=0.0184$ ). Yellow light stimulation was ON across all trials G. Number of blocks necessary to reach the 85% performance criterion in difficult learning task (Welch’s t-test,  $p=0.0429$ ). H. Mean reaction and first lick time for “Hit” trials in the limonene task (Mixed-effect analysis, group effect,  $p=0.8621$ ). I. Discrimination performance after reversal of the limonene learning task (S- becomes rewarded and S+ is no longer rewarded) (Mixed-effect analysis,  $p=0.4178$ ). J. Cumulative curve of mice reaching the 85% criterion in reversal task across time (Mantel-Cox test,  $p=0.2966$ ). K. Mean performance for 40 trials 24 hours after the last reinforcement (No reward, no stimulation) (Unpaired t-test,  $p=0.3949$ ). L. Mean performance for 40 trials 2 weeks after the last reinforcement (No reward, no stimulation) (Unpaired t-test,  $p=0.5636$ ). Data are shown as mean  $\pm$  SEM and individual data points.

## Optogenetic activation of AONp GABAergic fibers improves fine odor detection but disrupts odor-reward association

We then logically turned to optogenetic activation. We used Cre-dependent ChR2 expression in AONp inhibitory neurons that we stimulated by shedding blue light directly onto the passing axons in the peduncles, as previously described (Figure 8A-B). Light was triggered on odor onset and neurons were stimulated at 33Hz. This frequency was selected based on previous results showing that stimulation at this frequency increased beta synchronization in the OB (Mazo *et al.*, 2022).

Here, we found that similarly to inhibition, no effect was observed once a discrimination rule was already acquired, demonstrating that stimulation protocol does not disturb discrimination acuity or behavioural performance (Figure 8C). However, odor detection seemed increased by light stimulation, reflected by an increase in hits during catch trials using low suprathreshold concentrations (Figure 8D).

Intriguingly, stimulation of AONp GABAergic feedback had the same effect as inhibition, with an impairment in learning the discrimination task (Figure 8F) reflected by a significant increase in the number of blocks necessary to reach the criterion (Figure 8G). Again, no difference was apparent in reaction time or first lick between groups (Figure 8H).

Furthermore, contrasting the effect of inhibition, AONp fibers activation disrupted the ability of ChR2 mice to reverse the learnt rule (Figure 8I). Individuals quickly went back to chance level performance but almost never managed to reach the success criterion with the new rules (Figure 8J).

Finally, no effect of stimulation during learning was observed in memory formation since performance remained similar after 24 hours (Figure 8K) and 2 weeks (Figure 8L). Together with data obtained by inhibiting corticobulbar GABAergic inputs, these fibers do not seem necessary for olfactory memory encoding.

Collectively, these results point to the specific involvement of GABAergic corticobulbar fibers in fine odor detection. In addition, our data suggest that a physiological activation regime of

these fibers is necessary for the initial phases of odor-reward association, although its artificial activation is detrimental for value attribution to rewarded stimuli.

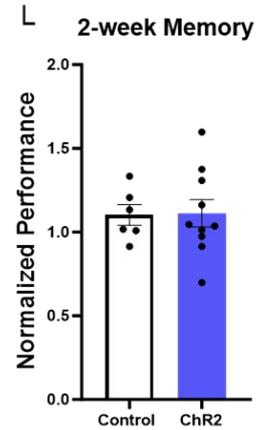
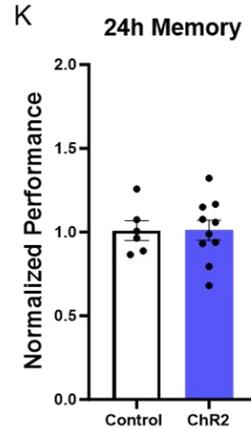
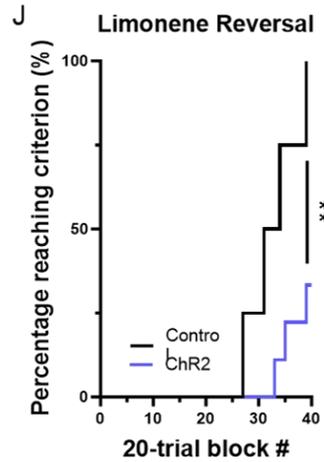
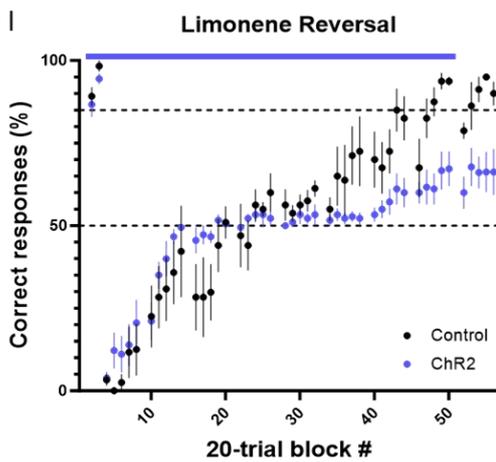
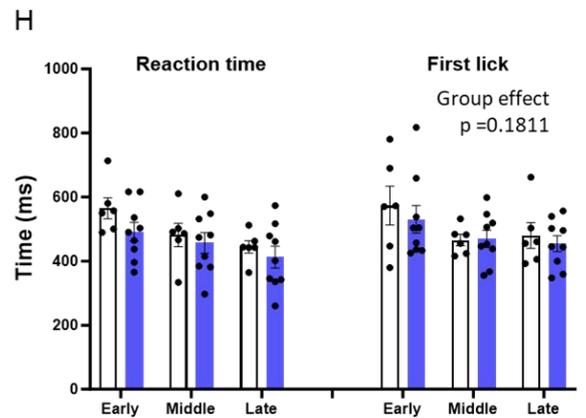
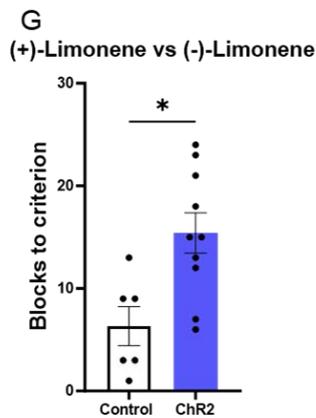
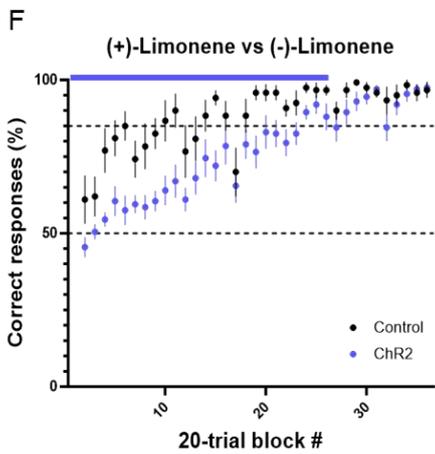
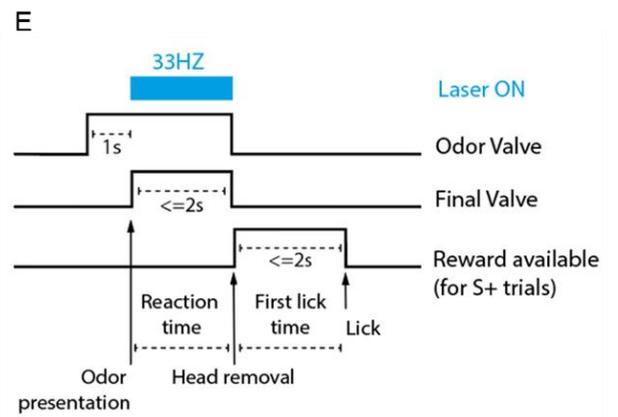
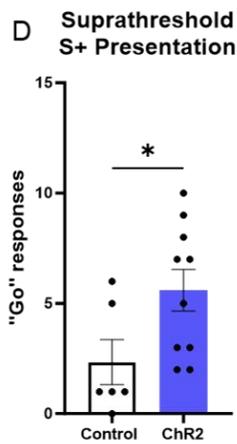
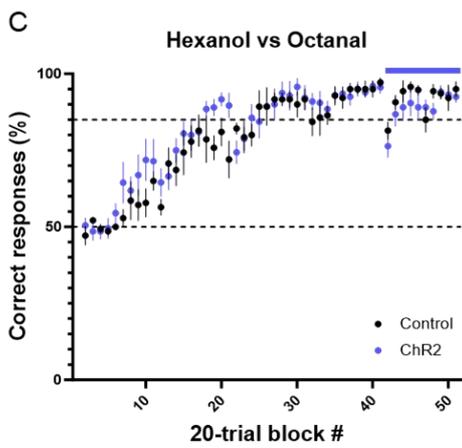
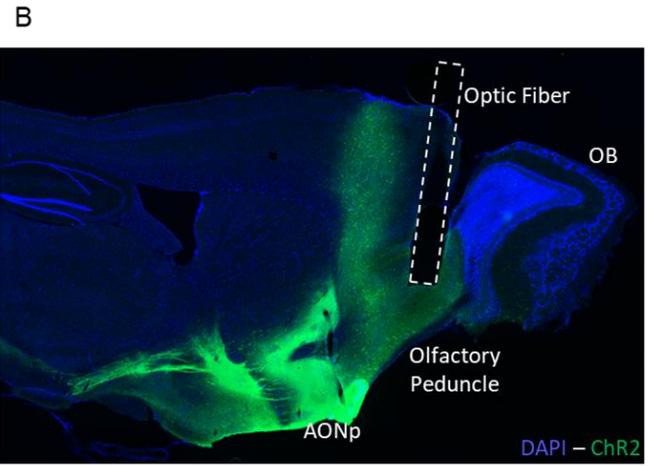
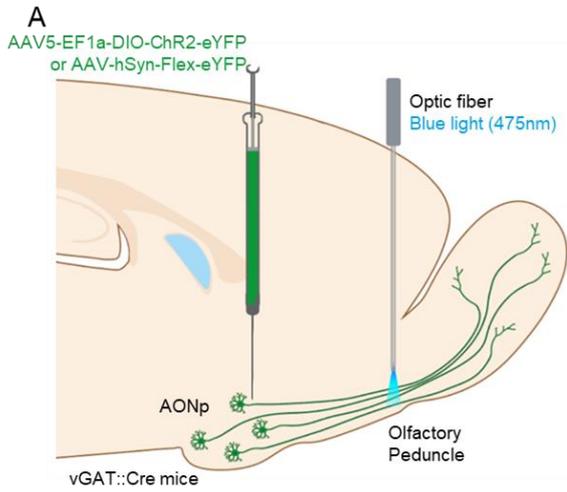
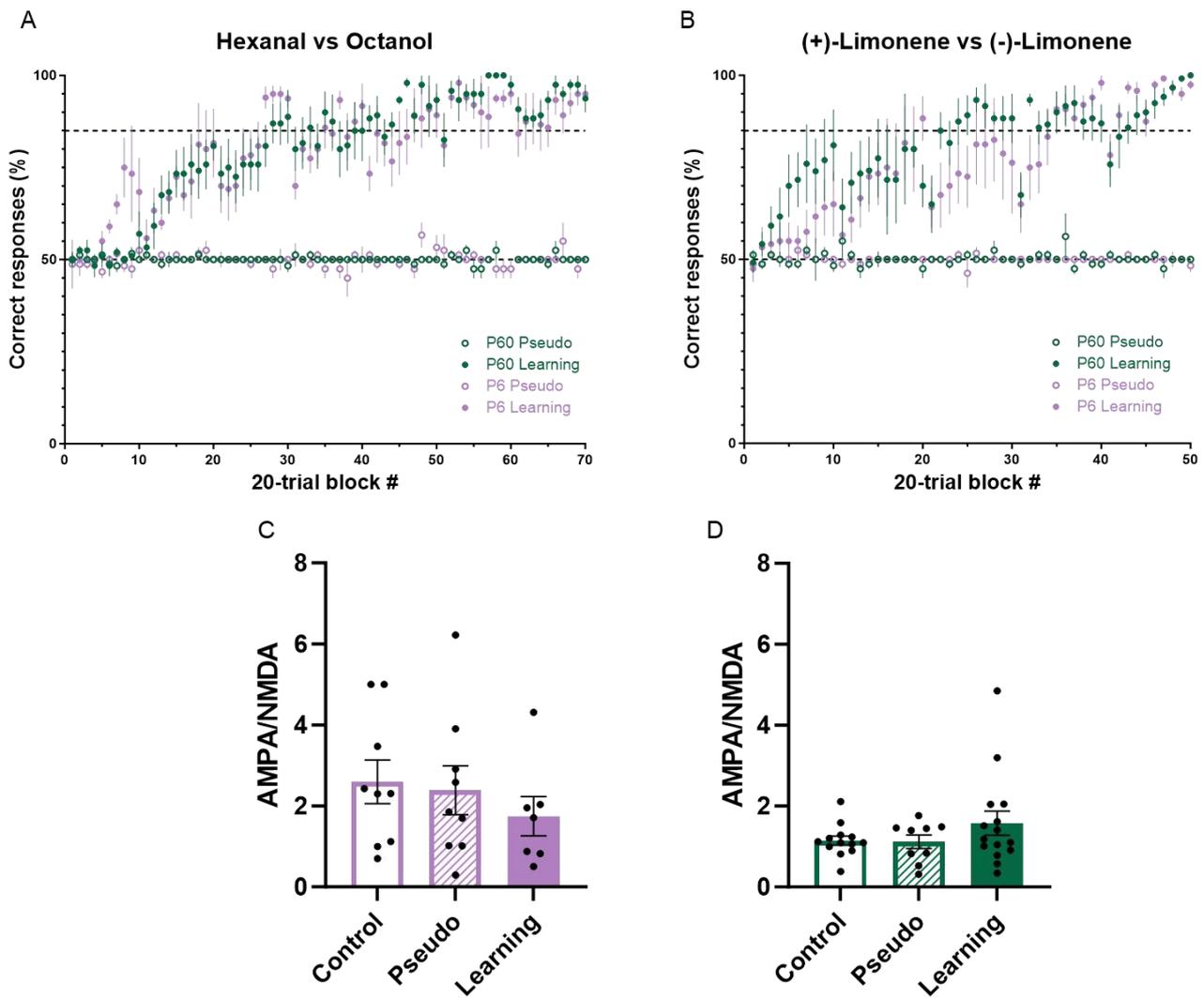
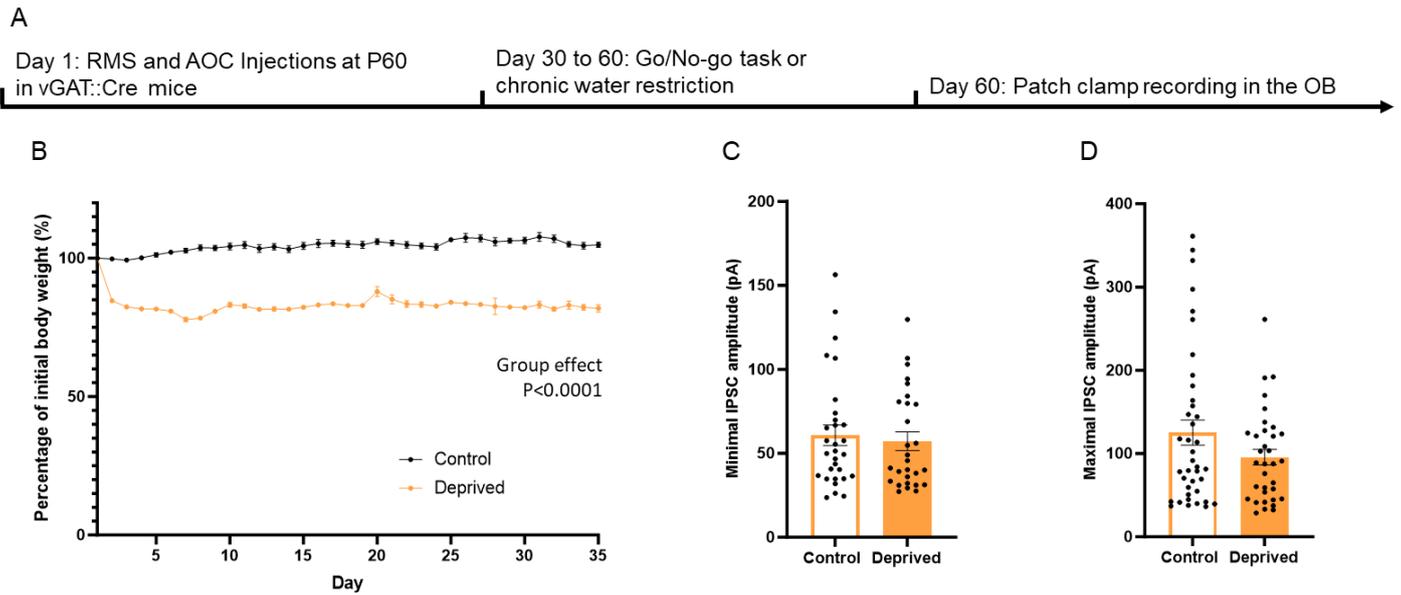


Figure 8. Optogenetic activation of AONp GABAergic fibers improves fine odor detection but disrupts odor-reward association. A. Stereotaxic injection of ChR2 or control vector in AONp and optic fiber implantation above the olfactory peduncle to allow stimulation of fibers projecting to the olfactory bulb. B. Histological verification of viral vector expression and optic fiber implantation. C. Learning performance of a discrimination task (Hexanol vs Octanal, 1/100) (Mixed-effect analysis,  $p=0.2536$ ,  $n=6-10$ ). Blue band represents trials when light was on (33Hz, 5ms pulse duration). D. Number of “go” responses when presented with a suprathreshold ( $10^{-7}$ ) S+ odor (Mann-Whitney test,  $p=0.0236$ ). E. Timeline of light stimulation during odor presentation in each trial. F. Learning performance of a second discrimination task ((+)-Limonene vs (-)-Limonene, 1/200) (Mixed-effect analysis,  $p=0.0003$ ). G. Number of blocks necessary to reach the 85% performance criterion in limonene learning task (Mann-Whitney test,  $p=0.0124$ ). H. Mean reaction and first lick time for “Hit” trials in the limonene task (Two-way ANOVA, group effect,  $p=0.1811$ ). I. Discrimination performance after reversal of the limonene learning task (S- becomes rewarded and S+ is no longer rewarded) (Mixed-effect analysis,  $p<0.0001$ ). J. Cumulative curve of mice reaching the 85% criterion in reversal task across time (Mantel-Cox test,  $p=0.0068$ ). K. Mean performance for 40 trials 24 hours after the last reinforcement (No reward, no stimulation) (Mann-Whitney test,  $p=0.8749$ ). L. Mean performance for 40 trials 2 weeks after the last reinforcement (No reward, no stimulation) (Mann-Whitney test,  $p=0.9813$ ). Data are shown as mean  $\pm$  SEM and individual data points.



Supplementary Figure 1. A-B. Discrimination performance across days for Hexanol/Octanol (A, Mixed-effect analysis for learning conditions,  $p=0.3058$ ) and (+)-Limonene/(-)-Limonene (B, Mixed-effect analysis for learning conditions,  $p=0.2078$ ) discrimination task. C. Neonatal cells AMPA/NMDA Ratio (Kruskal-Wallis test,  $p=0.4463$ ,  $n=9-9-7$ ). D. Adult-born cells AMPA/NMDA Ratio (Kruskal-Wallis test,  $p=0.6687$ ,  $n=13-9-15$ ). Data is shown as mean  $\pm$  SEM and individual data points.



Supplementary Figure 2. Water deprivation is not the cause for the observed effects of olfactory learning. A. Timeline of the experiments. B. Weight monitoring in control versus water-deprived animals (Mixed-effect analysis,  $p < 0.0001$  for group factor). C. Minimal light-evoked IPSCs amplitude (Mann-Whitney test,  $p = 0.5968$ ,  $n = 30-28$ ). D. Maximal light-evoked IPSCs amplitude (Mann-Whitney test,  $p = 0.3998$ ,  $n = 39-34$ ). Data are shown as mean  $\pm$  SEM and individual data points.



# Discussion

## On general results

### Learning-induced plasticity of adult-born neurons

#### General changes in abGCs responses after olfactory learning

Our results using patch clamp recordings demonstrated that fully mature abGCs in the OB displayed higher inhibitory responses to centrifugal fiber stimulation after associative olfactory learning (Mazo *et al.*, 2022) and that postnatally generated cells are not involved in this particular plasticity. This raises many questions about the role of adult neurogenesis and their control by top-down input for olfaction, and in a general neuroscientific view.

Previous studies reported an increase of cortical excitation on abGCs as a result of olfactory learning (Lepousez *et al.*, 2014a; Wu *et al.*, 2020). The fact that we do not observe the same results, although puzzling, can be explained by several differences in protocols. Most importantly, in both previous studies, analyzed cells were younger (32 to 35 days post-injection) than in our experiments (at least 60 dpi). Finally, results by Wu and colleagues were obtained with 2-photon microscopy, which is inherently different from acute slice electrophysiology on both kinetics (due to the use of GCaMP) and amplitude measurements, but allowed for precise follow-up of odor-responsive abGCs along the learning protocol.

#### Synaptic changes of abGCs

Our results show that the increased GABA response after learning may occur through at least 2 mechanisms. Firstly, an increase in the minimal IPSC amplitude (Figure 3), which could be explained by the presence of a greater number of receptors at the synapse. It is unlikely that a change in the presynaptic probability of GABA release explains the specific change observed in the learning condition (Figure 2), given that changes in short-term plasticity were reported in both the pseudo-learning and learning groups and seem to be explained by water

deprivation (Figure 4). Nevertheless, a more detailed analysis of changes in minimal responses should help us answer this question. On the other hand, histology labeling showed an increase in the number of synapses between GABAergic top-down projections and abGCs, suggesting more contacts between inhibitory cortical neurons and abGCs after olfactory learning. More experiments need to be performed to determine if the increase in putative GABAergic synapses involved connections with more cortical neurons or an increase in the number of contacts between the same pair of neurons. These two mechanisms result in the increase of general inhibition that abGCs receive from the cortex.

In a previous study (Lepousez et al., 2014), we showed that the density of putative GABAergic synapses (Gephyrin<sup>+</sup> puncta) was increased after olfactory learning, specifically on the proximal domain of GCs dendrites, as well as the frequency of spontaneous IPSCs. At the time, the existence of GABAergic projections from the cortex had not yet been described, and thus had not been specifically studied. It is possible that a similar increase in cortical GABAergic fibers after learning occurs both in young (Lepousez et al., 2014) and more mature abGCs (present study), which is not the case for cortical glutamatergic synapses that change only in young cells. Interestingly, we also demonstrated that the inhibitory centrifugal inputs are concentrated in the proximal and distal segments of abGCs dendrites, similar to what has been observed for excitatory top-downs (Lepousez *et al.*, 2014a). Although we detected numerous inhibitory synapses on the apical part of GCs where they make dendrodendritic contacts with M/TCs, almost no colocalisation was observed with GABAergic fibers coming from the AON.

This anatomical feature indicates that dendrites in the GCL receive both local and extrinsic cortical inhibitory inputs, whereas the IPL and the EPL are targeted by intrinsic GABAergic input provided by local interneurons including SACs and other GCs. Still, we would need to confirm whether this organization is similar with nnGCs.

But what are the consequences of higher inhibitory responses in abGCs? The precise location of inhibitory, together with excitatory, cortical feedback near the soma may make the cortico-bulbar inputs more likely to control action potential generation (Pressler and Strowbridge, 2019). Considering the location of these GABAergic inputs, we could argue that inhibition at the base of the dendrite would globally decrease the transmission of APs in the whole dendrite and thus impair the ability of the cells to inhibit M/TCs. Since GCs are well-known for

regulating the activation of principal cells in the OB, this would result in a disinhibitory mechanism that would allow better transmission by the M/TCs. Thus, the increase in inhibition could favor the transmission of the olfactory message through the cells connected to these abGCs. Our results suggest that both excitatory and inhibitory input are integrated in proximal dendritic domain (<100  $\mu\text{m}$ ), leading to spike initiation and then propagation of APs through the distal apical dendritic arbor.

Nevertheless, previous studies showed that learning can enhance the response of abGCs to the learnt odors (Breton-Provencher *et al.*, 2009; Grelat *et al.*, 2018; Mandairon *et al.*, 2018) and increase the connectivity of abGCs with MCs (Huang *et al.*, 2016). This suggests that learning establishes plasticity specifically on abGCs that respond to the learnt odors, which could then provide selective inhibition to responsive MCs. In this context, increase in inhibition on "non-responsive" abGCs could contribute to reinforce this plasticity mechanism in specific abGCs populations dedicated to learnt odors.

Even more, it has been demonstrated that excitatory cortical inputs onto GCs allowed stronger inhibition of MCs through potentiation of the dendrodendritic synapse in an NMDA-dependent manner (Balu, Pressler and Strowbridge, 2007; Restrepo *et al.*, 2009). We could then hypothesize that an increase in GABAergic input would balance this mechanism to further refine the tuning of MCs output.

The question remains that although we observe an increase in inhibition, functional studies showed that general activation of abGCs increased performance in olfactory discrimination (Grelat *et al.*, 2018). A possible explanation is the increase on a specific subset of abGCs to select MTCs causes transmission of a relevant message for the animal, although it needs to be formally examined.

To complete our understanding of this system, two aspects need to be studied:

On the first hand, we have not yet determined whether abGCs displaying higher inhibition from the cortex are actually involved in the information processing of the odors used in the learning task. Indeed, it seems when looking at the data that not all recorded GCs display the changes in GABA responses, and we are not able to specifically record those that are located near the activated glomeruli. As was mentioned before, inhibition of abGCs would result in

higher activity of connected M/T cells. Thus, we would expect the subpopulation displaying the increase in inhibitory drive to be involved in the treatment of learnt odors. To confirm this, we could use the specific mutant mouse line M72-IRES-ChR2-YFP which would allow the creation of association with light stimulation of a specific fluorescent glomerulus (Smear *et al.*, 2013). Optogenetic stimulation in acute slices would require red-shifted opsins such as Chrimson, but it would then be possible to decipher the cortical stimulation response amplitude according to the proximity of recorded GCs with the stimulated glomerulus.

On the other hand, it would be interesting to decipher whether long-term plasticity of these synapses is elicited by repetitive activation of the GABAergic fibers, and consequently to determine whether artificial activation could mimic the effects of learning. To assess this, *in vivo* artificial stimulation of GABAergic AONp neurons in the same way we did during our behavioral experiments, combined with acute slices recordings, would help determine whether light-evoked activation of these fibers is sufficient to induce similar changes in abGCs.

### Adult-born vs neonatal neurons

Comparing the synaptic properties of nnGCs with those of abGCs, we found a greater amplitude of glutamatergic and GABAergic currents from cortical inputs in nnGCs. This contrasts with a previous study that reported a stronger glutamatergic connection with abGCs (Wu *et al.*, 2020). In this study, recorded abGCs were only 2 weeks old, and cells in the control group could have been generated at any time and represent a very heterogeneous population, conversely to our P6-born condition. Finally, the higher excitation in abGCs in respect to nnGCs was reported by measuring the total charge resulting from a high-frequency light stimulation over a period of 1 second, as opposed to our analysis of individual responses to short pulses of light. For all these reasons, we cannot directly compare our results on centrifugal excitation with this study.

As we already discussed, adult neurogenesis is itself a form of plasticity that brings new functional units to an existing network, making it the substrate for new paths of message transmission and integration. Here, the fact that only this population, and maybe even a subset

of it, is modified by learning, supports the hypothesis that new neurons can serve as new a substrate available to introduce and conserve new information. This is reinforced by the fact that in the control condition (without learning), abGCs display significantly smaller inhibitory responses to cortico-bulbar inputs than postnatal ones, which raises the hypothesis of a higher dynamic range and a potential involvement in learning processes. This supports the fact that adult-born neurons, instead of helping the turn-over of bulbar interneurons, would rather increase the computational power of the bulb throughout life, as shown in (Platel *et al.*, 2019), and hints about why adult neurogenesis occurs particularly in this system, since novel stimuli can be encountered throughout the whole life. Considering that a critical period exists for plasticity during the maturation of new neurons (Gao and Strowbridge, 2009; Nissant *et al.*, 2009), along with changes in both GABA and glutamate inputs from the cortex (Tufo *et al.*, 2022), it seems necessary to constantly bring immature cells to allow adaptability of the individual to a changing environment.

However, in our study, abGCs were more than 10 weeks old at the time the mice learned the task; therefore, they had already reached their final excitability (Bardy *et al.*, 2010). Interestingly, it has been shown that OB adult-born neurons undergo experience-dependent plasticity long after maturation and integration, suggesting that the potential time window for plasticity of these neurons extends well into maturity (Breton-Provencher *et al.*, 2009; Livneh and Mizrahi, 2012; Grelat *et al.*, 2018). In this context, how different forms of learning-induced plasticity are generated and regulated during the maturation of abGCs is not clear yet.

Finally, previous studies from our lab showed that mature abGCs express unique features in their synaptic outputs that are less sensitive to GABA<sub>B</sub> receptor modulation than nnGCs, leading to their escape from autoinhibition (Valley *et al.*, 2013). This reduced autoinhibition could potentially explain the requirement of extra top-down inhibition control in respect to pre-existing neurons.

On the contrary, we did not observe any effect of learning on GCs that had been generated postnatally, which still displayed higher excitatory and inhibitory responses from top-down projections than abGCs. This could suggest other roles for nnGCs, such as responding to innately relevant odors, but can reinforce the hypothesis of adult neurogenesis as a mechanism providing higher dynamic range to a complex network. Moreover, we did not study immature

abGCs, which have already been shown to display long-term plasticity (Gao and Strowbridge, 2009; Nissant *et al.*, 2009) and experience-induced changes after learning (Lepousez *et al.*, 2014a; Wu *et al.*, 2020). Thus, an interesting experiment would be to examine the responses and learning-induced changes of these immature cells in response to GABAergic top-down projections.

### Neuromodulation of cortical inhibition on GCs

Our data showed that synapses between inhibitory cortical fibers and abGCs were influenced by neuromodulatory receptors such as CB1 or GABA<sub>B</sub> receptors. As we mentioned before, GABA<sub>B</sub>Rs were demonstrated to be expressed by both nnGCs and abGCs, although less present in the synapses of the latter (Valley *et al.*, 2013b). Moreover, these receptors can tune glutamatergic inputs from the cortex (Mazo *et al.*, 2022) and OSN synapses, with a reduced expression after olfactory fear learning (Bhattarai *et al.*, 2020). Thus, GABA<sub>B</sub>Rs are ubiquitous in the OB and seem to be involved in learning processes. It will be interesting to know which regime of GABAergic fiber activity leads to GABA<sub>B</sub> receptor activation, and in which physiological mechanisms it plays a role. On the other hand, CB1Rs have been highlighted both in excitatory cortical (Soria-Gómez *et al.*, 2014; Pouille and Schoppa, 2018) and inhibitory forebrain feedback (Zhou and Puche, 2021), where they regulate short-term plasticity. Consequently, we can hypothesize that their presence at the synapse between inhibitory AON fibers and abGCs could help tune the inhibition received by the OB projection neurons. Further experiments are needed to characterize the function of the endocannabinoid system at this synapse, its precise role in short- and long-term plasticity and in modulating top-down activity in various physiological contexts.

Finally, for GABA<sub>B</sub> and CB1 receptor-dependent modulation, it will be interesting to assess whether it exhibits specific characteristics in abGCs as a function of their maturity and how this compares with nnGCs.

## Effect of AONp GABAergic projections on olfactory perception and learning

### Activity of AONp projection during Go/no-Go task

As we already mentioned, long-range GABAergic inputs to the OB arise mainly from the AONp (Mazo *et al.*, 2022).

Using fiber photometry above the olfactory peduncle, we observed that these projections were activated during the whole duration of the Go/No-go trial, with an apparent evolution in activity after associative learning (Figure 6).

Interestingly, the activation when the mice arrived in the odor port seems to strengthen across learning, which could translate as an improved attention mechanism increased odor sampling, or stimulus expectation. However, this could also be due to odor residuals in the odor port, although this hypothesis is unlikely since this plateau was not observed in naïve mice. To confirm these observations, quantitative analysis and an increased number of individuals would need to be performed.

Previous studies showed that excitatory feedback from the APC were sharply activated during odor exposure and evolves with associative learning in a similar manner (Wu *et al.*, 2020). However, the 2-photon head-fixed protocol used in this study does not allow the study of pre-odor engagement that we observe in freely moving mice.

Another interesting aspect is the strong decrease in activity observed after the mice left the odor port. This hints for a role of the GABAergic AONp projections in odor processing, such as sensory gating, that does not need to be maintained after odor removal.

Finally, we did not observe any difference in activity between S+ and S- “Go” responses, but analyses remain to be performed to decipher any potential difference between correct responses and errors. Even more, analysis of S+ vs S-, in Go and No-go response are required to evaluate odor value encoding in these fibers.

Overall, preliminary observations suggest that AONp GABAergic projections to the OB are activated during the whole duration of the odor-guided task, irrespective of the odor, with a

probable increase and a change in kinetics during associative learning. However, fiber photometry only allows for the study of global population activity, and it remains possible that some heterogeneity exists, with some cells displaying suppressive responses as previously observed in other systems (Schroeder *et al.*, 2023). Thus, other experimental strategies such as 2-photon microscopy or micro-endoscope would prove crucial by providing cellular-resolution information.

### Optogenetic activity manipulation of AONp fibers during behavior

We found that manipulation, by either optogenetic activation or inhibition, of corticobulbar GABAergic fibers activity originating in the AONp resulted in delayed discrimination learning of odorants (see Figure 7F and Figure 8F), without affecting the discrimination acuity of already learnt odors (see Figure 7C and Figure 8C).

How could opposite activity changes produce similar behavioral effects? We know that precise firing and fine tuning of various bulbar subpopulations is necessary for correct discrimination through pattern separation (Gschwend *et al.*, 2015; Li *et al.*, 2018). Thus, it is possible that in physiological conditions, either only some parts/cell types of the bulb are inhibited by the studied projections, or subsets of AONp neurons are activated at a time. Indeed, inhibitory projecting neurons in the AONp are heterogeneous and could have different roles in bulbar modulation (Mazo *et al.*, 2022). Other studies showed that GABAergic top-down terminals from the subthalamic zona incerta to the neocortex display bidirectional learning-dependent modifications (Schroeder *et al.*, 2023).

However, this precise tuning was not allowed by our experiments, which caused broad activation or inhibition of projecting GABAergic fibers in the olfactory peduncle. This does not seem like a problem when the fibers were inhibited, since we only prevented them to fulfil their role in normal conditions, but optogenetic stimulation may have resulted in general inhibition of the OB circuitry, thus impairing olfactory bulb network computations required during odor-reward association.

In this work, we also evaluate how long-range GABAergic fibers could be implicated in fine odor detection as was preliminary suggested by our previous results (Mazo *et al.*, 2022). For

that, once mice reached learning criterion, animals were exposed to catch trials using a suprathreshold concentration of the S+ odor. Interestingly, silencing GABAergic top-down projections decreased the mouse's ability to detect weak odorants (Figure 7D), while their activation improved detection (Figure 8D). In this case, activation and inhibition produced opposite behavioral alterations, suggesting that odor detection is less sensitive to activation frequency and/or required homogeneous participation of different AON-projecting GABAergic fibers.

These results suggest that the GABAergic feedback from the AON, through inhibition of various cell-types in the OB, could help message transmission when the signal is very poor (low concentration) and would thus be involved in signal-to-noise control. This idea agrees with the proposed role of the AON on sensory gating (Brunert, Medinaceli Quintela and Rothermel, 2023) potentially acting through an AON-TCs loop (Chae *et al.*, 2022).

Moreover, optogenetic activation, but not inhibition, of GABAergic fibers reduced the behavioral flexibility of mice, as seen by their inability to reach the success criterion when the learning rule was reversed, echoing the latency to first learn the association. This hints that GABAergic top-down modulation could help in associating a value to a given stimulus. To refine this result, specific stimulation of the fibers only during S+ (or S-) could provide important information, especially since abGCs have been demonstrated to be predominant in the processing of the S+ odor (Grelat *et al.*, 2018).

Our experiments also showed that modulation of these fibers had no effect on 24h or 2-week memory of the task. Previous studies showed that the AON is greatly involved in olfactory-related memory (Aqrabawi and Kim, 2018a, 2020), but these functions seem to not involve the population of GABAergic centrifugal fibers.

The AON has also been deemed crucial for other odor-related behaviors such as social odor processing, interhemispheric communication, and sensory gating (Brunert, Medinaceli Quintela and Rothermel, 2023). Since the inhibitory top-down projections do not project contralaterally, it seems improbable that they take any part in interhemispheric communication, but our results seem to reinforce the role of this region as a control center of sensory inputs.

In summary, inhibition of these fibers impairs detection of low concentration odors and slows down olfactory learning. In agreement with this hypothesis, artificial activation improves the performance in catch trials using suprathreshold odor presentation. On the other hand, broad artificial activation seems to be detrimental for early phases of olfactory learning. Even more, this activation is probably affecting the odor-value assignment process, by impairing the reversal learning of already well-discriminated odors.

The AONp could serve as a powerful tool to drive strong silencing of various parts of the OB. As seen in previous studies, these neurons extend terminals mainly in deep layers, namely GCL, IPL and MCL, although TCs and eTCs show responses to cortical stimulation (Mazo *et al.*, 2022). The response amplitude was particularly strong in GCs and dSACs, suggesting a computing power essentially located in the GCL. Thus, optogenetic activation may have resulted in the general disinhibition of principal OB cells through the silencing of GCs. However, two-photon microscopy showed that activation of these projections results in inhibition of MCs and TCs, with a special influence of the pattern separation ability of TCs, although no distinction was made between APC and AON origin of the fibers (Mazo *et al.*, 2022). To better understand the effect of AONp inhibition, fiber photometry recordings in the bulb with optogenetic manipulation of these fibers, combined with olfactory learning, may give us insights on the actual result of our manipulation on various subpopulations of the bulb including abGCs, nnGCs and both MCs and TCs (which can be deciphered according to the position of the recording fiber, or through the use of specific genetic line such as *Tbet::Cre* mice that allow specific recombination only in MCs). In addition, future experiments using activity proxy markers, as cFos or pS6 (for mitral cells), will also be performed to evaluate the impact of optogenetic manipulation in different cell types.

Moreover, we cannot rule out that long-term repetitive stimulation of the fibers, roughly 200 times a day for several weeks, may have caused potentiation or depression of these synapses, which could have altered the results and flawed our conclusions. Thus, performing the experiments suggested above will be necessary to investigate this aspect.

## Water deprivation and the influence of internal state

Our data seem to provide insights on the effect of water/food deprivation on the processing of the olfactory message. Indeed, water-deprived mice also eat less, resulting in loss of bodyweight. Thus, the effect we show on synaptic strength, as a result of water restriction, might be due to lack of nutritional intake. Several explanations, not mutually exclusive, are foreseeable:

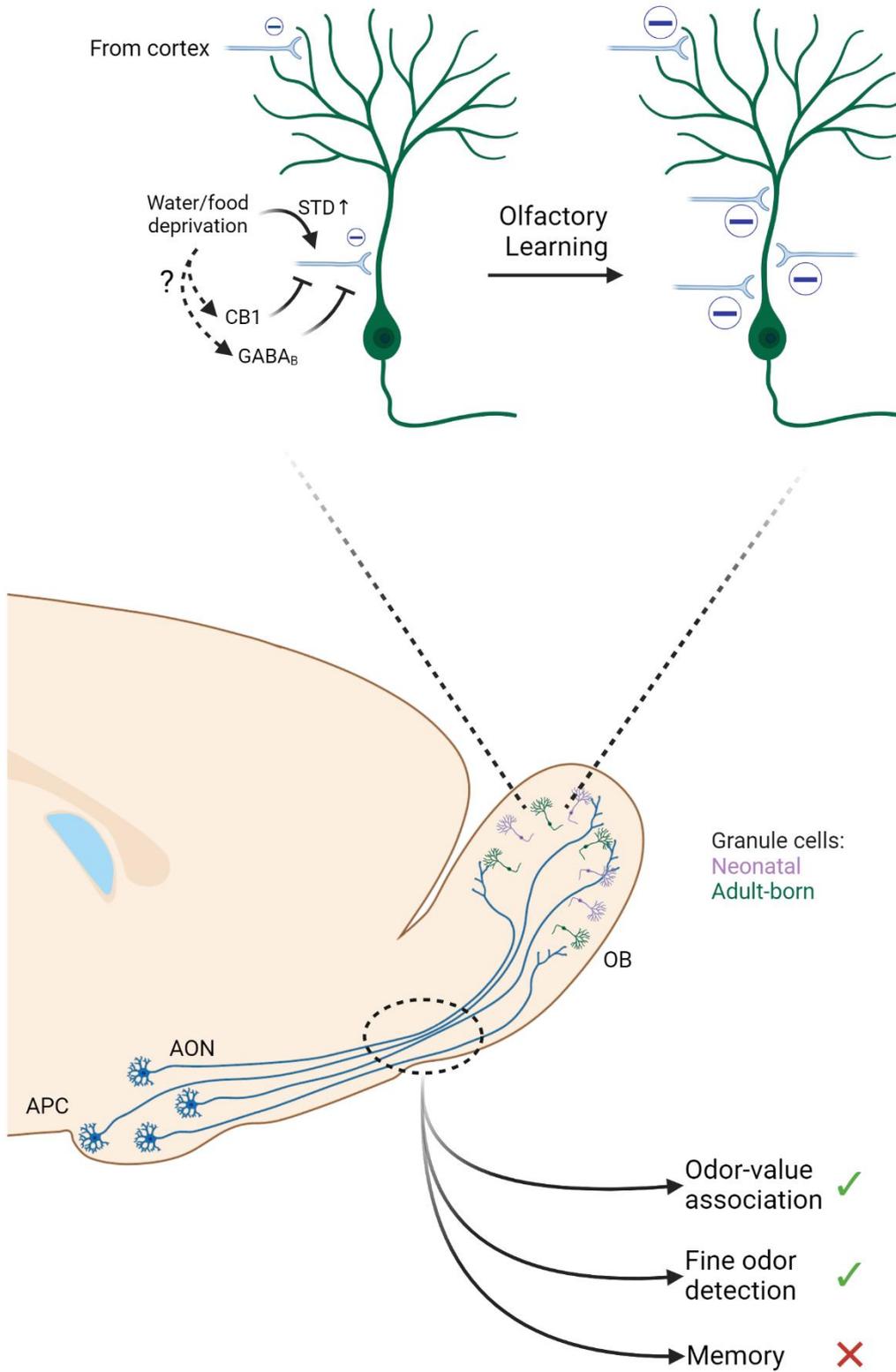
- Neuronal activity is the result of precise ion movements at very short time scales, and we know that the initial equilibrium of the involved ions – especially sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ) and chloride ( $\text{Cl}^-$ ) – can result in various effects on neuronal excitability, probability of neurotransmitter release and response amplitude. Moreover, the maintenance of the gradients, for example through the NaK ATPase pump, as well as neurotransmitters synthesis and migration to terminals, need massive amounts of energy. Since we already know that dehydration alters cognitive function (Faraco *et al.*, 2014), chronic water restriction, and by extension lack of ions and energy, could impair the fine homeostasis of the brain and cause neuronal transmission changes.
- Top-down inputs are a putative way for controlling sensory inputs according to activity in other brain centers. It would in consequence be logical that internal states such as hydric stress would influence the incoming message, in the ecological sense that food- and water- seeking behaviors must in this case be favored, or at least maintained at a reasonable level despite the lack of energy. We know that hunger tunes the activity of excitatory cortical feedback in the bulb in a CB1R-dependent manner, resulting in better odor detection (Soria-Gómez *et al.*, 2014). Thus, top-down inputs are known to translate internal states in the OB and actively participate in subsequent signal tuning, which could be what we observed in our experiment. However, one important point in our protocol is that water and food deprivation effects cannot be distinguished, and experiments with only food restriction would be necessary to decipher whether the observed effect is due to one or the other, or both.
- Recent work also supports internal-state dependent sensory processing by showing that food restriction induces loss of coding precision in the visual cortex to compensate

for energy-saving strategy through secretion of leptin (Padamsey *et al.*, 2022). In this study, the effect was shown to be mediated by a decrease in AMPA receptors conductance, a compensatory increase in input resistance, and a depolarized resting potential. We can imagine additional mechanisms affecting the mobilization of neurotransmitter pools and changing the dynamic of synaptic transmission. This of course remains hypothetical and needs to be studied, perhaps in other restriction states.

- A last aspect is the emotional state of chronically water-restricted animals. Indeed, it has early been shown that chronic water restriction disrupts circadian rhythm of corticosterone secretion, with high levels of circulating stress hormone right before water was given (Armario and Jolin, 1986). Today, we use chronic corticosterone administration as a model of depressive disorders in mice (David *et al.*, 2009), suggesting that restriction-induced high levels of this hormone could create a depressive-like state in our animals. Moreover, we know that corticosterone-induced chronic stress impairs olfactory function (Siopi *et al.*, 2016), and that general mood greatly influences olfactory processing (Kontaris, East and Wilson, 2020). Even more, recent results suggest that depressive state alter both odor hedonics and adult olfactory neurogenesis (Athanassi *et al.*, 2023). Thus, water-deprivation could also play a role on olfactory perception and synaptic transmission in the OB through induction of a chronic stress state in restricted animals.

These various hypotheses could explain the short-term dynamics observed in patch-clamp recordings of water-deprived animals. However, the fact that no change in current amplitudes were observed in non-learning, water-deprived animals, and pseudo-learning ones, suggests that the effect is associated with discrimination learning and thus gives robust insight into learning-induced network modifications.

## Adult-born granule cells



## Mitral vs tufted: Conclusions or mystery?

In the OB, message transmission to cortical regions is mediated by both MCs and TCs. However, their connectivity and functional differences are still poorly understood.

As we saw in our introduction, differences in anatomical projections have been described, with TCs projecting mainly to the most anterior regions of the olfactory system, especially the AON, while MCs preferentially target the APC and other cortical regions.

Functionally, MCs were previously thought to predominantly have a role in odor identification while TCs played a role in odor navigation through concentration processing, since these were the known roles of their preferred target (Rabell *et al.*, 2017; Bolding and Franks, 2018). However, a recent study elegantly showed that both MC and TC populations displayed activity linked to odor identity and concentration, with TC ensembles showing faster odor-evoked responses and greater ability to predict both of these features (Chae *et al.*, 2022).

Moreover, this study showed that the antero-posterior tropism seems conserved in the cortico-bulbar projections sent by APC and AON, which target interneurons that preferably tune MCs and TCs, respectively. Looking closely at the data, it seems in fact that ipsi-lateral AON still has a non-negligible influence on MC activity. Thus, only the ipsilateral APC and contralateral AON have selective effect on either MC or TC ensembles. Functionally, the authors show that APC cortical control tuned odor responses of MCs, while AON feedback only influenced the gain-control of TCs. Thus, it seems that the olfactory system presents two partially segregated loops of message transmission and feedback, which possess both anatomical and functional differences. The authors of the study hypothesize that TC-AON loop could be used in robust odor identification while the MC-APC loop would serve for flexible representations according to task difficulty and previous experience.

While this study looked at general inhibition of the AON and APC, with no distinction between excitatory and inhibitory feedback, it still gives us an intriguing insight into the role of these cortico-bulbar projections. Moreover, we have already discussed the fact that GCs in various layers of the GCL present different effects on MCs and TCs. Indeed, since the mean length of the apical dendrite does not vary significantly (Orona, Scott and Rainer, 1983),

superficial GCs must preferentially form reciprocal synapses with TCs in the EPL, while deeper GCs would target MCs in the MCL. Moreover, we know that adult neurogenesis produces new GCs that preferentially integrate in the deeper part of the GCL (Lemasson *et al.*, 2005; Breton-Provencher and Saghatelian, 2012). Thus, these characteristics suppose that abGCs preferentially target MCs, as seen previously (Bardy *et al.*, 2010). This implicates that the changes in cortical inputs to abGCs, but not neonatal ones, would display a stronger effect on the MC ensembles during odor processing than TC ones. Nonetheless, it needs to be noted that in the control condition, nnGCs receive higher inhibition than abGCs, suggesting that TCs are initially more impacted by these fibers, with learning triggering changes primarily on the MC population.

If this is the case, the role of AONp GABAergic feedback in complex discrimination learning would thus be more salient on MC populations message integration, which would be consistent with the fact that the MC-APC loop is hypothesized to be involved in specific modifications of odor representations during learning.

Thus, the TC-AON loop would convey odor identity in the discrimination task, with the ipsilateral AON projecting back to abGCs, reshaping the MC-APC pathway for associative learning and pattern separation of learnt odors.

Further important information is the fact that AONp GABAergic inputs reduce odor-evoked activity of both TCs and MCs, but this seems to increase the difference in the representation of two different odors only in the TC ensembles, as shown in our previous work (Mazo *et al.*, 2022). However, this experiment did not involve olfactory learning, which could change these representations.

The changes of MC and TC representations with learning have also been demonstrated in previous work, although MCs are particularly modulated by associative learning (Yamada *et al.*, 2017). Furthermore, MC ensembles were highly reshaped by the go/no-go olfactory task and resulted in weaker odor evoked-activity and higher suppression in a task-specific manner (Kudryavitskaya *et al.*, 2021). Here, however, the logical result of higher inhibition of GCs would be a stronger odor-evoked response in MCs, but we need to keep in mind that only a subset of recorded abGCs displayed high levels of inhibitory responses, as we discussed before. Thus, it remains possible that learning increases general suppression of MCs odor-evoked responses,

while a subset keeps high response amplitudes thanks to increased inhibition of precise GCs, suggesting that learning could shape the olfactory network to select specific information pathways of particular behavioral relevance. This idea is supported by data showing precise spatial survival and integration of abGCs after olfactory learning (Alonso *et al.*, 2006), while electrophysiological results show that only high frequency stimulation of abGCs inhibits MC populations (Alonso, Lepousez, Wagner, *et al.*, 2012). An increase in inhibition from the AON could then keep abGC activity at a low-frequency level, helping relevant message transmission by MCs during discrimination. Here, we may thus have unraveled a mechanism allowing specific patterns of activation in the OB as a result of associative learning, which remains to be demonstrated *in vivo*.

We know that abGCs can evoke inhibitory currents on virtually all cell types in the OB (Bardy *et al.*, 2010), but data show that most MCs display these responses, against roughly 50% of TCs. To refine our hypotheses, the experimental design described in the previous section of this discussion would also help for understanding their activity during the go/no-go task combined with AONp GABAergic fibers activation or silencing. Thus, although this study did not compare results to nnGCs, it hints that a difference in connectivity might be at play in differentially tuning the activity of output neurons in the OB.

## GABAergic long-range projections

### The influence of GABAergic long-range projections in the brain

Our work gives new insights on the role of long-range GABAergic projections in the brain. As opposed to the most commonly studied local inhibitory interneurons, long-range GABAergic neurons are defined as “inhibitory neurons connecting brains areas associated with distinct functions” (Caputi *et al.*, 2013b).

Interestingly, GABAergic projecting neurons are found in numerous systems such as the cerebellum, the hippocampus, and in our case, the olfactory system. Although anatomically described for a long time, their functional relevance remained elusive until the last decades. They have now been shown to be involved in associative learning (Brown *et al.*, 2012), reward encoding (Seo *et al.*, 2016; Sharpe *et al.*, 2017; Bouarab, Thompson and Polter, 2019; Schroeder *et al.*, 2023), and also adult-born neuron production and survival in both the hippocampus and OB (Bao *et al.*, 2017; Hanson, Swanson and Arenkiel, 2020), among other roles. However, how top-down afferents encode information that originated in several brain regions and how excitatory and inhibitory top-down signal converge with bottom-up representation is still unknown.

Our behavioral experiments, despite their lack of specificity, clearly showed that correct activity of GABAergic long-range inputs originating from the AON is necessary for fine odor detection, and also reversal of a learnt rule. However, we still need to decipher what controls their activity during odor encounter, and how this activity evolves with olfactory experience and learning. To understand how these cells are controlled, tracing experiments using pseudorabies viruses would allow the identification of upstream regions and neuronal types.

In fact, several operational scenarios are possible. Learning-dependent recruitment of long-range GABA can boost M/TCs activity, as was proposed for long-range GABA in the hippocampus (Basu *et al.*, 2016) and neocortex (Schroeder *et al.*, 2023).

In this line, HDB long-range GABAergic neurons were recently studied in an extensive manner, showing that long-range inhibition allowed modulation of odor-evoked activity in M/TCs, mainly resulting in an increase of the signal-to-noise ratio during olfactory perception (Böhm, Brunert and Rothermel, 2020).

However, computational modelling of these fibers showed that their stimulation generated a global silencing of both M/TCs and GCs (Mazo *et al.*, 2022), in accordance with our optogenetics results showing an impairment of discrimination learning, as already shown in the HDB (Nunez-Parra *et al.*, 2013). Overall, the respective effect on each population during odor perception remains to be deciphered, not to mention the effect of learning on this global OB inhibition.

This work shows that long range inhibition is not simply crucial for correct processing of the olfactory message in the OB, as previously shown, but also demonstrated that these fibers, while differentially targeting cell populations in the OB, display learning-induced plasticity. To complete our understanding of these fibers function, recording other cell types after associative learning is key, as well as understanding how the change in AON-to-abGCs inhibition tunes the global network and transforms the information before conveying it further to cortical areas.

### GABAergic long-range and oscillations in the brain

GABA long-range projections are also thought to participate in the generation of oscillation rhythms in the brain and consequently the synchronization of distant regions. Stimulation of basal forebrain GABAergic neurons displayed an increase in theta and gamma oscillation rhythms for local stimulation of terminals in the GL and the GCL, respectively (Villar, Hu and Araneda, 2021). Moreover, 33Hz stimulation of olfactory cortex inhibitory fibers increased beta-range synchronization of the OB network (Mazo *et al.*, 2022), which was shown to increase with learning processes in regard to centrifugal fibers (Martin *et al.*, 2004). Thus, various long-range inhibitory neurons seem to induce distinct oscillatory rhythms in the OB, according to both the region they originate and the localization of their terminals. This could be related to changes observed in gamma and beta oscillations during olfactory discrimination learning (Losacco *et al.*, 2020), although causality between long-range GABA projections and oscillations during learning remains to be formally established.

This also helps in reflecting upon the role of top-down projection in other sensory modalities. In the visual system, various centrifugal fibers are involved in learning and sensitivity

(Ruediger and Scanziani, 2020), while in the auditory system, top-down projections were shown to modulate the signal according to expectations (Asilador and Llano, 2021).

More recently, a study also described a long-range inhibitory pathway from the Zona Incerta (ZI) to the auditory cortex that transmits integrated top-down information essential for learning. Interestingly, these afferents preferentially target interneurons and bidirectionally encode learnt top-down relevance of sensory information (Schroeder *et al.*, 2023). This motif is similar to the connectivity found in the OB network, where GABAergic fibers from the AON preferentially target GCs. The authors propose this disinhibitory connectivity might provide a more flexible and dynamic substrate for circuit control than the direct excitation supplied by classical top-down afferents. In the case of the OB, the effects on M/TCs might depend not only on long-range afferent signaling itself, but also on the current activity patterns of the targeted GC types, which further control different somatodendritic domains, or even different types of principal neurons.

Besides, a subset of M/TCs also receive direct inputs from GABAergic fibers from the AON, as previously described, further enriching this circuit diagram. As proposed for ZI projections to the auditory cortex, the experience-dependent redistribution of inhibition likely enhances the computational outcomes for OB processing.

### GABAergic long-range projections and the predictive brain

One of the main roles proposed for top-down projections, is the ability to predict the incoming signal in what is called the “Bayesian brain”. The predictive capacity of the brain has been theorized for various contexts and results from the ability to anticipate stimuli according to the probability of possible outcomes and previous experiences (Nave *et al.*, 2020). Our work shows that top-down fibers are modulated not only by prior learning, but also internal information, and transmit them at least to the main coordinators of the OB, namely the GCs. This probably results in very early changes in sensory processing, thus making the perception of a given odor unique for each animal. Moreover, preliminary observations showed these fibers to seem to display activation prior to odor presentation in an operant conditioning task, which increased with knowledge of the task (Figure 6). This could putatively hint on a stimulus expectation mechanism transmitted to the OB through these projections.

Thus, top-down fibers allow tuning that influences the way the olfactory message is transmitted to regions where the odor will be identified, characterized, and associated with previous or new experiences. We could thus consider that, depending on various factors such as physiological state, previous experiences, or mood, each animal would perceive a slightly different odor, hinting on what we could define as subjectivity in sensory perception, or at least olfaction. Of course, this unique perception could be restrained to a subset of particularly relevant odors. Moreover, many other mechanisms supposedly participate in this modulation, such as adult neurogenesis, since we know that specific learning shapes the fate of new neurons and regulates their position in the OB (Alonso *et al.*, 2006; Mandairon *et al.*, 2006).

Last, but not least, top-down projections have been proposed, in a fragile balance with bottom-up processing, to be involved in various psychiatric disorders such as depression, bipolar disorders (LeDuke *et al.*, 2023), or schizophrenia (Zhu, Zikopoulos and Yazdanbakhsh, 2023). Thus, a better grasp of these mechanisms could allow for better comprehension of the pathophysiology of these disorders and, hopefully, later help in the support of patients.

## On the olfactory go/no-go task:

The behavioral task used in this work relies on the supposed association of a reward with a specific odor, and the various difficulty of discrimination between this odor and a second, non-rewarded one, according to their similarity, respective concentration, or proportion in a mix. It is usually accepted (Dolensek *et al.*, 2020) that giving a water drop to a thirsty animal is enough to create a positive valence which is associated to the corresponding odor. However, a question keeps returning when trying to interpret the results and understanding fine behavior: why does the animal go for the reward only when smelling the right odor? Despite the apparent naivety of the question, there is in my opinion something odd with the initial hypothesis of this paradigm: the proximity of the waterspout and the slight effort necessary to obtain the reward makes me wonder why the animal does not try to get the reward at every trial, which would simply occur with a 50% probability. Of course, this kind of reasoning relies on the supposition that mice can think about this problem in the same way I do.

As a supposition, I wish to propose a second hypothesis: in a sufficiently water-deprived state, the lack of reward after licking the spout drives a strong negative valence that gets associated to the non-rewarded odor, leading to correct rejections as a strategy for avoiding negative emotions. This can be supported by the dopaminergic hypoactivity demonstrated in positively conditioned animals when they do not receive a reward following a conditioned stimulus. Although this dopaminergic hypoactivity has been described and is necessary for all stimulus-reward association (Schultz, 2016), it could still explain why when an animal is not thirsty enough, but still wants water, it starts to lick the spout in most trials, since not getting the reward becomes less aversive (Schultz, Apicella and Ljungberg, 1993; Glimcher, 2011). This can be seen as a cancellation of the deprivation-induced shift of the valence level that occurs during water restriction (Dolensek *et al.*, 2020).

More precisely, thinking in terms of cost/benefit efficiency, one could argue that going for the reward no matter the perceived stimulus is a more efficient way of getting the maximal number of rewards, since the only risk of the “go” response is to experience disappointment (as discussed above), while the “no-go” response presents a non-negligible probability of

missing a reward. Thus, if the disappointment component is not salient enough, it is easy to understand that individuals have good reasons to go for the reward in every trial.

Some previous studies showed that performance was dependent on the deprivation level of animals and found that optimal performance occurred in the middle of the daily task, in what they call a “discrimination state”, where animals are not too thirsty but also not sated (Matteucci *et al.*, 2022). Sadly, the authors did not provide the various proportions of miss and false alarm responses explaining the drops in performance, but these results are encouraging for the hypothesis of optimal deprivation.

However, there is another aspect to consider. In the succession of events of our task, once the mouse leaves the odor port, it gets a 2-second window to either lick the spout, and maybe get a water drop, or refrain from it. There lies a crucial difference between the two outcomes: the false alarm trials are inherently shorter than the correct rejection ones, which translates to a shorter time to wait before the next. In a pure efficiency view, and supposing that mice are 1. motivated enough to get the rewards as fast as possible and 2. smart enough to understand this, it seems only logical that they would rather go for the reward every time rather than wait for the trial to be complete. In this view, the lick could be considered by the animal as a “reset” button that allows it to start the next trial faster. This calls for a true negative valence of the false alarm response for the mice to respond correctly, which can be impaired by a lack of thirst.

One could claim that no proof is shown that they understand this timing difference between S+ and S- trials, and more importantly that the mean reaction time of 500 ms suggests they still try to smell, and perhaps identify the odor, whereas they could simply go for a prospective lick as soon as the final valve opens. These are both valid arguments to acknowledge, keeping in mind that the situation described in the last paragraphs is considered in the case where the negative valence of not getting the reward is dampened (by a gain of weight for instance). Explanations for these arguments could be that smelling the odors becomes a puzzle for playful animals or simply that olfactory enrichment is appetitive for olfaction-driven animals such as rodents.

To assess the various aspects of this question, several experiments could be considered:

- First, the total duration of each trial should be the same no matter the response of the animal. Thus, we need to correct the triggering of the ITI in “go” trials that would start 2 seconds after the animal leaves the odor port and not as soon as it licks the spout.
- Secondly, in our setup, the use of the two-alternative choices (2AC) task, where mice have to lick the right or left spout depending on the odor, could help discard the default “go” response (Uchida and Mainen, 2003). Indeed, it would then be impossible to indifferently go for the reward without missing half of it, making the decision part of the task mandatory.
- It is also necessary to try to perform this experiment with real appetitive rewards such as saccharine water, hopefully in non-deprived animals. This would provide two main advantages: it would ensure that learning is actually induced by the association between the S+ odor and the reward but would also allow us to get rid of all the drawbacks of water deprivation which result in chronic weight-loss of animals, possibly non-physiological behavioral read outs, and changes in electrophysiological responses of neurons as seen in our results (Figure 4). Recent work explored deprivation-less associative learning in auditory tasks, which was made possible with stimulation of the Medial Forebrain Bundle, which is proposed as an alternative to deprivation (Verdier *et al.*, 2022). However, this is hardly compatible with all experiments requiring fiber implantations such as optogenetics or photometry, and thus still needs refinement.
- Lastly, to assess the actual valence association in our task and thus confirm or not the valence shifting hypothesis, recordings of dopaminergic activity would be required to decipher the respective value associated with each stimulus. Behaviorally, a test of odor preference could give relevant information about the associated valence of odors used in the discrimination task, along with analysis of facial expression during the task (Dolensek *et al.*, 2020).

These reflections upon the olfactometer task are simply putative and remain to be understood, and hopefully assessed at some point to refine our interpretation of behavior and limit the caveats of our experiments. However, whether this hypothesis is true, even partially, or not, it does not invalidate the fact that mice learn to discriminate odors in the olfactometers, as a combination of associative and perceptual learning. Thus, even if the conditioning is

negatively induced, the effects observed in adult-born neuron recordings are still the result of associative learning and can be correctly interpreted.

## Neurogenesis in adult humans

We have shown in this study that synapses between cortical fibers and adult-born neurons in the OB were specifically modified by discrimination learning, giving rise to hypotheses on how previous experiences shape early processing of olfactory stimuli. This suggests, along other studies, that neurogenesis, in its particular way of complexifying the existing network, is crucial in experience-dependent information integration in the brain (Grelat *et al.*, 2018).

Our model, the OB, is a place of great neuronal generation across life of rodents. However, how is this study relevant for other animals, especially humans?

Although adult neurogenesis in humans has been hypothesized as soon as the phenomenon was discovered in other animals, it took some more years to be demonstrated using BrdU (Eriksson *et al.*, 1998) or <sup>14</sup>C incorporation (Spalding *et al.*, 2013) in the human hippocampus and also in the striatum (Ernst *et al.*, 2014). However, a debate still exists on whether this phenomenon is truly occurring or not, the detractors stating that the generation of new neurons in the human hippocampus decreases sharply after a few years (Sorrells *et al.*, 2018), or that progenitors, although present in the adult hippocampus, do not generate new neurons (Cipriani *et al.*, 2018).

The diversity of results seems to be rooted in the post-mortem tissue processing variations across studies, resulting in contradictory results (Moreno-Jiménez *et al.*, 2021). Today, it appears that the debate is not about whether new neurons are brought to the network during adult life in humans, but rather if these new neurons originate from constantly dividing stem cells or slowly differentiating quiescent cell pools present in various regions (Zhou *et al.*, 2022).

Thus, despite the fact that actual adult neurogenesis has not been proven in the human OB, understanding the role of adult-born neurons in neurophysiology remains, regardless of the system, which is relevant for future work in the field of neurogenesis.

Moreover, another human adult neurogenesis mechanism is greatly overlooked, although it has been demonstrated with no following debate, and occurs in the olfactory system. Indeed, we know that new neurons are constantly produced in the human olfactory epithelium, which arise from epithelial stem cells and allow the expression of functional olfactory receptors

(Durante *et al.*, 2020). In rodents, epithelial neurogenesis allows for regeneration of the olfactory organ after lesions thanks to quiescent progenitors necessary for tissue maintenance (Leung, Coulombe and Reed, 2007). Since the sense of smell can be recovered after lesion of the olfactory nerve or epithelium alteration, it seems that neurogenesis could play a role in these mechanisms in humans.

The role of neurogenesis in other regions of the human brain still remains to be characterized. If the new neurons are generated in the hippocampus, we can suppose that hippocampal functions such as spatial learning and memory or contextual fear association can require these cells (Kempermann, 2022). Moreover, this mechanism seems particularly impaired in neurodegenerative disorders such as Alzheimer's Disease (Choi and Tanzi, 2023), and increase of adult neurogenesis in AD mouse models seem to partially rescue cognitive impairment (Richetin *et al.*, 2015; Choi *et al.*, 2018; Walgrave *et al.*, 2021; Mishra *et al.*, 2022). However, functional studies are very limited in humans and investigating specific neuronal populations in a behaving organism remains to this day only possible in animal models, although some magnetic resonance imaging techniques seem to be able to identify neural progenitors in live human brain (Manganas *et al.*, 2007; Sierra, Encinas and Maletic-Savatic, 2011). For now, we can only assume that findings in rodents or non-human primates could bring answers about the role of neurogenesis in humans, even though some functional studies in human aim to assess the hippocampal function (Riphagen *et al.*, 2020), which combined in particular with NPC-detecting MRI may one day assess adult-born neurons function. However, we know that several parameters increase hippocampal volume, such as physical exercise or cognitive stimulation (Fotuhi, Do and Jack, 2012), which could involve enhanced neurogenesis. Conversely, the size of the hippocampus decreases in some conditions, such as depression, while antidepressants appear to rescue this phenomenon by promoting neurogenesis and plasticity (Warner-Schmidt and Duman, 2006; Anacker *et al.*, 2011). Thus, adult hippocampal neurogenesis could play a role in protecting the brain against network impairment and cognitive dysfunction.

Nonetheless, we can summon up Dobzhansky's assertion that "Nothing in biology makes sense, except in the light of evolution", in order to better grasp understanding of the mechanisms at hand. Thus, another way to decipher the actual role of adult neurogenesis, but

also an explanation about why it occurs in such few places, resides in evolutionary aspects of this phenomenon.

## Evolutionary perspectives: why is adult neurogenesis conserved, yet so sparse?

Adult neurogenesis in the brain, although still debated in humans, has been demonstrated in various species across the animal phylogeny. It was demonstrated to occur in rodents and other mammals, such as pigs and non-human primates (Franjic *et al.*, 2022), but also in other vertebrates such as birds (Alvarez-Buylla *et al.*, 1998), reptiles (Pérez-Cañellas and García-Verdugo, 1996), or fish (Birse, Leonard and Coggeshall, 1980). Moreover, adult neurogenesis is present in invertebrates, as seen in the extensively studied *Drosophila* (Fernández-Hernández, Rhiner and Moreno, 2013) but also decapod crustaceans such as crayfish (Zhang *et al.*, 2009; Sandeman, Bazin and Beltz, 2011). Thus, adult neurogenesis is extensively found in the animal kingdom and, although it does not occur in the whole brain, it appears not to be anecdotic. Consequently, we can assume that this phenomenon must have been selected during evolutionary history.

Beyond the fact that adult neurogenesis exists in phylogenetically diverse taxa, it seems that new neurons produced during adult life always differentiate from glial cells present in neurogenic niches (Sullivan, Benton, *et al.*, 2007), which supports a common mechanism across the species and perhaps a shared origin. If we consider the hypothesis that adult neurogenesis allows the constant renewal of the brain networks and provides new substrates for novel information flow, it seems logical that this mechanism would have been selected during evolution, giving advantage to individuals in adaptation to changing environments for instance. However, since all biological processes come at a given energy cost, the production of new neurons could easily be present only in the parts of the brain that need permanent remodeling. So, what does this information suggest about the role of adult neurogenesis?

First, we can consider the need for some species to grow during their whole life. Indeed, examples in both invertebrates (crustaceans) and vertebrates (reptiles) display constant growth, even during adult life. Although this increase in size declines as the animal ages (luckily for us humans), it calls for the need to increase the brain size. Thus, in these species, adult neurogenesis could simply be seen as a necessary developmental process accompanying general body growth. However, it cannot be the sole explanation since it is also seen in non-growing animals, but this feature could help us understand at least part of the role of adult

neurogenesis: regeneration. Even if most animals don't need the constant increase of their neuronal population, lesions and neuronal loss can occur in a non-physiological manner. The ability to generate new neurons could then serve as a regeneration mechanism as seen in other tissues, which was demonstrated in *Drosophila* (Fernández-Hernández, Rhiner and Moreno, 2013). However, full regenerative ability would require a neurogenesis niche that is able to produce a considerable number of cells, but also the ability for these cells to travel and integrate in a greater range of brain areas than has been observed in most studies. This question has been reviewed by Kaslin and colleagues, who support this hypothesis with the fact that fish and amphibians present both widespread neurogenesis and better brain injury regeneration capacities (Kaslin, Ganz and Brand, 2007). Thereby, we can consider that places where neurogenesis actually occur in the mammalian brain are also prone to regeneration, with a good example found in the olfactory epithelium and its ability to overcome injury (Costanzo, 1991). Thus, neurogenesis might help physiological and pathological tissue regeneration, but this role seems very limited in the mammalian brain.

If we take a closer look at the regions where these new neurons differentiate and integrate, we can notice that numerous species display adult-born cells in their olfactory system. This has been shown in crayfish (Sullivan, Sandeman, *et al.*, 2007), turtles (Pérez-Cañellas, Font and García-Verdugo, 1997), and of course rodents. From a sensory point of view, olfaction is peculiar in the sense that the perceived stimuli are both more diverse and difficult to predict than other modalities such as vision or hearing. Thus, neurogenesis could help overcome the perpetual encounter of new stimuli and the necessary tuning of message processing in the OB. This is also supported by the lack of precise topological representation of odors in bulbar and cortical areas, as opposed to retinal topography conservation in the visual cortex, for example. New neurons could help shape the olfactory environment representation throughout life, either by replacing previous neurons or by adding complexity to the network.

The same hypothesis has been poised about hippocampal neurogenesis and spatial memory, where navigation in a changing environment would be eased by providing new functional units into the circuitry (Kempermann, 2012). However, hippocampal neurogenesis is mainly found in mammals and is suggested by the author as a late-evolved trait. Thus, we can probably distinguish adult neurogenesis in regions common to various taxa as early evolved (in the OB for example), while others might have developed more recently such as adult

hippocampal neurogenesis in mammals, allowing a greater diversity of colonized ecological niches (Kempermann, 2016).

In humans, these two mechanisms could have been at play: across the last thousands of years, it is possible that we took less and less advantage of our olfactory faculties. One could thus argue that, given the fact that proliferation, survival, and integration of new neurons is dependent on neuronal activity, neurogenesis in the olfactory system may have declined to subsist only in vestigial, barely detectable levels. However, hippocampal function has been extensively relevant in our evolutionary history and could consequently be conserved from a late-evolved traits present only in mammals.

This particular case of neurogenesis has been recently hypothesized to underly the Baldwin evolutionary effect, where the selection of individuals is not only the result of genetic traits, as seen in classical evolutionary views, but also emerges from the ability of the individual to adapt and learn during its whole life (Abrous, Koehl and Lemoine, 2022). This paper also argues that while adult hippocampal neurogenesis allows for behavioral and emotional flexibility, it comes at the cost of maladaptation, which may result in mental disorders, while a fixed postnatal network would prevent this kind of vulnerability.

In summary, hippocampal and olfactory neurogenesis appear to arise from temporally different evolutionary steps but enable the same kind of flexibility. While olfactory adult-born neurons may allow the correct perception and discrimination of new stimuli throughout life, thus being crucial for making organisms to correctly respond to a constantly changing environment, hippocampal ones seem to cover the need of behavioral and emotional adaptation in mammals.

# Conclusion

Overall, this work provides insights on two exciting topics.

On one hand, it reaffirms the fundamental role of neurogenesis in reward-associated learning, and states that cells emerging from this mechanism display a unique role in plasticity. Although this study focused on neurogenesis in the OB, which has not yet been proven in humans, it helps in raising questions about the specific role it could play in any other regions such as the hippocampus or the hypothalamus.

On the other hand, our results display cutting-edge insights into how top-down projections, and more specifically inhibitory ones, might integrate both experience-dependent and internal information to help in modulating ascending messages in early sensory processing. More work is still needed to decipher their precise activity in respect to various internal states or pathological conditions and their impact on the general message transmission, but they seem to play a great role in creating unique patterns of activation during odor discrimination tasks.

What is particularly interesting is the fact that both adult neurogenesis and top-down projections have been linked to psychiatric disorders such as depression, and could thus be candidates to, if not cure them, at least alleviate their impact on an individual's life. Indeed, sensory perception, and especially olfaction, is greatly modified by mood disorders, and modulation of top-down activity, particularly acting on adult neurogenesis, could be a promising perspective to untangle the physiopathology of these diseases to ultimately relieve suffering patients.



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# Abstract

Olfaction allows most animals to perceive volatile molecules filling the environment they roam. A complex neuronal network – the olfactory system – is required for an animal to detect, identify, and discriminate odors, as well as associate them with contextual cues, leading to adapted behavioral responses. The olfactory bulb is the first relay for information coming from the odor detection organ, but also integrates considerable inputs from various brain regions such as the olfactory cortex. Among them, we have recently uncovered a direct long-range inhibitory connection between the olfactory cortex and the olfactory bulb.

In addition, the past decades shed light on a previously refuted mechanism, adult neurogenesis. This process has been demonstrated in the hippocampus, hypothalamus, and olfactory bulb of rodents, and allows the integration of new functional neurons in the brain networks throughout the whole life of an individual. Moreover, it was shown that neurons born in the adult brain are uniquely involved in learning and memory processes. We thus sought to determine whether the inhibitory projections from the cortex to the adult-born neurons in the olfactory bulb were modified after olfactory learning, underlying entering signal modulation by previous experiences.

Combining an olfactory learning task with *ex vivo* electrophysiology, we showed that adult-born granule cells in the olfactory bulb exclusively received more inhibition from the olfactory cortex after learning. Furthermore, we demonstrated that this increase in inhibition was partly due to a greater number of synapses between cortical fibers and adult-born granule cells.

Furthermore, optogenetic manipulation of GABAergic top-down activity bidirectionally modulates fine odor detection and impairs olfactory learning, but not memory, revealing their significant impact on behavior.

Thus, we unraveled a mechanism where top-down projections might play a major role in sensory learning through increased inhibition, specific to adult-born neurons, and highlighted the relevance of this inhibitory feedback for olfactory processing.