UNIVERSITY OF CALIFORNIA, SAN DIEGO

The role of adult neurogenesis in facilitating olfactory information discrimination and its dependence on behavioral states

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in

Biology

by

Wankun Li

Committee in charge:

Professor Takaki Komiyama, Chair
Professor Kristin Baldwin
Professor Jeffry Isaacson
Professor Jill Leutgab
Professor Jing Wang

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The Dissertation of Wankun Li is approved, and is acceptable in quality and form for publication on microfilm and electronically:

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ABN	Adult-born neuron
BrdU	Bromodeoxyuridine
DCX	Doublecortin
EPL	External plexiform layer
GC	Granule cell
GCL	Granule cell layer
GFAP	Glial fibrillary acidic protein
Hipp	Hippocampus
IPSC	Inhibitory postsynaptic current
MC	Mitral cell
MCL	Mitral cell layer
OB	Olfactory bulb
OSN	Olfactory sensory neuron
PGC	Periglomerular cell
RMS	Rostral migratory stream
SVZ	Subventricular zone
TC	Tufted cell

LIST OF FIGURES

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As a person identified as an extrovert, I have come to the realization more and more that the innermost self refuses to go through peaks and valleys alone. I always consider myself extremely fortunate to be surrounded by people who truly love me and care for me and support me as who I am every step of the way. These supports go far beyond the realm of academic careers - they build me as a person.

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Chu MW, Li WL, Komiyama T. Balancing the Robustness and Efficiency of Odor Representations during learning; and

Chu MW, Li WL, Komiyama T. Lack of pattern separation in sensory inputs to the olfactory bulb during perceptual learning.

Chapter 2 is material currently being prepared for submission for publication. Li WL, Chu MW, Wu A, Suzuki Y, Imayoshi I, Komiyama T. Adult-born neurons facilitate olfactory bulb pattern separation during task engagement. The dissertation author was the primary investigator and author of this material.

VITA

2011 Bachelor of Science in Biological Sciences
Peking University, Beijing, China

2017 Doctor of Philosophy in Biology
University of California, San Diego, La Jolla, CA

PUBLICATIONS

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Professor Takaki Komiyama

ABSTRACT OF THE DISSERTATION

The role of adult neurogenesis in facilitating olfactory information discrimination and its dependence on behavioral states

by

Wankun Li

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Professor Takaki Komiyama, Chair

Stem cells that have the potential of proliferation are commonly seen in lower animals, as well as certain tissues in mammals such as the skin tissue or liver tissue. The mammalian brain, as a highly differentiated and complex organ, however, was thought to consist only post-mitotic neurons. Findings in recent decades have confirmed that, in mammals like rodents or human beings, neurons are continuously generated throughout the adulthood by stem cells residing in specific niches of the brain. One of the two major niches is the sub-ventricular zone, from where newly generated neurons migrate through the rostral migratory stream and arrive almost exclusively at the olfactory bulb (OB), the first olfactory information processing center.

Mice were used to study the role of adult-born neurons (ABNs) in olfaction. As creatures constantly exposed to complex olfactory stimuli and relying on olfaction to survive, their OB needs sufficient capacity to encode olfactory information, for example, discriminating highly similar stimuli. Olfactory information is converged in the OB, and then relayed by mitral cells (MCs), the excitatory principal neurons in the OB, to higher brain areas. Interneurons, mainly consisting of periglomerular cells (PGCs) and granule cells (GCs), do not send long-range projections, but to modulate activities of MCs. Such modulation is considered the key of plasticity of the OB in decoding and encoding olfaction information. More interestingly, interneurons receive centrifugal projections from multiple higher brain areas as well as neuromodulatory inputs, which make them ideal candidates to mediate top-down control of the olfactory perception. One widely studied top-down influence on peripheral sensory areas is related to the behavioral state.

Once entering the OB, ABNs develop into interneurons, and, within a critical period, display higher plasticity and morphological dynamics. These features indicate that ABNs are specially important in olfactory information processing. To investigate the role of ABNs, I used a genetic method to specifically ablate ABNs. The effect of missing ABNs on olfactory perception was studied using carefully designed behavioral tasks, and with the longitudinal two-photon microscopy technique, changes in MCs olfactory encoding in the absence of ABNs were discovered.

Chapter 1. Introduction

Does adult neurogenesis exist in mammals?

From bacteria to humans, cell proliferation takes place after the initial postnatal development and throughout the entire adulthood (Gabor and Hotchkiss 1979, Carlson 2007). It is considered a way of regeneration, and ultimately merely a coping method to body damages and a means to provide survival advantages in an inhospitable environment. Primitive animals such as leeches and planarians are well-known for regeneration (Alvarado 2000). Once wounded, their stem cells will be activated and, through signaling pathway magic, new organs or even new entire bodies will be recovered exactly as the original copy (Reddien and Alvarado 2004). People divided a planarian into staggering 279 pieces, and each piece grew back to a complete, functioning organism in the course of weeks (Handberg-Thorsager, Fernandez et al. 2008). Such phenomena are less seen in vertebrates, though with exceptions like salamanders who are the champions of organ-redevelopment research. Their limbs, tails, or even hearts and brains can be fully regenerated (Mladinic, Muller et al. 2009).

All these regeneration wonders would not happen without active pluripotent or unipotent stem cells and progenitors. For example in wounded salamanders, cells would first dedifferentiation into pluripotent stem cells, and then re-differentiate to desired tissues (Odelberg 2004). As species evolve, cells become more differentiated and complex, and as a result, for individuals, cells are losing their pluripotency or unipotency. Take human beings are an extreme example. Our skin

can renew itself thanks to rapid cell replication by skin stem cells, and our livers can grow back even after 3 quarters being removed in surgeries (Michalopoulos and DeFrances 1997). However, the capacity of proliferation and differentiation is highly limited. We can recover from wounds from a plastic surgery, but can our face grow back to exactly how it looked like just by itself after, say, severe burn? At the top of the degree of differentiation and complexity of mammalian organs lies the brain. For the longest time, our brain was considered entirely post-mitotic, meaning all the neurons in the central nervous system lose the ability to divide and differentiate after the initial postnatal development process. "Our skin is completely replaced every two weeks, which means after two weeks my lips will completely forget the kiss, but my brain will remember it forever", says the main character in the movie "Everything, everything". It is true that skin cells and liver cells have all the machineries to fulfill their respective functions pre-programmed in their genomes, which makes their location within the organs and the choice of their neighbors un-critical. However, for neurons inside the mammalian brains, their location largely reflects their functional identity. A neuron in the dorsal raphe neucleus can bring us a polar opposite mood than one in the substantia ngra (Graeff, Guimarães et al. 1996, Schultz 2015, Luo and Huang 2016, Yohn, Gergues et al. 2017); an excitatory pyramidal neuron in layer 5 of the motor cortex may have to submit to the command from another similar-looking one in layer 2/3 (Peters, Lee et al. 2017).

Not only their placement matters, but also their dynamic connections with other neurons through axons, dendrites and gap junctions are the central mechanism of how our brain processes and stores information. If we view lots of

stimuli-rich pictures and try to remember them, the connections between our Hippocampus (Hipp) and the visual cortex can be strengthened, but maybe not so much between the Hipp and the auditory cortex. If one eye of a cat is covered for a long time, the corresponding area on the visual cortex will experience degeneration due to a lack of inputs (Le Vay, Wiesel et al. 1980). Don't we wish our brains can grow back exactly what it was like before damage, so that people do not have be permanently paralyzed and live the rest of their lives in a wheelchair, or suffer from short term memory deficiency? Sadly, the neural plasticity that enables us to learn and to remember is what renders brain regeneration considered unpractical. If my brain would humble itself and allow me to compare it with a computer hard drive, once the hard drive is damaged, we can repair the hardware and replace the electronic units, or even to switch to a new one, but the data would not be recovered. It is long known that there are neuron deaths in the brain throughout our life (Kole, Annis et al. 2013), though because of the same logic, people have thought it makes sense that, even if there are new neurons generated, it is next to impossible for them to be perfectly located at the vacancy from the dead one and functionally integrate into the brain without disrupting existing circuits (Gage 2002). As a result, for the longest time, we accepted the fate that the neuron number count for our brain only ticks down as we get older.

Discovery of adult neurogenesis in the mammalian brain

The notion that all neuronal elements of the central nervous system were generated from precursor cells in the neuroepithelium during early development (Abney, Bartlett et al. 1981) and the mechanical problem of accommodating the dividing neurons (Gage 2002) were overcome by the discovery that neural stem cells did exist throughout life in the adult brain and could give rise to new neurons just as in the developing brain. A decade or so after the ascendance of the computer model of brain circuitry (Morris 1999), Altman (Altman 1962, Altman 1963, Altman and Das 1965) made his original claim of adult neurogenesis in rat and cat Hipp by intracranial or intraventricular injection of thymidine-H^3. Further evidence was accumulated by Kaplan and his colleagues Using the same labeling strategy paired with electron microscopy. The willingness to accept adult neurogenesis was further enhanced by the convincing evidence that fetal tissue could be grafted in the adult intact brain, and damaged adult brain and spinal cord allowed these newly grafted cells to survive and differentiate (Dunnett, Björklund et al. 1985). Direct proof that cells with stem cell properties existed in the adult mammalian brain was provided, first from the subventricular zone (SVZ) (Reynolds and Weiss 1992, Richards, Kilpatrick et al. 1992), and then in the dentate gyrus (DG) of the Hipp (Gage, Coates et al. 1995, Palmer, Takahashi et al. 1997), using dish culture of separated tissues. Comparing to the "local generation, local integration" feature of DG adult-born neurons (ABNs), the destination of ABNs generated from SVZ was a bit more challenging to confirm, as it was later revealed by electron microscopic analysis that neuroblasts migrated a long distance from SVZ through rostral migratory stream (RMS) all the way to the olfactory bulb (OB). Unlike early

development where radial glial or axonal fibers would provide guidance, there was a layer of GFAP (Glial Fibrillary Acidic Protein)-positive astrocytes ensheathing the chains of traveling neuroblasts (Lois and Alvarez-Buylla 1994, Lois, García-Verdugo et al. 1996, Gould 2007).

Up to this point it was still unclear whether this could be a residual phenomenon from early development or the newly-generated neurons actually had a physiological significance by actively participating in the brain function. In the 1980s, Burd & Nottebohm first described synapses formation of adult born neurons (Burd and Nottebohm 1985). More interestingly, the same lab reported the first indication that ABNs were recruited into functional circuits. They used thymidine-H^3 to label ABNs in song birds, gave sound stimulation, did post hoc HRP staining, and found co-localization of the two types of signals (Paton and Nottebohm 1984).

Two studies conducted by Kornack and Rakic discovered adult neurogenesis in monkey Hipp and OB (Kornack and Rakic 1999, Kornack and Rakic 2001). Given the resemblance of the central nervous system, people naturally wondered if adult neurogenesis existed in human beings as well. If proven true, it would be exciting not only in the indication that adult neurogenesis played a role in human brain function as well, but also because it could mean our brain had the ability to host neural stem cells and adult-born neurons, which then elicited the hope of stem cell therapy for damage repair. So it was not surprising that interest in adult neurogenesis markedly increased when it was demonstrated that new neurons were produced in the DG of adult humans (Eriksson, Perfilieva et al. 1998). They examined the brains of patients with cancer injected with BrdU (Bromodeoxyuridine)

(Kuhn, Dickinson-Anson et al. 1996) which provided a unique opportunity to look at adult neurogenesis in autopsy. DG and SVZ of humans were confirmed to also be the two niches of adult neurogenesis. No neocortical neurogenesis was detected in both healthy and cortically-injured (as some hoped that it could induce neurogenesis) adult humans (Bhardwaj, Curtis et al. 2006, Paridaen and Huttner 2014). Would the migration and integration trajectory from SVZ be preserved throughout evolution? Sanai et al discovered a ribbon of SVZ astrocytes lining the lateral ventricles of the adult human brain that proliferated in vivo and behaved as multipotent progenitor cells in vitro. However, no evidence was found proving that the migration target was the OB (Sanai, Tramontin et al. 2004). Curtis et al. later claimed to have observed OB ABNs (Curtis, Kam et al. 2007), but their data were questioned and their findings were defied as unsubstantiated (Sanai, Berger et al. 2007). More studies came out supporting the theory that the travel path took a turn in human beings. Wang et al. studied neuroblasts and migratory immature neurons identified in SVZ and RMS of adult monkey, fetal human and adult human and reported that no ABNs were found in the adult human OB (Wang, Liu et al. 2011). People also found the astrocytes corridor declined during adulthood, but there was no sign of ABNs destined for OB using ^{14}C dating (Sanai, Nguyen et al. 2011, Bergmann, Liebl et al. 2012). New proposal of the destination arose when new neurons were found in the striatum which was adjacent to the SVZ niche. The same study also reported the type of ABNs being exclusively interneurons, and the speed of adult neurogenesis declined with age (Ernst, Alkass et al. 2014).

After all, these findings suggest that adult olfactory bulb neurogenesis, contrary to most mammals, is absent in adult humans. Have we lost the ability to generate new neurons because our olfactory epithelium has regressed making odors less perceptible for us (Bergmann, Spalding et al. 2015)? ABN integration into Hipp DG is preserved from rodents to human, just as memory encoding and storage have been important across species. However, OB adult neurogenesis is lost in humans, which is parallel to our lower reliance on olfaction. This leads to the next question: why was there adult neurogenesis in the OB at first place? Does this evolutional change actually reflect, in a certain sense, the role of ABNs?

The olfactory system in rodents

Life-long neurogenesis is costly. To spend that amount of energy on olfaction, a seemingly less indispensible sense, the nature might be making a mistake by not optimizing resource utilization. Is it the case? To start off, I would like to do olfaction some justice. Olfaction, the sense of smell, is highly under-appreciated by humans. If we ask someone "among the five senses (sight, sound, touch, smell, taste), which would you rather lose", it would not be surprising for the most-picked to be "smell". In fact, not many people can afford the consequence of losing olfaction. Clinical studies of thousands of patients reportedly having distorted olfactory experience (phantosmia) or olfaction loss (hyposmia or anosmia) have shown an astonishingly high correlation between these disabilities and impairment in physical and psychological well-being including depression, appetite loss, weight drop or even suicidal tendency (Deems, Doty et al. 1991, Temmel, Quint et al. 2002, Blomqvist,

Bramerson et al. 2004, Gudziol, Wolff-Stephan et al. 2009, Nordin, Hedén Blomqvist et al. 2011, Croy, Negoias et al. 2012, Keller and Malaspina 2013). Like Bonnie Blodgett said in her famous memoir "Remembering Smell" recording her full experience of phantosmia and anosmia, "What is breathing, what is living, without smell?"

In his Nobel Prize acceptance speech, Richard Axel called olfaction "the primal sense". It is indeed true for rodents as well as numerous other mammalian species. Rodents heavily rely on olfaction for social behaviors: smelling predators to run, smelling the opposite sex and being attracted, smelling food and foraging, or smelling their own pups and taking them under her care (Schultz and Tapp 1973, Brennan and Keverne 2004, Keverne 2004, Restrepo, Arellano et al. 2004). Olfaction is also the primal sense in that, unlike vision, hearing or taste, the olfactory information flow does not go through thalamus (Shepherd 2005). Instead, the OB, serving as the first olfactory information processing center, directly projects to amygdala, which happens to be the nucleus known to be related to emotions like fear (Clark 1995, Davis and Whalen 2000, LeDoux 2003). Thus it would not be a stretch to think that animals rely on this primary sense for emotional homeostasis which is another critical aspect for survival besides meeting basic physical needs. Besides amygdala, the OB also projects to piriform cortex and entorhinal cortex, together called "primary olfactory cortex". They both have the 3-layer archicortex structure (Martinez, Blanco et al. 1987, Klingler 2017), which emerged prior to the 6-layer neocortex (such as visual cortex or auditory cortex) structure in the evolution history. These projections indicate that olfaction is more ancient than other senses.

Does the possible explanation of adult neurogenesis occurring in the OB solely lie in the importance of olfaction? It is believed that the complexity of olfactory inputs may also contribute to the reason (Petreanu and Alvarez-Buylla 2002). To have a better understanding of this postulation, it would be helpful to take a look at the fine structure of the OB (de Castro 2009). Odor molecules diffuse into the nasal cavity and bind to the olfactory sensory neurons (OSNs) in the olfactory epithelium. Each one of the approximately 5 million OSNs in a rodent nose expresses only one of the estimated 1300 different olfactory receptors with diverse tuning curve to different molecules (Buck and Axel 1991, Malnic, Hirono et al. 1999, Zhang and Firestein 2002). The axons of OSNs form the olfactory nerve which projects to the OB and forms synapses with the apical dendrites of mitral/tufted cells (MC/TCs), giving rise to the OB glomeruli (Ramon y Cajal 1890, Greer 1991). While those OSNs that express the same olfactory receptor gene are randomly distributed on the epithelium, their axons selectively project to only 1± 4 of the 2000 glomeruli in the OB, reflecting a precise convergence of olfactory information (Mombaerts, Wang et al. 1996, Xu, Greer et al. 2000). The convergence is retained at the principle neuron level where each MC/TC would receive inputs exclusively from one glomeruli. Then they relay information through long-range feed-forward projections to higher brains areas such as the anterior olfactory nucleus, the olfactory tubercle, the piriform cortex, amygdaloid nuclei and the entorhinal cortex (Devor 1976, Derer, Caviness et al. 1977, Scott, McBride et al. 1980, Schwob and Price 1984).

It seems that the olfactory perception is largely determined by the OSN projection "map" that is linearly transmitted into cortex. However, there is a

tremendous degree of flexibility and adaptivity of the OB circuitry odor representation in accordance with olfactory experience thanks to the largest population of neurons in the OB - interneurons. One characteristic of the OB circuitry is the high proportion of inhibitory to projection neurons (approximately 100:1) (Shepherd 2004). The activity of MC/TCs is regulated by dendro-dendritic synaptic contacts with periglomerular cells (PGCs) and granule cells (GCs), which are the two major types of interneurons in the OB (Lazarini and Lledo 2011). PGCs make synaptic connections with MC/TCs primary dendrites in the glomeruli and they include both GABAergic and dopaminergic subtypes. PGCs also form axodendritic connections outside glomeruli and dendro-axonic inhibitory synapses onto ORNs (Wachowiak and Cohen 1999, Aroniadou-Anderjaska, Zhou et al. 2000, Berkowicz and Trombley 2000, Ennis, Zhou et al. 2001). GCs form reciprocal dendro-dendritic contacts with TC/MC dendrites and they are believed to be exclusively GABAergic. Due to the difference in connectivity patterns, PGCs are considered mostly regulating intra-glomerular circuits, while GCs mediate interactions between TC/MCs receiving inputs from different glomeruli (interglomerular circuits) (Schoppa and Urban 2003). Principle neurons activated by odors would release glutamate at the reciprocal connection sites and activate GCs. The latter would in turn exert suppression through GABAergic synapses, onto not only the activating principle neurons, but also others they connect to, causing self-inhibition and lateral inhibition among TC/MCs. This process is thought to shape the tuning curve of TC/MCs, modulating neuron ensemble odor representation, and ultimately sculpting olfactory perception (Wellis and Scott 1990, Isaacson 1999, Luo and Katz 2001, Urban and

Sakmann 2002, Shepherd, Chen et al. 2004).

In the retina, a given photoreceptor activates inhibitory circuits that influence cells around it, producing the center-surround properties of the ganglion cell receptive fields. These receptive fields encode information along the dimensions of brightness, contrast, wavelength, saturation and so on. For auditory system, dimensions consisting the stimuli space, such as pitch, tone, and intensity, could be easily named as well. Unlike vision or hearing, olfaction has intrinsic high dimension (Hopfield 1991). As a consequence, in the OB, a given MC/TC needs an inhibitory network that influences other MC/TCs, not necessarily physically around them but in the more complex olfactory space (Giovanni and Randolf 2000, Sachse and Galizia 2002). That means that groups of MC/TCs far away from each other can be linked by inhibitory connections. Co-activation of glomeruli that respond to a single odor cannot be predicted on the basis of the physical distance with respect to one another (Kauer and White 2001). Thus, unlike other sensory systems, it might not be feasible to preassemble a unique inhibitory circuit that processes olfactory information optimally for all the possible odors that an animal might experience during development and in adult life. Therefore, it might be necessary to build the inhibitory circuit of the OB according to experience by the activity-dependent survival mechanism described here. The olfactory adult neurogenesis provides a rich potential for the OB to achieve so.

The olfactory adult neurogenesis in rodents

As mentioned above, PGCs and GCs are the two major types of interneurons in the OB, and their regulation can be critical for olfactory information processing. Intriguingly, all the ABNs generated from SVZ and migrating through RMS towards OB eventually become almost exclusively PGCs and GCs. However, in order to truly appreciate the fact that the subject of olfactory adult neurogenesis has drawn so much interest, it would be helpful to delve into existing results tackling questions such as: How does a young neuroblast integrate into a pre-existing and functional network? What determines its survival or death? What influences its integration? After being incorporated, what is the functional impact of this life-long supply of interneurons in the OB? Are there special characteristics of ABNs that are different from the mature interneurons? In the past two decades, many efforts have been made to tackle different parts of these questions, and we are able to have an increasingly clear picture when putting these puzzles together (Ming and Song 2011, Nissant and Pallotto 2011).

Maturation, inputs and outputs

ABNs mature as they migrate from SVZ, first tangentially, and then radially once reaching the OB. The maturation process can be marked by morphological and physiological changes into different stages (Nissant and Pallotto 2011).

Even at the precursors/neuroblasts stage in the RMS, adult-born cells are already sensitive to neurotransmitters (Platel, Stamboulian et al. 2010). Glutamate is released by the astrocytes surrounding the RMS, and the neuroblasts already express respond to glutamate through GluK5, AMPA and NMDA receptors (Platel,

Lacar et al. 2007, Platel, Heintz et al. 2008, Platel, Dave et al. 2010). Neuroblasts also release GABA through non-synaptic, non-vesicular mechanisms (Wang, Krueger et al. 2003, Bolteus and Bordey 2004, Liu, Wang et al. 2005, Platel, Lacar et al. 2007, Platel, Dave et al. 2010), and respond to GABA through $GABA_A$ receptors (Nguyen, Malgrange et al. 2003, Wang, Krueger et al. 2003, Bolteus and Bordey 2004, Liu, Wang et al. 2005).

An ABN can enter into the OB as fast as one day after being born, but on average it would take one week. Petreanu and Alvarez-Buylla (2002) were the first to describe five-stage maturation of newborn GCs from migrating neuroblasts to fully arborized mature neurons based on their age-post-birth, morphology, location, and physiological properties (Petreanu and Alvarez-Buylla 2002). This five-stage process can take place within the first 2-3 weeks after birth, during which neurons experience fast synaptogenesis and connection formation. Afterwards, there is a longer slow maturation phase, which can last until 8 weeks post-birth (Nissant and Pallotto 2011).

Once entering the OB, ABNs are immediately greeted with dense local GABAergic interneurons and centrifugal projections as well as glutamatergic principle neurons and other feedback projection fibers, which provide a rich array of stimulation for their survival and development, which will be further discussed in the next section. ABNs are shown to form the first GABAergic and glutamatergic input synapses within the first 24 h of radial migration (Panzanelli, Bardy et al. 2009, Katagiri, Pallotto et al. 2011). While receiving new synapses, they rapidly extend their apical dendrite through the GCL toward the EPL. Only a few days later, some

GCs are able to have a mature shape with dendritic branches in the EPL and dynamic spines (Panzanelli, Bardy et al. 2009). Studies have suggested that the receiving of GABAergic inputs and maturation of synapses occur very fast, and precedes glutamatergic input formation. One indication is that while dendrite grow rapidly and total number of inhibitory synapses increases, the density of GABAergic contacts, inhibitory postsynaptic currents (IPSCs) and short-term plasticity of evoked IPSCs remain unchanged (Panzanelli, Bardy et al. 2009). With a few days delay in the onset, the wave of proximal glutamatergic synapses formation comes quickly (Kelsch, Lin et al. 2008, Panzanelli, Bardy et al. 2009). This is accompanied by modifications at both presynaptic and postsynaptic sides (Katagiri, Pallotto et al. 2011). Overall, it may be beneficial for inhibitory inputs to procede excitatory ones so that the cells can develop in a controlled fashion.

The only reported synaptic output of GCs is through the dendro-dendritic synapses formed with the lateral dendrites of TC/MCs, which are located on the apical dendrites in the EPL (Rall, Shepherd et al. 1966, Price and Powell 1970, Whitman and Greer 2007). Panzanelli et al. (2009) described early steps of dendrodendritc synapse formation in detail. Their results suggested that these synapses formed first on the shaft before mature spines, and also the formation of unidirectional excitatory synapses from TC/MCs onto GCs might happen before they become bi-directional (Panzanelli, Bardy et al. 2009). Direct physiological evidence for active dendro-dendritic connections were demonstrated using electrophysiology recording and optogenetics (Isaacson and Strowbridge 1998, Bardy, Alonso et al. 2010).

After fast development of inputs and outputs, ABNs enter into a period of slow maturation, mainly referring to the fine development of dendrites and spines and enrichment of connectivity (Zucker and Regehr 2002, Mizrahi 2007, Nissant, Bardy et al. 2009, Panzanelli, Bardy et al. 2009). It is shown that there may be a transient overproduction of synaptic contacts, peaking at 4 weeks post-birth, followed by a selective elimination (Whitman and Greer 2007). The latter phase is thought to be a slow refinement process (Kano and Hashimoto 2009).

The most interesting part of this developmental timeline lies in the concept of the "critical period", typically considered 2-8 weeks after being born in SVZ. Within this time window, ABNs rapidly mature and integrate, and display higher plasticity and morphological dynamics comparing with mature counterparts. Meanwhile, they are also vulnerable to death as discussed in the following section, but the survival rate is activity-dependent. People also found that manipulating ABNs within this age range could bring an effect on olfactory behavior, as well as TC/MC odor representation. These discoveries are the foundation of the ABN-ablation timeline I adopted in my thesis study described in Chapter 2.

Activity-dependent control of survival and development

It is important to mention that almost half of the new ABNs are eliminated within a few weeks after their birth (Petreanu and Alvarez-Buylla 2002, Winner, Cooper-Kuhn et al. 2002). A company may invite candidates for interviews, but maintains an overall 50% hiring rate; however, this rate may vary depending on the need of the entity. Similarly, we may wonder: is there a relation between survival

rate of the ABNs and the demands of the system, for which centrifugal inputs reflect the brain state and MC inputs tell about the olfactory environment (Shepherd, Chen et al. 2004)? Studies have indeed reported a strong correlation between the circuit activity and the maturation and survival of these cells.

During migration in RMS before reaching the OB, neurotransmitters already have an effect on migration and survival. GABA negatively controls the proliferation of SVZ precursor cells (Liu, Wang et al. 2005) and degreases the speed of migration in the RMS (Bolteus and Bordey 2004, Platel, Heintz et al. 2008). Glutamate also slows down migration but promotes survival (Platel, Heintz et al. 2008, Platel, Dave et al. 2010).

The richness of sensory inputs can influence the survival of newborn cells. Olfactory deprivation can decrease the survival rate (Corotto, Henegar et al. 1994, Wilson and Sullivan 1995, Petreanu and Alvarez-Buylla 2002, Winner, Cooper-Kuhn et al. 2002, Mandairon, Stack et al. 2006, Bovetti, Veyrac et al. 2009), and conversely, olfactory enrichment can cause an increase (Rochefort, Gheusi et al. 2002, Breton-Provencher, Lemasson et al. 2009). The effects of these manipulations were specially obvious during the critical period after cell birth (2-4 weeks) (Yamaguchi and Mori 2005, Quast, Ung et al. 2016). More direct evidence was provided when researchers manipulated excitability of ABNs specifically and found a positive correlation between that and the newborn cell survival rate during the critical period (Kelsch, Lin et al. 2009, Lin, Sim et al. 2010). In fact, ABN survival is also spatially selective, meaning they survive primarily in the areas activated by odors (Mandairon and Linster 2009). Besides survival, sensory activity can also

affect synaptic integration of ABNs. It was demonstrated that olfactory deprivation affected the spatial pattern of excitatory synaptogenesis by decreasing apical dendrites within the EPL and increasing the proximal portion (Kelsch, Lin et al. 2009). This opposite effect could be a result of compensation by maintaining excitation level under a lack of MC inputs.

Interestingly, people found that temporary passive exposure to odors would not affect cell survival. However, when rodents were actively learning odor discrimination tasks, the survival rate increased (Alonso, Viollet et al. 2006). Further, this effect depended on the age of ABNs (Mouret, Gheusi et al. 2008). In my thesis research described in Chapter 2, we can see a comparison of the consequence of ABN ablation between active learning task engagement and passive exposure states. Learning can change both local TC/MC inputs (by deeper inhalation, for example) and centrifugal inputs. To emphasize the latter, the ability to perform associative discrimination tasks has been shown to depend on the integrity of cortical centrifugal projections (Martin, Gervais et al. 2004). It is very worth mentioning that newborn cell would receive proximal glutamatergic inputs from these centrifugal fibers before arrival the OB and making contacts with local neurons, and these top-down inputs can influence their survival and development. So survived ABNs can be important mediators for the control from higher brain areas onto olfactory perception at the early stage of the OB.

The role of OB ABNs in modulating odor discrimination

The most-studied functional impact of ABNs is within the context of discriminating between different odors, whether dissimilar or higher similar. Studies have found over a decade ago that the ability to discriminate two very close odorants was improved after chronic exposure to the same odorants (Wilson and Stevenson 2003, Mandairon, Stack et al. 2006, Mandairon, Stack et al. 2006). This experience-dependent discriminability change is called perceptual learning (Gilbert, Kesner et al. 2001). Considering MCs are the only output neurons from the OB transmitting sensory inputs into higher brain areas, it is reasonable to hypothesize that perceptual improvement would be companied by differentiated MC population odor representation. "Pattern separation", a term first proposed in studies of Hipp (Leutgeb, Leutgeb et al. 2007, Bakker, Kirwan et al. 2008), is defined as "the process by which overlapping or similar inputs (representations) are transformed into less similar outputs" (Colgin, Moser et al. 2008, Wilson 2009). There has been accumulation of evidence for pattern separation in MCs (Abraham, Spors et al. 2004, Doucette and Restrepo 2008, Abraham, Egger et al. 2010, Li, Gire et al. 2015, Chu, Li et al. 2016). During my graduate school career, I participated in two coherent studies focusing on the properties of MC pattern separation over courses of odor discrimination learning (Figure 1.1,1.2). The discriminability of MC ensembles, reflected by the fraction of divergent MCs as well as the decoder accuracy, significantly increased when the animals were encouraged to discriminate between two highly similar odors (Figure 1.1c,e,g), but significantly decreased when the two odors were very different (Figure 1.1d,f,h) (Chu, Li et al. 2016). Furthermore, this phenomenon of bidirectional change was not observed in OSN inputs (Figure 2),

which strengthened the argument that pattern separation did happen at the MC population level (Chu, Li et al. 2017).

As modulators of MC activities, is there an impact of ABNs on pattern separation? To answer this question, the effect of adult neurogenesis on pattern separation has been studied in DG as well as OB, and the consensus at this point seems to be that ABNs are important for pattern separation in both locations (Aimone, Deng et al. 2011, Sahay, Wilson et al. 2011). In DG, ablation of newborn GCs was reported to impair normal spatial pattern separation (Clelland, Choi et al. 2009), while increasing hippocampal adult neurogenesis was sufficient to improve pattern separation (Sahay, Wilson et al. 2011). In OB, activation of inhibitory neurons, including young ABNs, can accelerate perceptual learning (Abraham, Egger et al. 2010), while suppression of them can reduce MC pattern separation (Gschwend, Abraham et al. 2015). For ABNs specifically, some studies have shown that newborn cells are indispensible for odor discrimination on the behavioral level (Gheusi, Cremer et al. 2000, Enwere, Shingo et al. 2004, Bath, Mandairon et al. 2008, Moreno, Linster et al. 2009, Alonso, Lepousez et al. 2012). However, others have reached the opposite conclusion, where little effects of ABN manipulation on odor discrimination was found (Imayoshi, Sakamoto et al. 2008, Breton-Provencher, Lemasson et al. 2009, Lazarini, Mouthon et al. 2009). The discrepancy can be due to differences in the efficiency and timeline of manipulation methods, as well as in the behavioral context.

Conclusion

In an adult rodent brain, neurogenesis supplies for two areas, DG and the OB, where there is a constant, high demand of plasticity to process complex information from the external world. For olfactory adult neurogenesis, new neurons become PGCs and GCs, the major types of inhibitory interneurons. With the first few weeks as a critical period of development, these ABNs display high plasticity and sensitivity to olfactory experiences in terms of survival probability and synapses formation between both centrifugal projections and local principle neurons, which make them ideal candidates to modulate TC/MCs odor responses and ultimately, olfactory perception.

Many questions remain unanswered. Is mammalian adult neurogenesis merely an evolution residue, or an active measure emerged based on the need? Do the ABNs regulate the OB circuit in a global manner, such as altering oscillatory patterns and excitability, or a stimulus-specific manner, such as adjusting connectivity only with MC/TCs responsive to a new odorant? How would the odor-evoked activity patterns change for ABNs throughout maturation - further, is the direction of such changes homogenous or heterogeneous? How do these changes contribute to odor encoding accordingly? On the cellular level, what are the unique properties and functional purposes for different types of inputs onto and outputs from ABNs during their development?

Ultimately, it is our hope that the knowledge accumulated from the study of adult neurogenesis would help us understand how to control proper synaptic integration and long-term survival of cells in pathological conditions.

Perspective

Looking back on studies that advanced our understanding of mammalian adult neurogenesis, we ought to appreciate the importance of techniques and tools. Without the invention of microscopes, Cajal would not be able to draw the circuitry structure of the OB more than a century ago. Without the discovery and detectability of radioactive isotopes like ^{14}C or ^{3}H, people could not have direct evidence for the occurrence of mitosis in the adult brain, which implied the existence of neural stem cells. Without the set up for tissue separation and culture, we would not be able to directly identify the hidden stem cells that had been searched for. Without the combination of electrophysiology, immunohistology and pharmacology, it would be impossible for the maturation process of neurotransmission, inputs and outputs of single ABNs to be studied so carefully and thoroughly.

Within the past decade, we witnessed a flourishing season of technology development. A rapid advancement of multi-photon microscopy and optogenetics allowed us to not only passively record *in vivo*, but also actively manipulate, neuron populations with unprecedented sizes. Exponential growth in the amount of engineered viruses and transgenic animals has given us opportunities to record and manipulate with high specificity on both neuron type level and projection level. My thesis study described in Chapter 2 is just another tribute to this era - using transgenic mice to achieve specific and efficient ablation of ABNs, and two-photon microscopy technique to record simultaneously and longitudinally MC populations. It only gets faster: the interdisciplinary researches are generating more powerful tools to record simultaneously even bigger neuron populations of multiple brain areas

(Sofroniew, Flickinger et al. 2016, Jun, Steinmetz et al. 2017), or to alter genetic codes in a finer temporally and spatially controlled manner (Cong, Ran et al. 2013, Hsu, Lander et al. 2014).

However, this also poses a challenge for us -- to balance between the quest for scientific answers and the pursuit of cutting-edge techniques. As I see, the update of technologies is way faster than the turnover of on our knowledge. A tool can be outdated within a few years, but a piece of newly discovered knowledge can last forever. The science community altogether should not lose sight of what is important, and what has a long-lasting impact. Yes, the availability of new techniques and tools can open up a whole new reservoir of scientific questions we can now answer; but ultimately, asking the right questions, and use or invent the right tools to answer them is what perpetuates the betterment of scientific research.

Figure 1.1 Comparison of Mitral cell odor responses during easy and difficult discrimination task

Mean ± S.E.M. for all error bars. (**a**) Schematic of the olfactory bulb. AAV2.10FLEX-hsyn-GCaMP6f was injected into the right olfactory bulb of Pcdh21-cre mice to express GCaMP6f specifically in mitral cells. (**b**) Trial structure of the discrimination task. (**c,e,g**) Difficult discrimination task (%52 Heptanal %48 Ethyl-tiglate vs. %48 Heptanal %52 Ethyl-tiglate). (**d,f,h**) Easy discrimination task (%100 Heptanal vs. %100 Ethyl-tiglate). (**c**) Behavioral performance task for each session (day) during the difficult discrimination task (n = 10 mice). (**d**) Behavioral performance task for each session (day) during the easy discrimination task (n = 10 mice). (**e**) Fraction of divergent neurons out of responsive neurons increases throughout difficult discrimination training (Pearson correlation; $r = 0.41$, $p < 0.001$). (**f**) Fraction of divergent neurons out of responsive neurons is maintained throughout easy discrimination training (Pearson correlation; $r = -0.03$, $p = 0.82$). (**g**) Population decoder accuracy is enhanced during difficult discrimination training (Pearson correlation; $r = 0.38$, $p < 0.01$). (**h**) Decoder accuracy during easy discrimination training significantly decreases (Pearson correlation; $r = -0.42$, $p < 0.01$).

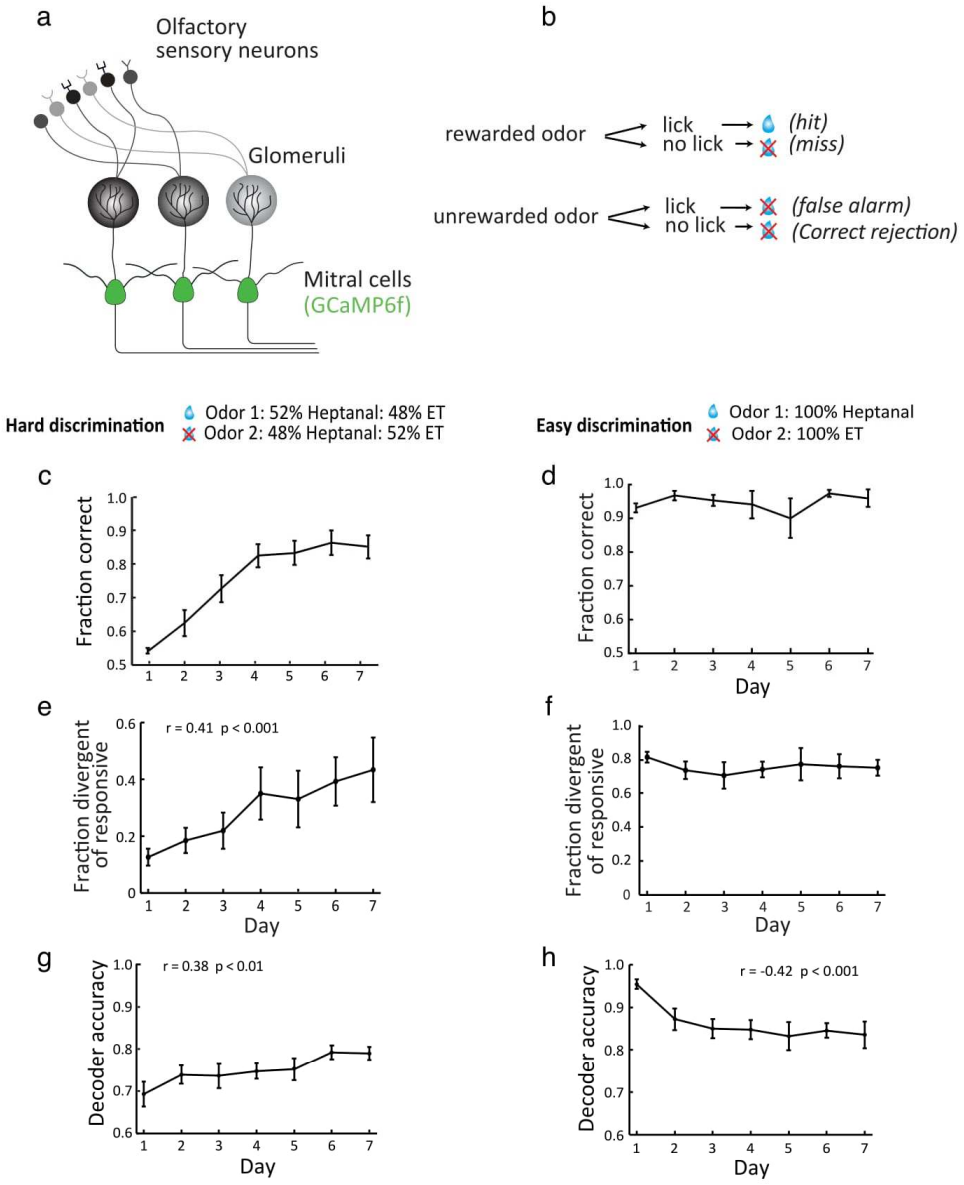
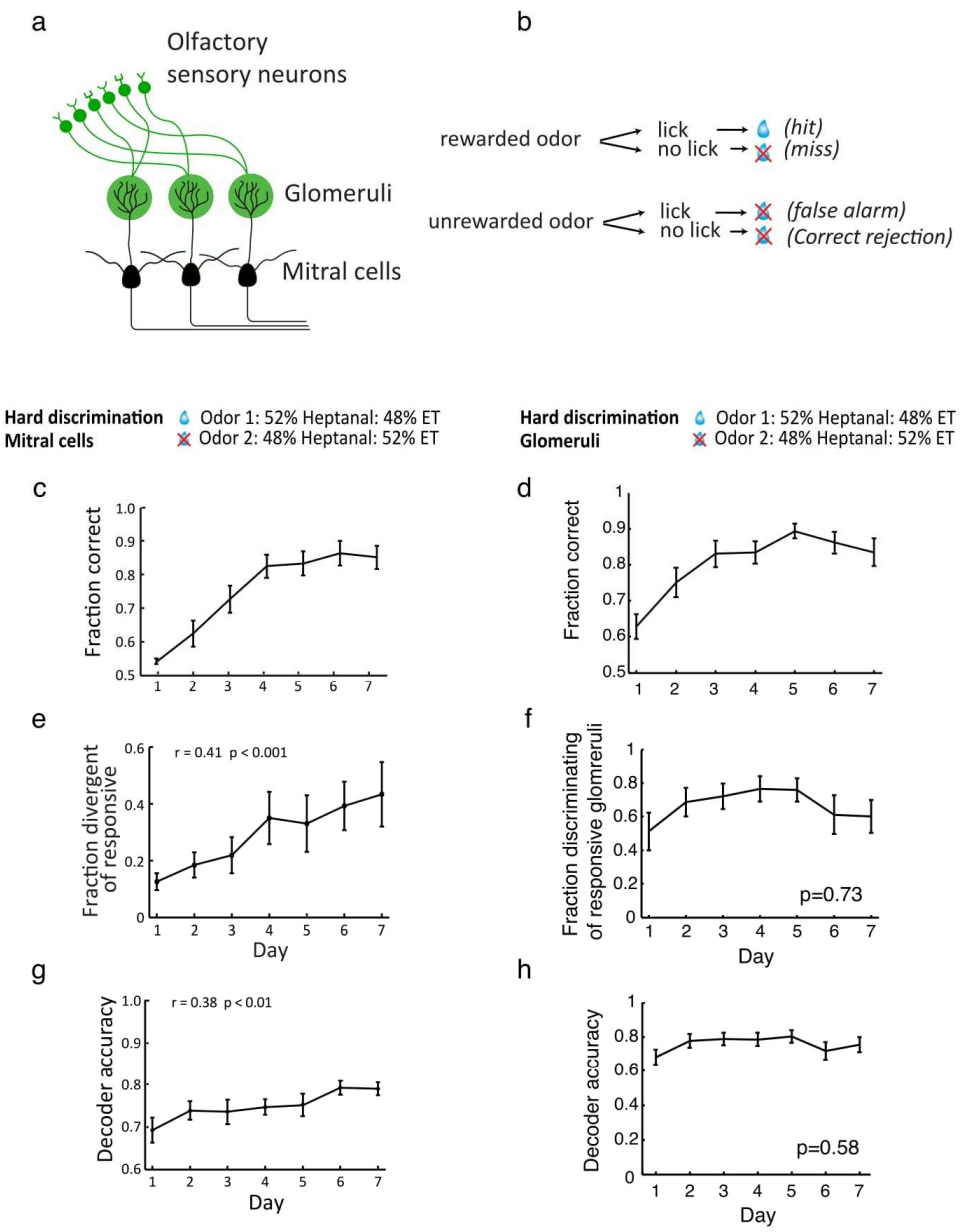

a
Olfactory sensory neurons
Glomeruli
Mitral cells (GCaMP6f)
b
rewarded odor
lick
no lick
(hit)
(miss)
unrewarded odor
lick
no lick
(false alarm)
(Correct rejection)
Hard discrimination
Odor 1: 52% Heptanal: 48% ET
Odor 2: 48% Heptanal: 52% ET
Easy discrimination
Odor 1: 100% Heptanal
Odor 2: 100% ET
c
Fraction correct
d
Fraction correct
Day
e
Fraction divergent of responsive
r = 0.41 p < 0.001
Day
f
Fraction divergent of responsive
Day
g
Decoder accuracy
r = 0.38 p < 0.01
Day
h
Decoder accuracy
r = -0.42 p < 0.001
Day

Figure 1.2 Comparison of glomerular vs. mitral cell odor responses during difficult discrimination task

Mean ± S.E.M. for all error bars. (a) Schematic of the olfactory bulb. Two photon imaging of glomerular responses was performed in OMP-tTA::tetO-GCaMP6s mice, in which ORNs express GCaMP6s. (b) Trial structure of the discrimination task. (c,e,g) Mitral cell responses during difficult discrimination task (%52 Heptanal %48 Ethyl-tiglate vs. %48 Heptanal %52 Ethyl-tiglate). See Figure 1.1 legends for detailed description. (d,f,h) Glomerular responses during difficult discrimination task. (d) Behavioral performance task for each session (day) during the difficult discrimination task (n = 13 mice). (f) Fraction of divergent neurons out of responsive neurons does not significantly change throughout difficult discrimination training (Pearson correlation; $p = 0.73$). (h) Decoder accuracy does not change during difficult discrimination training(Pearson correlation; $p = 0.87$).


a
Olfactory
sensory neurons
Glomeruli
Mitral cells
b
rewarded odor
lick
no lick
(hit)
(miss)
unrewarded odor
lick
no lick
(false alarm)
(Correct rejection)
Hard discrimination
Mitral cells
Odor 1: 52% Heptanal: 48% ET
Odor 2: 48% Heptanal: 52% ET
Hard discrimination
Glomeruli
Odor 1: 52% Heptanal: 48% ET
Odor 2: 48% Heptanal: 52% ET
c
Fraction correct
d
Fraction correct
e
Fraction divergent of responsive
r = 0.41 p < 0.001
Day
f
Fraction discriminating of responsive glomreruli
p=0.73
Day
g
Decoder accuracy
r = 0.38 p < 0.01
Day
h
Decoder accuracy
p=0.58
Day

References

Abney, E. R., P. P. Bartlett and M. C. Raff (1981). "Astrocytes, ependymal cells, and oligodendrocytes develop on schedule in dissociated cell cultures of embryonic rat brain." Developmental Biology **83**(2): 301-310.

Abraham, N. M., V. Egger, D. R. Shimshek, R. Renden, I. Fukunaga, R. Sprengel, P. H. Seeburg, M. Klugmann, T. W. Margrie, A. T. Schaefer and T. Kuner (2010). "Synaptic Inhibition in the Olfactory Bulb Accelerates Odor Discrimination in Mice." Neuron **65**(3): 399-411.

Abraham, N. M., H. Spors, A. Carleton, T. W. Margrie, T. Kuner and A. T. Schaefer (2004). "Maintaining accuracy at the expense of speed: Stimulus similarity defines odor discrimination time in mice." Neuron **44**: 865-876.

Aimone, James B., W. Deng and Fred H. Gage (2011). "Resolving New Memories: A Critical Look at the Dentate Gyrus, Adult Neurogenesis, and Pattern Separation." Neuron **70**(4): 589-596.

Alonso, M., G. Lepousez, W. Sebastien, C. Bardy, M. M. Gabellec, N. Torquet and P. M. Lledo (2012). "Activation of adult-born neurons facilitates learning and memory." Nat Neurosci **15**(6): 897-904.

Alonso, M., C. Viollet, M. M. Gabellec, V. Meas-Yedid, J. C. Olivo-Marin and P. M. Lledo (2006). "Olfactory discrimination learning increases the survival of adult-born neurons in the olfactory bulb." J Neurosci **26**: 10508-10513.

Altman, J. (1962). "Are New Neurons Formed in the Brains of Adult Mammals?" Science **135**(3509): 1127-1128.

Altman, J. (1963). "Autoradiographic investigation of cell proliferation in the brains of rats and cats." Anat Rec **145**(4): 573-591.

Altman, J. and G. D. Das (1965). "Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats." J Comp Neurol **124**(3): 319-335.

Alvarado, A. S. (2000). "Regeneration in the metazoans: why does it happen?" BioEssays **22**(6): 578-590.

Aroniadou-Anderjaska, V., F.-M. Zhou, C. A. Priest, M. Ennis and M. T. Shipley (2000). "Tonic and Synaptically Evoked Presynaptic Inhibition of Sensory Input to the Rat Olfactory Bulb Via GABA_BHeteroreceptors." Journal of Neurophysiology **84**(3): 1194.

Bakker, A., C. B. Kirwan, M. Miller and C. E. L. Stark (2008). "Pattern Separation in the Human Hippocampal CA3 and Dentate Gyrus." Science **319**(5870): 1640-1642.

Bardy, C., M. Alonso, W. Bouthour and P.-M. Lledo (2010). "How, When, and Where New Inhibitory Neurons Release Neurotransmitters in the Adult Olfactory Bulb." The Journal of Neuroscience **30**(50): 17023.

Bath, K. G., N. Mandairon, D. Jing, R. Rajagopal, R. Kapoor, Z. Y. Chen, T. Khan, C. C. Proenca, R. Kraemer, T. A. Cleland, B. L. Hempstead, M. V. Chao and F. S. Lee (2008). "Variant Brain-Derived Neurotrophic Factor (Val66Met) Alters Adult Olfactory Bulb Neurogenesis and Spontaneous Olfactory Discrimination." J Neurosci **28**(10): 2383-2393.

Bergmann, O., J. Liebl, S. Bernard, K. Alkass, Maggie S. Y. Yeung, P. Steier, W. Kutschera, L. Johnson, M. Landén, H. Druid, Kirsty L. Spalding and J. Frisén (2012). "The Age of Olfactory Bulb Neurons in Humans." Neuron **74**(4): 634-639.

Bergmann, O., K. L. Spalding and J. Frisén (2015). "Adult Neurogenesis in Humans." Cold Spring Harbor Perspectives in Biology **7**(7).

Berkowicz, D. A. and P. Q. Trombley (2000). "Dopaminergic modulation at the olfactory nerve synapse." Brain Research **855**(1): 90-99.

Bhardwaj, R. D., M. A. Curtis, K. L. Spalding, B. A. Buchholz, D. Fink, T. Björk-Eriksson, C. Nordborg, F. H. Gage, H. Druid, P. S. Eriksson and J. Frisén (2006). "Neocortical neurogenesis in humans is restricted to development." Proceedings of the National Academy of Sciences **103**(33): 12564-12568.

Blomqvist, E. H., A. Bramerson, P. Stjarne and S. Nordin (2004). "Consequences of olfactory loss and adopted coping strategies." Rhinol Intl **42**(4): 189-194.

Bolteus, A. J. and A. Bordey (2004). "GABA Release and Uptake Regulate Neuronal Precursor Migration in the Postnatal Subventricular Zone." The Journal of Neuroscience **24**(35): 7623.

Bovetti, S., A. Veyrac, P. Peretto, A. Fasolo and S. de Marchis (2009). "Olfactory enrichment influences adult neurogenesis modulating GAD67 and plasticity-related molecules expression in newborn cells of the olfactory bulb." PLoS ONE **4**.

Brennan, P. A. and E. B. Keverne (2004). "Something in the Air? New Insights into Mammalian Pheromones." Current Biology **14**(2): R81-R89.

Breton-Provencher, V., M. Lemasson, M. R. Peralta and A. Saghatelyan (2009). "Interneurons produced in adulthood are required for the normal functioning of the olfactory bulb network and for the execution of selected olfactory behaviors." J Neurosci **29**(48): 15245-15257.

Buck, L. and R. Axel (1991). "A novel multigene family may encode odorant receptors: A molecular basis for odor recognition." Cell **65**(1): 175-187.

Burd, G. D. and F. Nottebohm (1985). "Ultrastructural characterization of synaptic terminals formed on newly generated neurons in a song control nucleus of the adult canary forebrain." J Comp Neurol **240**(2): 143-152.

Carlson, B. (2007). "Principles of regenerative biology " Elsevier.

Chu, Monica W., Wankun L. Li and T. Komiyama (2016). "Balancing the Robustness and Efficiency of Odor Representations during Learning." Neuron **92**(1): 174-186.

Chu, M. W., W. L. Li and T. Komiyama (2017). "Lack of Pattern Separation in Sensory Inputs to the Olfactory Bulb during Perceptual Learning." eneuro.

Clark, G. A. (1995). "Emotional Learning: Fear and loathing in the amygdala." Current Biology **5**(3): 246-248.

Clelland, C. D., M. Choi, C. Romberg, G. D. Clemenson, A. Fragniere, P. Tyers, S. Jessberger, L. M. Saksida, R. A. Barker, F. H. Gage and T. J. Bussey (2009). "A Functional Role for Adult Hippocampal Neurogenesis in Spatial Pattern Separation." Science **325**(5937): 210-213.

Colgin, L. L., E. I. Moser and M.-B. Moser (2008). "Understanding memory through hippocampal remapping." Trends in Neurosciences **31**(9): 469-477.

Cong, L., F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. D. Hsu, X. Wu, W. Jiang, L. A. Marraffini and F. Zhang (2013). "Multiplex Genome Engineering Using CRISPR/Cas Systems." Science **339**(6121): 819-823.

Corotto, F. S., J. R. Henegar and J. A. Maruniak (1994). "Odor deprivation leads to reduced neurogenesis and reduced neuronal survival in the olfactory bulb of the adult mouse." Neuroscience **61**(4): 739-744.

Croy, I., S. Negoias, L. Novakova, B. N. Landis and T. Hummel (2012). "Learning about the Functions of the Olfactory System from People without a Sense of Smell." PLOS ONE **7**(3): e33365.

Curtis, M. A., M. Kam, U. Nannmark, M. F. Anderson, M. Z. Axell, C. Wikkelso, S. Holtås, W. M. C. van Roon-Mom, T. Björk-Eriksson, C. Nordborg, J. Frisén, M. Dragunow, R. L. M. Faull and P. S. Eriksson (2007). "Human Neuroblasts Migrate to the Olfactory Bulb via a Lateral Ventricular Extension." Science **315**(5816): 1243-1249.

Davis, M. and P. J. Whalen (2000). "The amygdala: vigilance and emotion." Molecular Psychiatry **6**: 13.

de Castro, F. (2009). "Wiring Olfaction: The Cellular and Molecular Mechanisms that Guide the Development of Synaptic Connections from the Nose to the Cortex." Frontiers in Neuroscience **3**: 52.

Deems, D. A., R. L. Doty, R. Settle and et al. (1991). "Smell and taste disorders, a study of 750 patients from the university of pennsylvania smell and taste center." Archives of Otolaryngology–Head & Neck Surgery **117**(5): 519-528.

Derer, P., V. S. Caviness and R. L. Sidman (1977). "Early cortical histogenesis in the primary olfactory cortex of the mouse." Brain Research **123**(1): 27-40.

Devor, M. (1976). "Fiber trajectories of olfactory bulb efferents in the hamster." J Comp Neurol **166**(1): 31-47.

Doucette, W. and D. Restrepo (2008). "Profound context-dependent plasticity of mitral cell responses in olfactory bulb." PLoS Biol **6**(10): e258.

Dunnett, S., A. Björklund, F. H. Gage and U. Stenevi (1985). Transplantation of mesencephalic dopamine neurons to the striatum of adult rats.

Ennis, M., F.-M. Zhou, K. J. Ciombor, V. Aroniadou-Anderjaska, A. Hayar, E. Borrelli, L. A. Zimmer, F. Margolis and M. T. Shipley (2001). "Dopamine D2 Receptor–Mediated Presynaptic Inhibition of Olfactory Nerve Terminals." Journal of Neurophysiology **86**(6): 2986.

Enwere, E., T. Shingo, C. Gregg, H. Fujikawa, S. Ohta and S. Weiss (2004). "Aging Results in Reduced Epidermal Growth Factor Receptor Signaling, Diminished Olfactory Neurogenesis, and Deficits in Fine Olfactory Discrimination." J Neurosci **24**(38): 8354-8365.

Eriksson, P. S., E. Perfilieva, T. Björk-Eriksson, A.-M. Alborn, C. Nordborg, D. A. Peterson and F. H. Gage (1998). "Neurogenesis in the adult human hippocampus." Nature Medicine **4**: 1313.

Ernst, A., K. Alkass, S. Bernard, M. Salehpour, S. Perl, J. Tisdale, G. Possnert, H. Druid and J. Frisén (2014). "Neurogenesis in the Striatum of the Adult Human Brain." Cell **156**(5): 1072-1083.

Gabor, M. H. and R. D. Hotchkiss (1979). "Parameters governing bacterial regeneration and genetic recombination after fusion of *Bacillus subtilis* protoplasts." J Bacteriol **137**(3): 1346-1353.

Gage, F. H. (2002). "Neurogenesis in the Adult Brain." The Journal of Neuroscience **22**(3): 612-613.

Gage, F. H., P. W. Coates, T. D. Palmer, H. G. Kuhn, L. J. Fisher, J. O. Suhonen, D. A. Peterson, S. T. Suhr and J. Ray (1995). "Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain." Proceedings of the National Academy of Sciences of the United States of America **92**(25): 11879-11883.

Gheusi, G., H. Cremer, H. McLean, G. Chazal, J. D. Vincent and P. M. Lledo (2000). "Importance of newly generated neurons in the adult olfactory bulb for odor discrimination." Proc. Natl. Acad. Sci. U. S. A. **97**(4): 1823-1828.

Gilbert, P. E., R. P. Kesner and I. Lee (2001). "Dissociating hippocampal subregions: A double dissociation between dentate gyrus and CA1." Hippocampus **11**(6): 626-636.

Giovanni, G. C. and M. Randolf (2000). "Odour perception in honeybees: coding information in glomerular patterns." Current Opinion in Neurobiology **10**(4): 504-510.

Gould, E. (2007). "How widespread is adult neurogenesis in mammals?" Nat Rev Neurosci **8**: 481-488.

Graeff, F. G., F. S. Guimarães, T. G. C. S. De Andrade and J. F. W. Deakin (1996). "Role of 5-HT in stress, anxiety, and depression." Pharmacology Biochemistry and Behavior **54**(1): 129-141.

Greer, C. A. (1991). "Structural organization of the olfactory system. In et al. T.V.Getchell(ed): Smell and taste in health and disease." New York: Raven Press: 65-81.

Gschwend, O., N. M. Abraham, S. Lagier, F. Begnaud, I. Rodriguez and A. Carleton (2015). "Neuronal pattern separation in the olfactory bulb improves odor discrimination learning." Nat Neurosci **18**(10): 1474-1482.

Gudziol, V., S. Wolff-Stephan, K. Aschenbrenner, P. Joraschky and T. Hummel (2009). "ORIGINAL RESEARCH—PSYCHOLOGY: Depression Resulting from Olfactory Dysfunction is Associated with Reduced Sexual Appetite—A Cross-Sectional Cohort Study." The Journal of Sexual Medicine **6**(7): 1924-1929.

Handberg-Thorsager, M., E. Fernandez and E. Salo (2008). "Stem cells and regeneration in planarians." Front Biosci **13**: 6374-6394.

Hopfield, J. J. (1991). "Olfactory computation and object perception." Proceedings of the National Academy of Sciences **88**(15): 6462-6466.

Hsu, P. D., E. S. Lander and F. Zhang (2014). "Development and Applications of CRISPR-Cas9 for Genome Engineering." Cell **157**(6): 1262-1278.

Imayoshi, I., M. Sakamoto, T. Ohtsuka, K. Takao, T. Miyakawa, M. Yamaguchi, K. Mori, T. Ikeda, S. Itohara and R. Kageyama (2008). "Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain." Nat Neurosci **11**(10): 1153-1161.

Isaacson, J. S. (1999). "Glutamate Spillover Mediates Excitatory Transmission in the Rat Olfactory Bulb." Neuron **23**(2): 377-384.

Isaacson, J. S. and B. W. Strowbridge (1998). "Olfactory reciprocal synapses: dendritic signaling in the CNS." Neuron **20**(4): 749-761.

Jun, J. J., N. A. Steinmetz, J. H. Siegle, D. J. Denman, M. Bauza, B. Barbarits, A. K. Lee, C. A. Anastassiou, A. Andrei, Ç. Aydın, M. Barbic, T. J. Blanche, V. Bonin, J. Couto, B. Dutta, S. L. Gratiy, D. A. Gutnisky, M. Häusser, B. Karsh, P. Ledochowitsch, C. M. Lopez, C. Mitelut, S. Musa, M. Okun, M. Pachitariu, J. Putzeys, P. D. Rich, C. Rossant, W.-l. Sun, K. Svoboda, M. Carandini, K. D. Harris, C. Koch, J. O'Keefe and T. D. Harris (2017). "Fully integrated silicon probes for high-density recording of neural activity." Nature **551**: 232.

Kano, M. and K. Hashimoto (2009). "Synapse elimination in the central nervous system." Current Opinion in Neurobiology **19**(2): 154-161.

Katagiri, H., M. Pallotto, A. Nissant, K. Murray, M. Sassoè-Pognetto and P.-M. Lledo (2011). "Dynamic development of the first synapse impinging on adult-born neurons in the olfactory bulb circuit." Neural Systems & Circuits **1**(1): 6.

Kauer, J. S. and J. White (2001). "Imaging and Coding in the Olfactory System." Annual Review of Neuroscience **24**(1): 963-979.

Keller, A. and D. Malaspina (2013). "Hidden consequences of olfactory dysfunction: a patient report series." BMC Ear, Nose, and Throat Disorders **13**: 8-8.

Kelsch, W., C.-W. Lin and C. Lois (2008). "Sequential development of synapses in dendritic domains during adult neurogenesis." Proceedings of the National Academy of Sciences **105**(43): 16803-16808.

Kelsch, W., C. W. Lin, C. P. Mosley and C. Lois (2009). "A Critical Period for Activity-Dependent Synaptic Development during Olfactory Bulb Adult Neurogenesis." J Neurosci **29**(38): 11852-11858.

Keverne, E. B. (2004). "Importance of olfactory and vomeronasal systems for male sexual function." Physiology & Behavior **83**(2): 177-187.

Klingler, E. (2017). "Development and Organization of the Evolutionarily Conserved Three-Layered Olfactory Cortex." eneuro **4**(1).

Kole, A. J., R. P. Annis and M. Deshmukh (2013). "Mature neurons: equipped for survival." Cell Death &Amp; Disease **4**: e689.

Kornack, D. R. and P. Rakic (1999). "Continuation of neurogenesis in the hippocampus of the adult macaque monkey." Proc. Natl. Acad. Sci. U. S. A. **96**(10): 5768-5773.

Kornack, D. R. and P. Rakic (2001). "Cell Proliferation Without Neurogenesis in Adult Primate Neocortex." Science **294**(5549): 2127-2130.

Kuhn, H. G., H. Dickinson-Anson and F. H. Gage (1996). "Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation." The Journal of Neuroscience **16**(6): 2027.

Lazarini, F. and P.-M. Lledo (2011). "Is adult neurogenesis essential for olfaction?" Trends in Neurosciences **34**(1): 20-30.

Lazarini, F., M. A. Mouthon, G. Gheusi, F. de Chaumont, J. C. Olivo-Marin, S. Lamarque, D. N. Abrous, F. D. Boussin and P. M. Lledo (2009). "Cellular and behavioral effects of cranial irradiation of the subventricular zone in adult mice." PLoS One **4**(9): e7017.

Le Vay, S., T. N. Wiesel and D. H. Hubel (1980). "The development of ocular dominance columns in normal and visually deprived monkeys." J Comp Neurol **191**(1): 1-51.

LeDoux, J. (2003). "The emotional brain, fear, and the amygdala." Cell Mol Neurobiol **23**(4-5): 727-738.

Leutgeb, J. K., S. Leutgeb, M.-B. Moser and E. I. Moser (2007). "Pattern Separation in the Dentate Gyrus and CA3 of the Hippocampus." Science **315**(5814): 961-966.

Li, A., D. H. Gire and D. Restrepo (2015). "ϒ Spike-Field Coherence in a Population of Olfactory Bulb Neurons Differentiates between Odors Irrespective of Associated Outcome." The Journal of Neuroscience **35**(14): 5808.

Lin, C.-W., S. Sim, A. Ainsworth, M. Okada, W. Kelsch and C. Lois (2010). "Genetically Increased Cell-Intrinsic Excitability Enhances Neuronal Integration into Adult Brain Circuits." Neuron **65**(1): 32-39.

Wang, C., F. Liu, Y-Y. Liu, C-H. Zhao, Y. You, L. Wang, J. Zhang, B. Wei, T. Ma, Q. Zhang, Y. Zhang, R. Chen, H. Song and Z. Yang (2007). "Identification and characterization of neuroblasts in the subventricular zone and rostral migratory stream of the adult human brain." Cell Research **21**: 1534-1550.

Liu, X., Q. Wang, T. F. Haydar and A. Bordey (2005). "Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors." Nature Neuroscience **8**: 1179.

Lois, C. and A. Alvarez-Buylla (1994). "Long-distance neuronal migration in the adult mammalian brain." Science **264**(5162): 1145-1148.

Lois, C., J.-M. García-Verdugo and A. Alvarez-Buylla (1996). "Chain Migration of Neuronal Precursors." Science **271**(5251): 978-981.

Luo, M. and L. C. Katz (2001). "Response Correlation Maps of Neurons in the Mammalian Olfactory Bulb." Neuron **32**(6): 1165-1179.

Luo, S. X. and E. J. Huang (2016). "Dopaminergic Neurons and Brain Reward Pathways: From Neurogenesis to Circuit Assembly." The American Journal of Pathology **186**(3): 478-488.

Malnic, B., J. Hirono, T. Sato and L. B. Buck (1999). "Combinatorial Receptor Codes for Odors." Cell **96**(5): 713-723.

Mandairon, N. and C. Linster (2009). "Odor Perception and Olfactory Bulb Plasticity in Adult Mammals." Journal of Neurophysiology **101**(5): 2204.

Mandairon, N., C. Stack, C. Kiselycznyk and C. Linster (2006). "Broad activation of the olfactory bulb produces long-lasting changes in odor perception." Proceedings of the National Academy of Sciences **103**(36): 13543-13548.

Mandairon, N., C. Stack and C. Linster (2006). "Olfactory enrichment improves the recognition of individual components in mixtures." Physiology & Behavior **89**(3): 379-384.

Martin, C., R. Gervais, P. Chabaud, B. Messaoudi and N. Ravel (2004). "Learning-induced modulation of oscillatory activities in the mammalian olfactory system: The role of the centrifugal fibres." Journal of Physiology-Paris **98**(4): 467-478.

Martinez, M. C., J. Blanco, B. M. M and A. F. J (1987). "Structure of the piriform cortex of the adult rat. A Golgi study." J Himforsch **28**(3): 341-348.

Michalopoulos, G. K. and M. C. DeFrances (1997). "Liver Regeneration." Science **276**(5309): 60-66.

Ming, G. L. and H. Song (2011). "Adult neurogenesis in the mammalian brain: significant answers and significant questions." Neuron **70**(4): 687-702.

Mizrahi, A. (2007). "Dendritic development and plasticity of adult-born neurons in the mouse olfactory bulb." Nat Neurosci **10**: 444-452.

Mladinic, M., K. J. Muller and J. G. Nicholls (2009). "Central nervous system regeneration: from leech to opossum." J Physiol **587**(12): 2775-2782.

Mombaerts, P., F. Wang, C. Dulac, S. K. Chao, A. Nemes, M. Mendelsohn, J. Edmondson and R. Axel (1996). "Visualizing an Olfactory Sensory Map." Cell **87**(4): 675-686.

Moreno, M. M., C. Linster, O. Escanilla, J. Sacquet, A. Didier and N. Mandairon (2009). "Olfactory perceptual learning requires adult neurogenesis." Proc. Natl. Acad. Sci. U. S. A. **106**(42): 17980-17985.

Morris, R. G. M. (1999). "D.O. Hebb: The Organization of Behavior, Wiley: New York; 1949." Brain Research Bulletin **50**(5): 437.

Mouret, A., G. Gheusi, M. M. Gabellec, F. de Chaumont, J. C. Olivo-Marin and P. M. Lledo (2008). "Learning and survival of newly generated neurons: when time matters." J Neurosci **28**(45): 11511-11516.

Nguyen, L., B. Malgrange, I. Breuskin, L. Bettendorff, G. Moonen, S. Belachew and J.-M. Rigo (2003). "Autocrine/Paracrine Activation of the GABA_A Receptor Inhibits the Proliferation of Neurogenic Polysialylated Neural Cell Adhesion Molecule-Positive (PSA-NCAM⁺) Precursor Cells from Postnatal Striatum." The Journal of Neuroscience **23**(8): 3278.

Nissant, A., C. Bardy, H. Katagiri, K. Murray and P. M. Lledo (2009). "Adult neurogenesis promotes synaptic plasticity in the olfactory bulb." Nat Neurosci **12**(6): 728-730.

Nissant, A. and M. Pallotto (2011). "Integration and maturation of newborn neurons in the adult olfactory bulb - from synapses to function." Euro J Neuro **33**(6): 1069-1077.

Nordin, S., E. Hedén Blomqvist, P. Olsson, P. Stjärne and A. Ehnhage (2011). "Effects of smell loss on daily life and adopted coping strategies in patients with nasal polyposis with asthma." Acta Oto-Laryngologica **131**(8): 826-832.

Odelberg, S. J. (2004). "Unraveling the Molecular Basis for Regenerative Cellular Plasticity." PLOS Biology **2**(8): e232.

Palmer, T. D., J. Takahashi and F. H. Gage (1997). "The Adult Rat Hippocampus Contains Primordial Neural Stem Cells." Molecular and Cellular Neuroscience **8**(6): 389-404.

Panzanelli, P., C. Bardy, A. Nissant, M. Pallotto, M. Sassoè-Pognetto, P.-M. Lledo and J.-M. Fritschy (2009). "Early Synapse Formation in Developing Interneurons of the Adult Olfactory Bulb." The Journal of Neuroscience **29**(48): 15039.

Paridaen, J. T. M. L. and W. B. Huttner (2014). "Neurogenesis during development of the vertebrate central nervous system." EMBO Reports **15**(4): 351-364.

Paton, J. and F. Nottebohm (1984). "Neurons generated in the adult brain are recruited into functional circuits." Science **225**(4666): 1046-1048.

Peters, A. J., J. Lee, N. G. Hedrick, K. O'Neil and T. Komiyama (2017). "Reorganization of corticospinal output during motor learning." Nature Neuroscience **20**: 1133.

Petreanu, L. and A. Alvarez-Buylla (2002). "Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction." J Neurosci **22**(14): 6106-6113.

Platel, J.-C., K. A. Dave, V. Gordon, B. Lacar, M. E. Rubio and A. Bordey (2010). "NMDA Receptors Activated by Subventricular Zone Astrocytic Glutamate Are Critical for Neuroblast Survival Prior to Entering a Synaptic Network." Neuron **65**(6): 859-872.

Platel, J.-C., S. Stamboulian, I. Nguyen and A. Bordey (2010). "Neurotransmitter signaling in postnatal neurogenesis: The first leg." Brain Research Reviews **63**(1): 60-71.

Platel, J. C., T. Heintz, S. Young, V. Gordon and A. Bordey (2008). "Tonic activation of GLUK5 kainate receptors decreases neuroblast migration in whole-mounts of the subventricular zone." J Physiol **586**: 3783-3793.

Platel, J. C., B. Lacar and A. Bordey (2007). "GABA and glutamate signaling: homeostatic control of adult forebrain neurogenesis." J Mol Histol **38**(6): 602-610.

Price, J. L. and T. P. S. Powell (1970). "The Synaptology of the Granule Cells of the Olfactory Bulb." Journal of Cell Science **7**(1): 125.

Quast, K. B., K. Ung, E. Froudarakis, L. Huang, I. Herman, A. P. Addison, J. Ortiz-Guzman, K. Cordiner, P. Saggau, A. S. Tolias and B. R. Arenkiel (2016). "Developmental broadening of inhibitory sensory maps." Nat Neurosci **20**: 189-189.

Rall, W., G. M. Shepherd, T. S. Reese and M. W. Brightman (1966). "Dendro-dendritic synaptic pathway for inhibition in the olfactory bulb." Experimental Neurology **14**(1): 44-56.

Ramon y Cajal, S. (1890). "Origen y Terminación de las Fibras Nerviosas Olfatorias." Bacelona:GacSan: 1-21.

Reddien, P. W. and A. S. Alvarado (2004). "Fundamentals of planarian regeneration." Annu Rev Cell Dev Biol **20**: 725-757.

Restrepo, D., J. Arellano, A. M. Oliva, M. L. Schaefer and W. Lin (2004). "Emerging views on the distinct but related roles of the main and accessory olfactory systems in responsiveness to chemosensory signals in mice." Hormones and Behavior **46**(3): 247-256.

Reynolds, B. and S. Weiss (1992). "Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system." Science **255**(5052): 1707-1710.

Richards, L. J., T. J. Kilpatrick and P. F. Bartlett (1992). "De novo generation of neuronal cells from the adult mouse brain." Proceedings of the National Academy of Sciences of the United States of America **89**(18): 8591-8595.

Rochefort, C., G. Gheusi, J. D. Vincent and P. M. Lledo (2002). "Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory." J Neurosci **22**(7): 2679-2689.

Sachse, S. and C. G. Galizia (2002). "Role of Inhibition for Temporal and Spatial Odor Representation in Olfactory Output Neurons: A Calcium Imaging Study." Journal of Neurophysiology **87**(2): 1106.

Sahay, A., Donald A. Wilson and R. Hen (2011). "Pattern Separation: A Common Function for New Neurons in Hippocampus and Olfactory Bulb." Neuron **70**(4): 582-588.

Sanai, N., M. S. Berger, J. M. Garcia-Verdugo and A. Alvarez-Buylla (2007). "Comment on "Human Neuroblasts Migrate to the Olfactory Bulb via a Lateral Ventricular Extension"." Science **318**(5849): 393-393.

Sanai, N., T. Nguyen, R. A. Ihrie, Z. Mirzadeh, H.-H. Tsai, M. Wong, N. Gupta, M. S. Berger, E. Huang, J.-M. Garcia-Verdugo, D. H. Rowitch and A. Alvarez-Buylla (2011). "Corridors of Migrating Neurons in Human Brain and Their Decline during Infancy." Nature **478**(7369): 382-386.

Sanai, N., A. D. Tramontin, A. Quiñones-Hinojosa, N. M. Barbaro, N. Gupta, S. Kunwar, M. T. Lawton, M. W. McDermott, A. T. Parsa, J. Manuel-García Verdugo, M. S. Berger and A. Alvarez-Buylla (2004). "Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration." Nature **427**: 740.

Schoppa, N. E. and N. N. Urban (2003). "Dendritic processing within olfactory bulb circuits." Trends in Neurosciences **26**(9): 501-506.

Schultz, E. F. and J. T. Tapp (1973). "Olfactory control of behavior in rodents." Psychological Bulletin **79**(1): 21-44.

Schultz, W. (2015). "Neuronal Reward and Decision Signals: From Theories to Data." Physiological Reviews **95**(3): 853-951.

Schwob, J. E. and J. L. Price (1984). "The development of axonal connections in the central olfactory system of rats." J Comp Neurol **223**(2): 177-202.

Scott, J. W., R. L. McBride and S. P. Schneider (1980). "The organization of projections from the olfactory bulb to the piriform cortex and olfactory tubercle in the rat." J Comp Neurol **194**(3): 519-534.

Shepherd, G. M. (2004). "The synaptic organization of the brain." Oxford University Press, New York.

Shepherd, G. M. (2005). "Perception without a Thalamus: How Does Olfaction Do It?" Neuron **46**(2): 166-168.

Shepherd, G. M., W. R. Chen and G. C. A (2004). "Olfactory bulb. In the synaptic organization of the brain, 5th edn. Shepherd, G.M." Oxford University Press, New York **165-216**.

Sofroniew, N. J., D. Flickinger, J. King and K. Svoboda (2016). "A large field of view two-photon mesoscope with subcellular resolution for in vivo imaging." eLife **5**: e14472.

Temmel, A. P., C. Quint, B. Schickinger-Fischer, L. Klimek, E. Stoller and T. Hummel (2002). "Characteristics of olfactory disorders in relation to major causes of olfactory loss." Archives of Otolaryngology–Head & Neck Surgery **128**(6): 635-641.

Urban, N. N. and B. Sakmann (2002). "Reciprocal intraglomerular excitation and intra- and interglomerular lateral inhibition between mouse olfactory bulb mitral cells." J Physiol **542**(2): 355-367.

Wachowiak, M. and L. B. Cohen (1999). "Presynaptic Inhibition of Primary Olfactory Afferents Mediated by Different Mechanisms in Lobster and Turtle." The Journal of Neuroscience **19**(20): 8808.

Wang, D. D., D. D. Krueger and A. Bordey (2003). "GABA depolarizes neuronal progenitors of the postnatal subventricular zone via GABAa receptor activation." J Physiol **550**(3): 785-800.

Wellis, D. P. and J. W. Scott (1990). "Intracellular responses of identified rat olfactory bulb interneurons to electrical and odor stimulation." Journal of Neurophysiology **64**(3): 932.

Whitman, M. C. and C. A. Greer (2007). "Synaptic Integration of Adult-Generated Olfactory Bulb Granule Cells: Basal Axodendritic Centrifugal Input Precedes Apical Dendro-dendritic Local Circuits." The Journal of Neuroscience **27**(37): 9951.

Wilson, D. A. (2009). "Pattern Separation and Completion in Olfaction." Annals of the New York Academy of Sciences **1170**: 306-312.

Wilson, D. A. and R. J. Stevenson (2003). "Olfactory perceptual learning: the critical role of memory in odor discrimination." Neuroscience & Biobehavioral Reviews **27**(4): 307-328.

Wilson, D. A. and R. M. Sullivan (1995). "The D2 antagonist spiperone mimics the effects of olfactory deprivation on mitral/tufted cell odor response patterns." The Journal of Neuroscience **15**(8): 5574.

Winner, B., C. M. Cooper-Kuhn, R. Aigner, J. Winkler and H. G. Kuhn (2002). "Long-term survival and cell death of newly generated neurons in the adult rat olfactory bulb." European Journal of Neuroscience **16**(9): 1681-1689.

Xu, F., C. A. Greer and G. M. Shepherd (2000). "Odor maps in the olfactory bulb." J Comp Neurol **422**(4): 489-495.

Yamaguchi, M. and K. Mori (2005). "Critical period for sensory experience-dependent survival of newly generated granule cells in the adult mouse olfactory bulb." Proc. Natl. Acad. Sci. U. S. A. **102**(27): 9697-9702.

Yohn, C. N., M. M. Gergues and B. A. Samuels (2017). "The role of 5-HT receptors in depression." Molecular Brain **10**(1): 28.

Zhang, X. and S. Firestein (2002). "The olfactory receptor gene superfamily of the mouse." Nature Neuroscience **5**: 124.

Zucker, R. S. and W. G. Regehr (2002). "Short-Term Synaptic Plasticity." Annual Review of Physiology **64**(1): 355-405.

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Chapter 2. Adult born neurons facilitate olfactory bulb pattern separation in a state-dependent manner

Summary

The rodent olfactory bulb incorporates thousands of newly generated inhibitory neurons each day throughout adulthood, but the role of adult neurogenesis in olfactory processing is not fully understood. To address this issue, we adopted a genetic method to inducibly suppress adult neurogenesis and investigated its effect on behavior and bulbar activity. Mice without young adult-born neurons (ABNs) showed normal ability in discriminating very different odorants but were impaired in fine discrimination. Furthermore, two-photon calcium imaging of mitral cells (MCs), the principal neurons of the bulb, revealed that MC ensemble odor representations of similar odorants were more ambiguous in the ablation animals. This increased ambiguity was primarily due to a decrease in MC suppressive responses. Intriguingly, these deficits in MC encoding were only observed during task engagement but not passive exposure. Our results indicate that young ABNs in the olfactory bulb are essential for the enhancement of MC pattern separation in a task engagement-dependent manner, potentially functioning as a gateway for inhibitory top-down modulation from higher brain centers.

Introduction

The brain remains plastic throughout life. A dramatic example of neural circuit plasticity during adulthood comes in the form of adult neurogenesis (Altman and Das 1965). The subventricular zone (SVZ) is one of the two main loci in the

rodent brain where adult neurogenesis takes place (Zhao, Deng et al. 2008). In the SVZ, many thousands of new neurons are produced each day throughout adulthood, and these new neurons migrate through the rostral migratory stream to the olfactory bulb (Lois and Alvarez-Buylla 1994), the first olfactory center of the brain. Once in the olfactory bulb, about 95% of the adult-born neurons (ABNs) differentiate into granule cells (GCs) and the majority of the remaining differentiate into periglomerular cells (Lledo, Alonso et al. 2006), both of which are GABAergic local inhibitory neurons. GCs inhibit mitral cells (MCs), the principal neurons of the olfactory bulb, through their dendro-dendritic reciprocal connections (Shepherd 1963, Rall, Shepherd et al. 1966, Isaacson and Strowbridge 1998). This inhibition of MCs can sparsen odor representations and enhance the signal-to-noise ratio (Yokoi, Mori et al. 1995, Koulakov and Rinberg 2011, Yu, Migliore et al. 2014). Consistent with this idea, general activation of bulbar inhibitory neurons can accelerate learning (Abraham, Egger et al. 2010), while suppression of inhibitory neuron activity can increase the excitability of MCs and reduce MC pattern separation (Gschwend, Abraham et al. 2015). Thus, local inhibitory neurons in the olfactory bulb, including ABNs, likely control olfactory perception by providing inhibition onto MCs.

As ABNs integrate into local circuits, they display higher levels of morphological and functional plasticity during the first ~8 weeks after their birth compared to their later mature stage (Mizrahi 2007, Kelsch, Lin et al. 2009, Nissant, Bardy et al. 2009, Sailor, Valley et al. 2016). Furthermore, the spine dynamics, synaptic plasticity, sensory response pattern, as well as survival rate of ABNs are

influenced by olfactory experience during this early period (Petreanu and Alvarez-Buylla 2002, Rochefort, Gheusi et al. 2002, Lemasson, Saghatelyan et al. 2005, Yamaguchi and Mori 2005, Alonso, Viollet et al. 2006, Mouret, Gheusi et al. 2008, Lepousez, Nissant et al. 2014, Livneh, Adam et al. 2014, Quast, Ung et al. 2016). These unique and plastic features of young ABNs make it likely that they play a unique role in the processing of the complex and dynamic olfactory environment. Indeed, some studies have shown that ABNs are essential for certain olfactory behaviors such as odor discrimination and association reversal learning (Gheusi, Cremer et al. 2000, Enwere, Shingo et al. 2004, Bath, Mandairon et al. 2008, Moreno, Linster et al. 2009, Alonso, Lepousez et al. 2012, Sakamoto, Leki et al. 2014). However, other studies have found little effects of ABN manipulation on odor discrimination (Imayoshi, Sakamoto et al. 2008, Breton-Provencher, Lemasson et al. 2009, Lazarini, Mouthon et al. 2009). Thus, ABNs are not essential for all olfactory processing. Instead, the inconsistencies between these results raise the possibility that the impact of ABNs may depend on the behavioral context.

Consistent with the idea that the functions of ABNs are context-dependent, GCs including ABNs receive abundant glutamatergic centrifugal inputs from higher brain areas such as anterior olfactory nucleus, piriform cortex and entorhinal cortex (Kiselycznyk, Zhang et al. 2006, Balu, Pressler et al. 2007, Boyd, Sturgill et al. 2012, Markopoulos, Rokni et al. 2012, Chapuis, Cohen et al. 2013, Nunez-Parra, Maurer et al. 2013, Rothermel, Carey et al. 2014, Boyd, Kato et al. 2015, Otazu, Chae et al. 2015). GCs also express a variety of neuromodulator receptors, which provide additional avenues for top-down modulation (Shipley, Halloran et al. 1985,

Castillo, Carleton et al. 1999, Devore and Linster 2012, Ma and Luo 2012, Moreno, Bath et al. 2012, Rothermel, Carey et al. 2014). Furthermore, anesthesia inactivates feedback fibers and reduces GC activity while increasing MC activity (Kato, Chu et al. 2012, Rothermel and Wachowiak 2014). Thus, GCs may function as a mediator of feedback regulation to shape MC odor encoding (Markopoulos, Rokni et al. 2012). Consistent with this notion, it has been reported that cortical feedback can decorrelate MC odor representations (Otazu, Chae et al. 2015). Similarly, neuromodulatory projections can modulate MC activity (Rothermel and Wachowiak 2014) and improve perceptual learning (Ma and Luo 2012). However, the role of young ABNs in mediating context-dependent modulation of MC activity has not been fully explored.

To assess the role of ABNs in the olfactory bulb, we adopted a genetic method to inducibly suppress adult neurogenesis. We reasoned that this chronic ablation allows us to probe for the functions of ABNs which cannot be compensated for by other inhibitory neurons. Behavioral experiments showed that ABN ablation animals were impaired in fine, but not coarse, odor discrimination. Neither suppression of hippocampal ABNs alone nor non-selective ablation of a comparable number of bulbar neurons caused the same behavioral deficit, highlighting the unique importance of young ABNs. Two-photon calcium imaging revealed that the behavioral deficit was accompanied by a decreased separation of MC ensemble responses to similar odorants in ablation animals. This decreased separation was largely due to a reduction in suppressive odorant responses by MCs. Interestingly,

this difference in suppressive responses was only observed when animals were actively engaged in the task.

Results

Ablation of adult-born neurons

To investigate the role of adult neurogenesis in olfactory processing, we adopted the transgenic mouse line GFAP-TK (Snyder, Soumier et al. 2011). In this line, herpes simplex virus Thymidine Kinase (TK) is expressed under the Glial Fibrillary Acidic Protein (GFAP) promoter, rendering mitotic neural stem cells sensitive to the antiviral drug Valganciclovir (VGCC) (Figure 2.1a). This gives us a means to specifically suppress adult neurogenesis in an inducible manner.

We sought to investigate the consequences of eliminating young ABNs that are 8 weeks old and younger. To achieve this, we treated GFAP-TK mice with VGCC, starting 8 weeks prior to the beginning of the behavioral experiments and continuing throughout the duration of the experiments (Figure 2.1b). Hereafter we refer to these animals as the 'ablation' animals. The ablation of adult neurogenesis in the olfactory bulb was nearly complete, shown by post-hoc BrdU labeling ($p < 0.0001$, Wilcoxon rank sum test, GFAP-TK$^+$ vs. GFAP-TK$^-$; Figure 2.1c) as well as immunostaining for Doublecortin, a marker for immature neurons (Figure 2.1d). Importantly, there was no difference in the density of GFAP-positive astrocytes in the olfactory bulb of VGCC-treated GFAP-TK$^+$ and GFAP-TK$^-$ mice ($p = 0.9911$, Wilcoxon rank sum test; Figure 2.1e), consistent with a previous report (Snyder, Soumier et al. 2011)). Another previous report also showed that regeneration of

olfactory sensory neurons is intact in this mouse line (Cummings, Snyder et al. 2014). Ablation mice showed no obvious general health impairment, and demonstrated mobility and anxiety levels comparable to control mice in an open field test (average speed: $p = 1.0000$, center time fraction: $p = 0.7984$, Wilcoxon rank sum test; Figure 2.1f).

ABN ablation impairs fine odor discrimination

Equipped with this effective and specific method of inducible adult neurogenesis ablation, we explored how the absence of young ABNs could affect olfactory behavior. We compared the behavioral performance of the ablation animals and littermate controls (control: $n = 22$; ablation: $n = 23$). Both groups were treated identically including VGCC administration, and experimenters were blind to their genotypes during experiments. Mice were trained in a two-alternative-choice olfactory discrimination task under head-fixation. In this task, a certain odorant was delivered in each trial for 4 seconds, followed by an answer period of 2 seconds during which mice were required to lick -either the left or right port according to the odorant cue to receive a water reward (Figure 2.2a). Mice were trained daily, one session per day, and each session consisted of 144.4 ± 17.4 trials for the control group and 146.4 ± 13.8 trials for the ablation group.

After the initial pre-training period (Methods), mice were trained in a relatively easy discrimination task in which mice were required to discriminate between conspicuously different binary mixtures (Left lick: 80 % heptanal and 20 % ethyl-tiglate (80H20E); right lick: 20 % heptanal and 80 % ethyl-tiglate (20H80E), all

mixture percentages are of a total concentration of 100 ppm; Figure 2.2b). Both ablation and control animals achieved expertise in this task (defined as >80 % success rate) in equivalent durations of training (number of sessions, control: 4.18 ± 0.21, ablation: 4.39 ± 0.25, mean ± S.E.M.; p = 0.4523, Wilcoxon rank sum test; Figure 2.2c). Thus, we conclude that young ABNs are not required for the performance of this easy discrimination task.

Given these results, we asked whether finer discrimination would reveal a deficit caused by ABN ablation. To address this question, we devised a difficult discrimination task in which 1 of 8 very similar mixtures, each with slightly varying ratios of ethyl-tiglate and heptanal, was presented in each trial. 4 of the 8 mixtures signaled left lick trials, while the other 4 mixtures signaled right lick trials (Figure 2.2d). After mice achieved expertise in the easy discrimination task, they were trained with this difficult discrimination task over 10 sessions. Although the performance of control animals was initially at chance level, it consistently improved over 10 sessions to achieve a success rate of 0.760 ± 0.017 in session 10. In contrast, ablation animals showed slower learning (comparison of linear regression slopes in individual animals, p = 0.0100, Wilcoxon rank sum test), and their performance was significantly lower than that of the control animals (p(group) < 0.0001, p(session) < 0.0001, two-way ANOVA; Figure 2.2e). The deficits observed in ablation animals were unlikely due to problems in motivation or licking ability, as both groups had the comparable fractions of answered trials (control vs. ablation, p(group) = 0.4101, two-way ANOVA; Figure 2.2f) and the comparable licking rates during reward consumption (control vs. ablation, p(group) = 0.1036, two-way

ANOVA; Figure 2.2g). To confirm that mice were performing the task by using odorant stimuli as the cues and not other cues (such as potential differences in sounds of different odorant valves), we performed an additional session after the 10th session. In this test session, all odorants were replaced with the same 50H50E mixture while the contingency between odorant valves and correct lick side was maintained. In this test session, the performance of both groups dropped to chance level (control: $p = 0.7241$, ablation: $p = 0.6925$, t test with chance level (0.5); Figure 2.2e, "50:50"), indicating that they were indeed relying on odorants as the cue. In conclusion, mice without young ABNs are impaired in the difficult discrimination task requiring fine odorant discrimination.

Hippocampal ABN suppression alone or random ablation of GCs did not impair fine discrimination

The results above suggest that ABNs in the olfactory bulb are critical for fine odorant discrimination. However, the dentate gyrus (DG) of hippocampus is the other major niche for adult neurogenesis (Ming and Song 2011, Gonçalves, Schafer et al. 2016), and the GFAP-TK method suppresses adult neurogenesis in both the SVZ and DG. To address this issue, we adopted another transgenic method previously described that could specifically suppress postnatally-born DG neurons (mGFAP-Cre::VGLUT1-LSL-TeNT, hereafter referred to as 'DG suppression') (Sakamoto, Leki et al. 2014). During the same easy and difficult discrimination tasks described in Figure 2.2, the DG suppression mice showed comparable performance to the control group in both easy and difficult discrimination tasks (control vs. DG

suppression; easy discrimination: number of sessions to reach expertise, p = 0.8407, Wilcoxon rank sum test; difficult discrimination: p(group) = 0.2663, p(session) < 0.0001, 2-way ANOVA; mean ± S.E.M.; Figure 2.3a,b). These results indicate that DG ABNs are not essential for fine olfactory discrimination and the behavioral impairment in GFAP-TK^{+} ablation group was primarily caused by the absence of ABNs in the olfactory bulb.

Next we considered two alternative possibilities underlying the behavioral deficit in the ablation animals. First, it is possible that ablation of any inhibitory neurons in the olfactory bulb may lead to similar deficits. Second, olfactory fine discrimination may be particularly sensitive to ABN ablation. To distinguish these possibilities, we sought to ablate a random subset of GCs regardless of their age. To this goal, we first estimated the degree of neuron loss in the ABN ablation animals by quantifying cell density in the granule cell layer (GCL) using DAPI labeling. This indicated a 10.02% reduction of total cell numbers through ABN ablation by the end of the behavioral tasks (control: n = 3, ablation: n = 4; Figure 2.3c,d). To achieve a similar level of neuron ablation without specifically targeting ABNs ('random ablation'), we bilaterally injected a mixture of diluted AAV2/1-CMV-Cre and AAV2/1-EF1a-FLEX-tCaspase3 (Yang, Chiang et al. 2013) into the center of the olfactory bulb. Post hoc DAPI staining and quantification ~1 month after injections revealed a 19.66% reduction in GCL cell density compared with un-injected animals (injected (n = 11) vs. un-injected control (n = 7): p < 0.0001, Wilcoxon rank sum test; Figure 2.3e,f), with no significant change in the width of GCL (injected vs. un-injected control: p = 0.9499, Wilcoxon rank sum test; Figure

2.3g). Thus, our random ablation eliminated a larger number of cells than our ABN ablation. Importantly, we found no change in Doublecortin immunostaining, indicating that our random ablation did not affect subsequent adult neurogenesis (injected (n = 7) vs. uninjected (n = 7) control, p = 0.9015, Wilcoxon rank sum test; Figure 2.3h,i). We trained these random ablation animals starting at 1 month after the injections. Random ablation animals exhibited normal performance in the easy discrimination task (control vs. random ablation, number of sessions to reach expertise, p = 0.4430, Wilcoxon rank sum test; mean ± S.E.M., Figure 2.3j). In the difficult discrimination task, the random ablation group initially showed slower learning than the control group, but they eventually reached the performance level that was statistically indistinguishable from the controls and significantly better than ABN ablation animals (Sessions 1-10: random ablation (n = 11) vs. control (n = 22), p = 0.0034, random ablation vs. ABN ablation, p < 0.0001; Sessions 1-5: random ablation vs. control, p = 0.0039, random ablation vs. ABN ablation, p = 0.4405; Sessions 6-10: random ablation vs. control, p = 0.2028, random ablation vs. ABN ablation, p < 0.0001; 2-way ANOVA; mean ± S.E.M.; Figure 2.3k). Together with the observation that the random ablation eliminated more cells than in ABN ablation, these results support the notion that young ABNs have a privileged role in mediating fine olfactory discrimination.

ABN ablation affects MC population coding during difficult discrimination

To investigate the neural basis of the impaired discrimination in ABN ablation animals, we monitored the activity of MCs in ablation and control animals using two-

photon calcium imaging. We utilized the transgenic mouse line Pcdh21-Cre, which expresses Cre specifically in the olfactory bulb principal neurons. We injected AAV1-hsyn-FLEX-GCaMP6f in the right olfactory bulb of GFAP-TK$^{+/-}$::Pcdh21-Cre (ablation) or GFAP-TK$^{-/-}$::Pcdh21-Cre (control, littermates) animals to specifically express GCaMP6f in mitral/tufted cells (Figure 2.4a). After training with the easy discrimination task, these mice were trained with the difficult discrimination task while we imaged the ensemble activity of MCs (control: n = 12, ablation: n = 10; Figure 2.4b,c).

Individual MCs showed odorant-specific responses with an increase or decrease in GCaMP6f fluorescence (Figure 2.4d). To quantify the discriminability of the 8 mixtures by the MC ensembles, we performed decoder analysis (Chu, Li et al. 2016) which attempts to decode the odorant on each trial based on the population activity of individual MCs during the odorant period (Methods). If the decoded odorant matched the actual odorant, the trial was scored as correct. We found that decoder accuracy was significantly better than chance (0.125) in both control and ablation groups (control: $p < 0.001$, ablation: $p < 0.001$, Student's t-test; Figure 2.4e). However, the decoder accuracy was higher in control animals than in ablation animals (control vs. ablation, $p(group) < 0.05$, two-way ANOVA; Figure 2.4e). These results indicate that MC responses to different mixtures are more ambiguous in ablation animals than in control. Next we asked whether the separation of mitral cell odor representations is sensitive to the similarity of odor mixtures. To address this, we performed a pairwise decoder analysis in which we built a decoder to decode the mixture identity for each pair of the 8 mixtures. Here we defined the "contrast"

between each pair of mixtures as the difference in the percentage of heptanal (Figure 2.4f). For example, the contrast between 52H48E and 48.5H51.5E is 3.5 (= 52 - 48.5). We found that within each of the control and ablation groups, there was a positive correlation between pairwise decoder accuracy and the contrast between the mixtures (control: $r = 0.1862$, $p < 0.01$, ablation: $r = 0.3029$, $p < 0.0001$, Pearson correlation; Figure 2.4g). Although the decoder performance of the control group was generally better than the ablation group, the difference was more prominent in mixture pairs with smaller contrasts (≤ 3) (control vs. ablation, pairs with contrasts ≤ 3: $p(\text{group}) < 0.001$, pairs with contrasts > 3: $p(\text{group}) = 0.2530$, two-way ANOVA; Figure 2.4g). These results suggest that ABN ablation causes the separation of MC population responses to similar odorants to be less robust, possibly underlying the behavioral deficits in fine discrimination. Consistent with this notion, the decoder accuracy of individual animals positively correlated with their behavioral performance ($p < 0.05$, Pearson correlation; Figure 2.4h).

ABNs are essential for suppressive responses of mitral cells

To investigate the basis for the decreased decoder accuracy in ABN ablation animals, we analyzed the responses of individual mitral cells to the 8 mixtures. We quantified two measures; the first is the fraction of MCs that responded to at least one mixture, and the second is the fraction of responsive MC-odorant pairs out of all MC-odorant pairs. We found that the fraction of MCs responsive to at least one mixture and the fraction of responsive MC-odorant pairs were both consistently lower in ablation animals compared to control (control vs. ablation, fraction of cells:

$p < 0.001$, fraction of cell-odorant pairs: $p < 0.01$, two-way ANOVA; Figure 2.5a). As a MC can respond to an odorant with increased or decreased activity, we next quantified excitatory and suppressive responses separately. This analysis showed that the excitatory response fraction was not significantly affected by ABN ablation (control vs. ablation, fraction of cells: $p = 0.0813$, fraction of cell-odorant pairs: $p = 0.6039$, two-way ANOVA; Figure 2.5b). Instead, the decreased responses in ablation animals were primarily due to decreases in suppressive responses (control vs. ablation, fraction of cells: $p < 0.0001$, fraction of cell-odorant pairs: $p < 0.0001$, two-way ANOVA; Figure 2.5c), suggesting that the net effect of ABNs on MC ensembles is inhibitory.

The decrease in suppressive but not excitatory responses of MCs in ablation animals raises the possibility that the decreased suppressive responses may underlie the reduced decoder accuracy in ablation animals. Therefore we explored the relationships of excitatory and suppressive MC responses with decoder accuracy and behavior. We found that the fraction of total (excitatory and suppressive) responses positively correlates with decoder accuracy (fraction of cells: $p < 0.01$, fraction of cell-odorant pairs: $p < 0.01$, Pearson correlation) and behavioral performance (cells: $p < 0.01$; cell-odorant pairs: $p < 0.05$; Figure 2.6a,b). When we only included excitatory responses, however, this relationship was not significant (decoder accuracy, cells: $p = 0.2089$, cell-odorant pairs: $p = 0.1272$; performance, cells: $p < 0.05$, cell-odorant pairs: $p = 0.1459$; Figure 2.6c left and Figure 2.6d left). Instead, the fraction of suppressive responses significantly correlated with both decoder accuracy and behavioral performance (decoder

accuracy, cells: $p < 0.01$, cell-odorant pairs: $p < 0.01$; performance, cells: $p < 0.05$, cell-odorant pairs: $p < 0.05$; Figure 2.6c right and Figure 2.6d right). Together these results suggest that young ABNs are essential for high levels of suppressive responses of MCs, which significantly contribute to odorant discriminability by MC population responses.

The necessity of ABNs depends on task engagement

Inhibitory neurons in the olfactory bulb, including ABNs, are major targets of extensive glutamatergic and neuromodulatory projections from higher brain areas. These centrifugal projections are suggested to be sensitive to brain states (Gilbert and Sigman 2007, Rothermel and Wachowiak 2014). Therefore it is tempting to hypothesize that the impact of ABN functions is sensitive to behavioral states such as task engagement. We reasoned that, if this is the case, the differences in the MC responses of control and ablation animals described above would be less pronounced when the mice were not engaged in the task.

To test this idea, we performed a new experiment in which another cohort of mice were passively exposed to odorants (control passive: $n = 10$; ablation passive: $n = 7$). Except for the lack of task engagement, all the other conditions were kept identical to the task condition, including VGCC treatment, water restriction, odorant stimulation protocol, and odorant identity (4 sessions of 2 ‘easy discrimination’ odorants followed by 10 sessions of 8 ‘difficult discrimination’ odorants). Imaging was performed during the passive experience of the 8 difficult discrimination odorants that are identical to the task condition. Strikingly, in this passive condition,

the fractions of MCs showing excitatory and suppressive responses were no longer statistically distinguishable between ablation and control animals (control passive vs. ablation passive, fraction of cells: total, $p = 0.6162$; excitatory, $p = 0.7625$, suppressive, $p = 0.2620$; fraction of cell-odorant pairs: total, $p = 0.2043$; excitatory, $p = 0.8691$, suppressive, $p = 0.0625$, two-way ANOVA; Figure 2.7a-c). These results suggest that MC responses, mainly suppressive responses, are increased in a task engagement-dependent manner. We further tested this notion with a linear regression model (Methods). The interaction term for the genotype (control vs. ablation) and the condition (task vs. passive) was statistically significant for suppressive (fraction of cells: $p = 0.0101$; fraction of cell-odorant pairs: $p = 0.0239$), but not excitatory (fraction of cells: $p = 0.1162$; fraction of cell-odorant pairs: $p = 0.7509$) responses. This result indicates that the effect of task engagement on suppressive, but not excitatory, responses is significantly larger in control animals than in ablation animals, supporting a role for young ABNs in enhancing suppressive responses in a task engagement-dependent manner.

The state-dependence of MC suppressive responses led us to a final question; does wakefulness influence MC suppressive responses? To address this question, we re-analyzed data from our previous study in which MC responses to a panel of odorants were directly compared in the same Pcdh21-Cre animals before and after the induction of anesthesia. In the previous study that originally reported these experiments (Kato, Chu et al. 2012), we only analyzed excitatory responses and showed that anesthesia enhanced excitatory responses (Figure 2.8a,b). In our new analysis of these previous data, we found that suppressive responses showed

a dramatic decrease upon the induction of anesthesia (fraction of cell-odorant pairs: $p < 0.05$; fraction of responses to each odor: $p < 0.0001$, Wilcoxon signed rank test; Figure 2.8a-c).

Based on these observations, we propose that behavioral states strongly modulate MC activity and in particular suppressive responses in a graded manner (from anesthetized to awake passive to task-engaged), facilitating olfactory discrimination during task engagement. Importantly, this state-dependent enhancement of suppressive responses during task engagement requires young ABNs.

Discussion

In this study, we ablated young ABNs in adult mice and investigated the consequences on their behavior and MC activity to probe the functional significance of adult neurogenesis. To our knowledge, this is the first study to record MC activity in ABN ablation animals during a behavioral task that reveals their impaired discrimination ability. The results provide a glimpse of the specific functions of ABNs.

Behavioral consequences of adult neurogenesis ablation

Previous studies on the effect of ABN ablation on olfactory behavior have reported inconsistent results. These discrepancies may be due to differences in ablation methods as well as task demands. In this study, we adopted an inducible, genetic ablation method and we confirmed that this method almost completely

eliminated ABNs. We and others also have found no evidence of non-specific effects on other cell types (Snyder, Soumier et al. 2011, Cummings, Snyder et al. 2014). Thus, this method can ablate young ABNs with high specificity and efficiency, allowing us to investigate its consequences on olfactory behavior and odor representations.

We established an olfactory discrimination two alternative choice task with two levels of difficulty. The operant and symmetric nature of the task allowed us to focus on the discrimination ability of individual animals, as opposed to spontaneous discrimination or asymmetric go/no-go tasks in which motivational states are difficult to control. Ablation mice were perfectly capable of discriminating conspicuously different odorants in this task, indicating that young ABNs are not necessary for basic odor processing, consistent with many previous studies. For difficult discrimination, we used 8 binary mixtures of similar ratios applied pseudorandomly in each trial. Previous studies showed that tasks involving multiple similar odorants (Uchida and Mainen 2003, Rinberg, Koulakov et al. 2006) delivered randomly (Zariwala, Kepecs et al. 2013) are more difficult than two-odorant tasks. This difficult condition revealed a robust impairment of ABN ablation animals in odor discrimination.

Importantly, our ablation method affects adult neurogenesis in both SVZ and hippocampus DG. However, DG-only suppression of ABNs did not produce the deficits in olfactory discrimination. Therefore, we conclude that young ABNs in the olfactory bulb are essential for fine discrimination of odorants.

Ablation of any neurons in the olfactory bulb may be expected to lead to deficits in olfactory behaviors. We find that ABNs less than 10-12 weeks old constitute ~10% of all GCs, consistent with a previous report (Imayoshi, Sakamoto et al. 2008). When we ablated a larger fraction (~20%) of GCs randomly without regard to their age, majority of which were presumably mature GCs, the behavioral impairment was much more subtle. These results support the notion that young ABNs have a unique role in fine olfactory discrimination.

Incorporation of inhibitory ABNs facilitate olfactory bulb pattern separation

To explore the potential neural basis underlying the behavioral impairment of ABN ablation animals, we used two-photon imaging to record the activity of populations of MCs (Kato, Chu et al. 2012) during the task performance, and analyzed data within the entire 4-s stimulus period. We are aware of the previous reports demonstrating the importance of finer time-scale dynamics of MC responses (Uchida and Mainen 2003, Abraham, Spors et al. 2004, Rinberg, Koulakov et al. 2006, Wachowiak 2011, Resulaj and Rinberg 2015), which is not accessible with the temporal resolution of our approach. In fact, in certain reaction time tasks, the responses within the first 100 ms of odorant onset are sufficient for discrimination. However, we argue that it is unlikely that the responses within the first 100 ms of odorant onset explain the entirety of odor representations important for odor perception. This may especially be the case in conditions such as the task used here in which mice are not encouraged to react as quickly as possible. Furthermore, our approach affords a unique opportunity to record the activity of a few dozens of

MCs longitudinally, allowing us to assess MC ensemble coding in ablation and control animals.

We performed MC calcium imaging during the difficult discrimination task to explore the potential neural basis underlying the behavioral impairment in ablation animals. This experiment revealed that the behavioral impairment in ablation animals accompanied a reduced separation of representations of similar odorants by MC ensembles as shown by the decoder analysis. Moreover, we found that the reduced separation of odor representations in ablation animals involved a preferential reduction of MC suppressive responses. The degree of reduction is related to the decoder performance, as the fraction of MC suppressive responses significantly correlated with the decoder accuracy and behavioral performance, supporting the importance of MC suppressive responses. Considering that ABNs exert inhibitory modulation onto MCs, these results together suggest that the ablation of ABNs caused a reduction in MC suppressive responses, which in turn affected the discriminability of MC ensembles. In normal animals, excitatory inputs from sensory neurons combined with local inhibitory control would allow MCs to respond to odorants in both excitatory and suppressive manners (Yokoi, Mori et al. 1995). The bidirectionality of responses effectively increases the dynamic range of MC responses and would contribute to an enhanced separation of representations of similar odors.

Consistently, a modeling study simulated the effect of adult neurogenesis on the OB circuitry with excitatory sensory inputs, local GC inhibition and MC outputs and predicted that a constant arrival and activity-dependent survival of ABNs are

sufficient to separate MC representations of very similar odorants in an experience-dependent manner (Cecchi, Petreanu et al. 2008). A continuous recruitment of new ABNs allows the bulb to adapt to changes in the olfactory environment. Our current results indicate that such a mechanism is particularly sensitive to behaviorally significant experience such as engagement in difficult discrimination.

We note that the decoder performance was relatively stable throughout imaging, in contrast to our recent report (Chu, Li et al. 2016). The apparent discrepancy likely stems from the fact that Chu et al. investigated changes of representations of novel odorants over time, while in the current study, mice had already been familiarized with the odorants, albeit at different mixture ratios, during the easy discrimination task prior to imaging.

Olfactory bulb as a state-dependent filter of odorant information

We found that the abundance of MC suppressive responses is highly sensitive to behavioral states. MC suppressive responses are increased during wakefulness compared to anesthetized states, and task engagement further enhances suppressive responses. This is consistent with a previous report stating that suppressive responses in MCs became more prominent during task engagement as opposed to passive exposure (Fuentes, Aguilar et al. 2008). Our results further extend these findings and demonstrate that the task engagement-dependent enhancement of suppressive responses is facilitated by young ABNs. During task engagement, ABN ablation animals have fewer suppressive responses than control animals. This difference in suppressive responses was absent during

passive exposure. The sensitivity to task engagement may explain the findings from a previous study that broad GC inactivation has only mild effects on MC responses under anesthesia and passive wakefulness (Fukunaga, Herb et al. 2014). It is known that ABNs receive centrifugal synaptic and neuromodulatory inputs from multiple brain areas, and these inputs can vary depending on brain states. Thus, the state-dependence of the functional role of ABNs can be better appreciated considering previously reported phenomena that synaptic inputs onto developing ABNs within different dendritic compartments formed in a sequential manner (Kelsch, Lin et al. 2008), with the formation of centrifugal inputs preceding local dendro-dendritic inputs (Whitman and Greer 2007). It has been shown that the survival rate of ABNs is sensitive to sensory experiences, which then are reflected by neuronal activities. Therefore, we postulate that ABNs that are strongly activated by centrifugal inputs may have a higher chance to survive, which can explain the state-dependent requirement of ABNs for MC suppressive responses that we have observed.

The activity of inhibitory circuits has been shown to be sensitive to behavioral states in various brain areas. Intracellular recordings from excitatory neurons in the primary visual cortex revealed that inhibitory inputs are more prevalent in the awake state than in anesthesia (Haider, Häusser et al. 2013). In the primary auditory cortex, task engagement suppresses sound-evoked responses and sharpens the tuning of excitatory neurons (Otazu, Tai et al. 2009, Lee and Middlebrooks 2011). This effect is mediated by subtype-specific modulation of local inhibitory neurons (Kuchibhotla, Gill et al. 2017). In the olfactory bulb, anesthesia suppresses local inhibitory

neurons (Kato, Chu et al. 2012, Wachowiak, Economo et al. 2013). Together with the current study, these results suggest that state-dependent engagement of inhibitory circuits and suppression of excitatory responses may be a common principle conserved across brain areas.

We also note that task engagement does not only affect suppressive responses of MCs. In passive exposure, excitatory responses were also reduced compared to the task condition, although this effect was insensitive to ABN ablation (Extended Figure 2.1a,b). Therefore, it appears that there are additional, ABN-independent mechanisms modulating MC responses in a state-dependent manner. These probably include various feedback systems, which can modulate MC activity and olfactory behavior (Castillo, Carleton et al. 1999, Linster, Garcia et al. 2001, Chaudhury, Escanilla et al. 2009, Escanilla, Arrellanos et al. 2010, Ma and Luo 2012, Nunez-Parra, Maurer et al. 2013, Rothermel, Carey et al. 2014, Kapoor, Provost et al. 2016). It is likely that some of the functions of these systems are independent of ABNs.

Taken together, we propose a model that task engagement increases the dynamic range of MC responses through top-down modulation from higher brain areas, which acts at least partially through young ABNs. Consistent with this notion, inactivation of piriform cortex, a main source of feedback projections to the olfactory bulb, enhances excitatory MC responses (Otazu, Chae et al. 2015), supporting the inhibitory role of cortical feedback. These dynamics of ABNs may underlie the observations that experience and learning profoundly shape the representations of odorant stimuli by olfactory bulb principal neurons (Doucette and Restrepo 2008,

Kato, Chu et al. 2012, Gschwend, Abraham et al. 2015, Chu, Li et al. 2016, Yamada, Bhaukaurally et al. 2017). Thus, the olfactory bulb functions as a dynamic, adaptive filter for incoming odorant information depending on behavioral demands, and adult neurogenesis is essential for this adaptive role of the olfactory bulb.

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Author Contributions

The project was conceived by T.K., I.I. and W.L.L. Hippocampal DG suppression experiments were performed by Y.S. and I.I. All other experiments were performed by W.L.L. with assistance from I.I., M.W.C., and A.W. and analyzed by W.L.L. and T.K. The manuscript was written by T.K. and W.L.L. with inputs from all other authors.

Methods

Subjects

All procedures were in accordance with protocols approved by the Institutional Animal Care and Use Committee at UCSD or Kyoto University and guidelines of the National Institute of Health.

For all experiments, mice were housed in plastic cages with standard bedding in a room with a reversed light cycle (12h-12h), and all experiments were performed during the dark period.

All experiments except hippocampal ABN suppression were performed at UCSD. GFAP-TK mice were generous gifts from H. Cameron with ICR background. Pcdh21-Cre mice were originally acquired from RIKEN Brain Research Center and backcrossed at least 4 generations to C57bl/6. Only male mice were used. All littermates were used for experiments, roughly 50% of which were positive for GFAP-TK, and the GFAP-TK negative mice served as control. The experimenters were blinded to the genotype of each mouse until the end of the experiments. The genotypes were confirmed by both PCR and post hoc Doublecortin immunostaining, which were always consistent with each other (PCR negative mice always showed Doublecortin signals and vice versa).

Hippocampal ABN suppression experiments were performed at Kyoto University. mGFAP-Cre mice (Garcia, Doan et al. 2004) were crossed with VGLUT1-LoxP-TeNT mice (Sakamoto, Leki et al. 2014). Both strains were maintained on the C57BL/6J background. The experimenters were blind to the genotype of each mouse during the experiments, after which double transgenic

mice were identified by PCR. VGLUT1-LoxP-TeNT single transgenic mice served as control. No behavioral abnormalities were observed in the mGFAP-Cre and VGLUT1-LoxP-TeNT single transgenic mice. All behavioral tests were carried out with 3-months-old male mice.

Valganciclovir (VGCC) treatment

VGCC (Genentech) was dissolved in drinking water at 0.63 mg/ml before water restriction, and mixed with powdered food (Harlan) at 0.44 mg/g during water restriction, to achieve approximately 0.1 mg/g body weight / day. Mice were 10-12 weeks old at the beginning of VGCC treatment.

Surgeries

After 6 weeks of continuous VGCC treatment, mice were anesthetized with isoflurane (3% induction, 0.7-2% maintenance) and surgeries were performed as previously described (Kato, Chu et al. 2012). Briefly, a stainless-steel custom headplate was secured onto the skull with cyanoacrylate glue, and an optical glass window (1×2 mm, oval) was implanted above the right olfactory bulb craniotomy and was secured by dental cement.

Viral injection

To express GCaMP6f in mitral cells, a viral vector containing a Cre-dependent, GCaMP6f-expressing construct (AAV2.1 hsyn-FLEX-GCaMP6f, UPenn

Vector Core, 1:11 diluted in saline) was injected into the craniotomy (20 nl / site, 4 sites, 250 μm depth).

To ablate a random subset of cells in GCL, a mixture of viruses containing Cre-expressing construct (AAV2.1-CMV-PI-Cre-rBG, UPenn Vector Core, 1:10 dilution in saline) and Cre-dependent modified Caspase3 (Yang, Chiang et al. 2013), AAV2.1-EF1a-FLEX-taCasp3-TEVp, custom prep by UPenn Vector Core, 1:1 dilution in saline) was injected into the olfactory bulb (300 nl or 500 nl, 1 site, 0.75 mm M-L, 0.8 mm anterior from the inferior cerebral vein, 1.5 mm D-V, injection speed: 100 nl / min) through a small craniotomy. For all behavioral experiments and a subset of histology experiments, the injections were bilateral. For the other histology experiments, the injections were unilateral and the uninjected hemisphere served as control.

BrdU treatment

To validate the effectiveness of adult neurogenesis ablation, after 6 weeks of continuous VGCC treatment, mice (6 control, 6 ablation) were treated with BrdU for 3 consecutive days, and were sacrificed 7 days later for immunostaining. BrdU powder was dissolved in drinking water at 1 mg/ml to achieve approximately 0.2 mg/g body weight / day.

Immunostaining and cell counting

30 μm-thick olfactory bulb coronal sections were prepared with a microtome (Thermo Fisher) and mounted on pre-coated slides. Immunostaining was then

performed with overnight primary antibody and 2-hr secondary antibody incubation. For BrdU staining, sections were incubated at 37 °C in HCl (6% in water) for 30 min, and neutralized by borate acid buffer (0.5 M) for 10 min prior to incubation with the primary antibody. Both primary and secondary antibodies were diluted in blocking buffer (0.3% TritonX-100, 1% serum from the same species as secondary antibody, 0.1% bovine serum albumin, 0.1 M ph7.4 PBS). BrdU: primary (rat, AbD serotec), 1:500, secondary (goat, Alexa 488, Thermo Fisher), 1:1000. Doublecortin: primary (goat, Santa Cruz), 1:400, secondary (donkey, Alexa 488, Thermo Fisher), 1:1000. GFAP: primary (goat, Santa Cruz), 1:400, secondary (same as doublecortin). NeuN: primary (mouse, Millipore), 1:400, secondary (goat, Alexa 488, Thermo Fisher), 1:1000. DAPI: 1:10,000 (Invitrogen) for Figure 2.1c,d,e, and Vectashield mounting medium (Vector Labs) for Figure 2.3c,e.

GFAP, BrdU, NeuN and DAPI quantification was performed manually using ImageJ. Representative sections (~4 for each animal) were chosen, and in each section, four rectangle areas were selected for counting, each encompassing the entire depth of the GC layer from dorsal, ventral, medial and lateral sides where signals were relatively homogenous. For GFAP signals, only complete structures containing soma were counted. For BrdU, all clearly visible puncta were included. To measure GCL width, 3-4 coronal sections from the widest segment of each OB were selected, and the distances between the central line of ventricle to the mitral cell layer on both medial and lateral sides were measured using ImageJ, and then averaged.

Odorant delivery

Odorants (Sigma) were diluted in mineral oil (Thermo Fisher) to a calculated vapor pressure of 200 ppm. A custom-built olfactometer mixed saturated odorant vapor 1:1 with filtered, humidified air for a final concentration of 100 ppm. Air flow rate was controlled at 1 L / min by a mass flow controller (Aalborg). Heptanal and Ethyl-tiglate were selected based on their structural dissimilarity and strong odorant-evoked responses in dorsal olfactory bulb.

Behavior

Water restriction started ~1 week after surgery and 14-18 days prior to the start of behavioral training. Mice were given at least 1 ml of water per day to maintain the body weight ≥ 80% of the initial value. The behavioral program was controlled by a real-time system (C. Brody). Two lick ports with infrared beam detector were available for left and right licks. A correct trial (determined by the first lick during the answer period) was rewarded with ~6 µl of water. Each daily training session consisted of 150 trials unless mice disengaged earlier.

Pre-training

In the first session, mice were rewarded for both left and right licks during a 2-s answer time. The inter-trial interval (ITI) was increased from 1 s to 3 s. In the second session, 80% Heptanal + 20% Ethyl-tiglate (80H20E) mixture was delivered for 4 s in each trial, followed by a 2s answer period during which a left lick was rewarded. Right lick during the answer period would terminate the trial without reward or punishment. ITI was increased by 3 s every ~20 trials up to 15 s and was

fixed at 15 s for all the following sessions. In the following session, 20% Heptanal + 80% Ethyl-tiglate (20H80E) mixture was delivered in each trial to train right licks.

Easy discrimination

Once mice could perform correctly for >90% of 60 consecutive trials in both the left- and right-lick pre-training sessions, we began the easy discrimination task in which 80H20E and 20H80E were pseudo-randomly delivered in each trial with no more than 3 successive trials of the same mixture. 80H20E and 20H80E signaled left and right lick trials, respectively. Incorrect responses terminated the trials without reward or punishment. Mice were trained with this easy discrimination task until they achieved >80% success rate in an entire session.

Difficult discrimination

In each trial, one of the eight mixtures (left lick: 54H46E, 52H48E, 51.5H48.5E and 51H49E; right lick: 49H51E, 48.5H51.5E, 48H52E, 46H54E) was pseudo-randomly delivered so that no consecutive trials were of the same mixture and each mixture was delivered at about the same frequency. Mice were trained with this difficult discrimination task for 10 sessions.

Passive exposure

A separate cohort of mice went through a passive experience paradigm, where they experienced the same odorants through the same timeline (pre-training, easy discrimination to difficult discrimination) with the same trial structure and session duration (150 trials) passively without task engagement. The number of easy discrimination sessions (4) was determined based on the median of session numbers during the task engagement experiment.

Open field test

An open field test was performed on a subset of mice who had completed the behavioral training. An enclosed cubic box (edge: 40 cm) made with black acrylic boards was used as the open field. Each mouse was placed in the center of the box floor, and was allowed to explore freely for 5 minutes. An infrared camera (29 frames / s) was secured at the center of the box ceiling to record the location of the mouse. Speed, distance and location were analyzed on a frame-by-frame basis using custom code in MATLAB. The center area was defined as the 20 × 20 cm area in the center of the floor.

Image acquisition

Two-photon imaging was performed with a commercial microscope (B-scope, Thorlabs) with 925 nm laser excitation (Mai-Tai, Spectra-physics) at the frame rate of 26-28 Hz. Each frame was 512 × 512 pixels with the average field of view of 546 × 467 μm. Imaging was performed continuously within each of 4000-frame (~44 s) segments, which were separated by a 6 s inter-segment interval. Trials that overlapped with these intervals were discarded. The average image from the first imaging session was used as a template to identify the same imaging field in the following sessions.

Data analysis

The image time series were first processed for full-frame motion correction with a custom program in MATLAB.

ROIs

Regions of Interest (ROIs) were manually drawn around each mitral cell with a custom MATLAB program on the average image of each session. ROIs were added or removed by comparing across all imaging sessions to make sure all analyzed cells were visible and appeared healthy in every session. A background ROI was also manually drawn in an area adjacent to each cell body ROI without cellular structures. The values of the pixels within each cell body and background ROI were averaged to generate two fluorescence time series (F). For each trial, (F(background) - mean(F(background)) was subtracted from F(cell body) to derive the final cell activity trace. The 5 s period before odorant onset was used as baseline for each trial and the activity trace for each trial was normalized to the mean of the baseline period to calculate F/F_0 and dF/F. The total number of mitral cells and mice imaged were: control: 703 cells in 12 mice; ablation: 540 cells in 10 mice; control passive: 416 cells in 10 mice; ablation passive: 298 cells in 7 mice.

Defining responsive cells

Responsive mitral cells were defined in each session as previously described (Chu, Li et al. 2016) using trial traces smoothed with MATLAB 'smooth' function (smooth factor = 6). A mitral cell was classified as responsive to a given odorant mixture if both of the following criteria were met:

Criterion 1: F/F_0 is significantly different by Wilcoxon rank sum test ($p < 0.05$) between each time point (frame) of all trials and baseline frames of all trials for at

least 75% of the time points within any 0.5-s time window during the 4-s odorant period.

Criterion 2: The difference between trial-averaged F/F_0 and the grand average of baseline frames of all trials exceeds 0.20 in at least one frame during the 0.5-s window that meets Criterion 1.

Decoder analysis

Mitral cell population response in each trial was expressed as a population activity vector by averaging F/F_0 values 0-2 s and 2-4 s of odorant period for each cell and concatenating these two values across all cells. For each mouse, every decoding process ran 100 iterations. For decoding using all mitral cells, 20 cells were randomly selected from all cells in each iteration. This number (20 cells) was decided based on the mouse with the smallest number of imaged mitral cells. '8-odorant decoding': in each iteration, centroids for all 8 odorants were calculated by averaging activity vectors of all trials for each respective odorant excluding the test trial. Euclidian distances between the test trial and all centroids were calculated using the MATLAB function 'pdist', and the centroid with the shortest distance defined the decoded odorant. If the decoded odorant matches the actual odorant delivered for that trial, the trial was considered correctly decoded. The final decoder accuracy of each mouse was the result of averaging fraction of correctly decoded trials across 100 iterations. 'Pairwise decoding' (Figure 2.4g): the same decoder analysis was also performed in a pairwise fashion for every pair of the 8 odorants in the difficult discrimination.

Calculating correlation coefficients

To calculate correlation coefficients between behavioral performance, decoder accuracy and response level described in Figure 2.4h and Figure 2.6, the MATLAB function 'corrcoef' was used.

Linear regression model

To test whether the effect of task engagement on mitral cell responses is different between control vs. ablation animals, matrices (number of mice × number of sessions) of responsive fractions for control task, ablation task, control passive and ablation passive were constructed and fit with the linear model: *Responsive fraction = a×genotype (control/ablation) + b×condition (task/passive) + c×session + d×genotype × condition + d* using Matlab function 'fitlm'.

Imaging under anesthesia

Anesthesia data from Kato et al. (2012) was reanalyzed. Briefly, mice were anesthetized by intraperitoneal injections of mixtures of urethane/chlorprothixene (1.5 g / kg and 2 mg / kg, respectively) or ketamine/xylazine (100 mg / kg and 8 mg / kg, respectively), and odorant responses were imaged while mice were kept on a thermal blanket at 37°C. ROC analysis was performed on mitral cell responses (as in Kato et al.) during the 4 second odorant period to determine the threshold for classifying responses as either excitatory (2.6 × baseline standard deviation) or suppressive (1.7 × baseline standard deviation).

Figure 2.1 Inducible ablation of adult neurogenesis.

(**a**) Pharmacogenetic ablation of ABNs. Valganciclovir (VGCC) induces apoptosis of GFAP-expressing mitotic neural stems cells, blocking the generation of ABNs. (**b**) Experimental timeline. Adult mice underwent 8 weeks of VGCC treatment before starting the behavioral task and imaging. (**c,d**) VGCC administration results in a near-complete ablation of ABNs in the olfactory bulbs of GFAP-TK+ (ablation) mice. (**c**) BrdU labeling of olfactory bulbs of GFAP-TK- (control) and ablation mice administered with VGCC. Green: BrdU; Blue: DAPI; MCL: mitral cell layer; GCL: granule cell layer; EPL: external plexiform layer. Right: quantification of the BrdU-labeled cell density in control (n = 4, black) and ablation (n = 4, red) ($p < 0.0001$, Wilcoxon rank sum test). Asterisks represent means. (**d**) Doublecortin (DCX) labeling of immature neurons in the olfactory bulbs showed similar results to BrdU labeling. Green: DCX; Blue: DAPI. (**e**) VGCC administration in ablation mice does not affect the density of GFAP+ astrocytes in the olfactory bulb. Left: GFAP immunostaining of astrocytes in the olfactory bulbs of control mice (left panel) and ablation mice (right panel) after 8 weeks of continuous treatment of VGCC. Arrows show examples of GFAP+ astrocyte cell bodies. Green: GFAP; Blue: DAPI. Right: No significant difference in the density of GFAP+ astrocytes in VGCC-administered control mice (n = 3, black) and ablation mice (n = 3, red) (mean ± S.E.M.; $p=0.9911$, Wilcoxon rank sum test). Asterisks represent means. (**f**) Open field test shows no deficiency in mobility in ablation mice (n = 8, red) compared to controls (n = 8, black). All error bars: mean ± S.E.M. Left: average speed (cm/s) ($p = 1.0000$, Wilcoxon rank sum test). Right: fraction of time spent in the center area ($p = 0.7984$, Wilcoxon rank sum test).

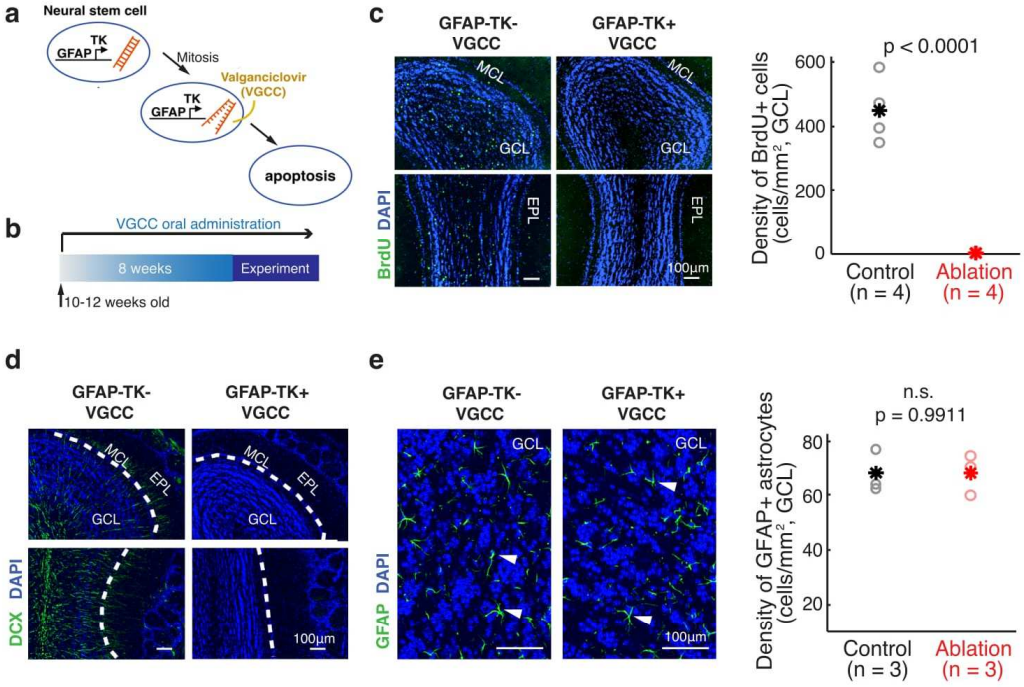

a
Neural stem cell
TK
GFAP
Mitosis
Valganciclovir
(VGCC)
apoptosis
b
VGCC oral administration
8 weeks
Experiment
10-12 weeks old
c
GFAP-TK-
VGCC
GFAP-TK+
VGCC
MCL
GCL
EPL
BrdU DAPI
100μm
p < 0.0001
Density of BrdU+ cells
(cells/mm², GCL)
600
400
200
0
Control
(n = 4)
Ablation
(n = 4)
d
GFAP-TK-
VGCC
GFAP-TK+
VGCC
MCL
EPL
GCL
DCX DAPI
100μm
e
GFAP-TK-
VGCC
GFAP-TK+
VGCC
GCL
GFAP DAPI
100μm
n.s.
p = 0.9911
Density of GFAP+ astrocytes
(cells/mm², GCL)
80
60
40
20
Control
(n = 3)
Ablation
(n = 3)
f
n.s.
p = 1.0000
Average speed (cm/s)
600
400
200
0
Control
(n = 8)
Ablation
(n = 8)
n.s.
p = 0.7984
Fraction of time
in the center
0.6
0.4
0.2
0
Control
(n = 8)
Ablation
(n = 8)

Figure 2.2 Ablation mice are impaired in difficult but not easy discrimination task.

(**a**) Trial structure. (**b**) Easy discrimination task. Mice are trained to lick left in response to H80E20 (a mixture of 80% heptanal and 20% ethyl-tiglate) and lick right to H20E80. (**c**) Number of sessions required to reach expertise (>80 % success rate) in the easy discrimination task. There is no significant difference between control (n = 22) and ablation (n = 23) groups (p = 0.4523, Wilcoxon rank sum test). Learning curves are not shown since each animal was trained until expertise, resulting in varying durations of training. (**d**) Difficult discrimination task. One of the 8 mixtures is presented pseudorandomly in each trial. (**e**) Fraction of correct trials over 10 sessions in the difficult discrimination task. Ablation animals exhibit impaired learning compared to control animals (p(group) < 0.0001, p(session) < 0.0001, two-way ANOVA). Both groups had equal and chance-level success rate in the test session with identical H50E50 mixtures, indicating that they were using odorants to guide their choice (described in Results). (**f**) Fraction of trials with answers (correct or incorrect) throughout the difficult task sessions. There is no difference between control and ablation (p = 0.4101, two-way ANOVA). (**g**) Control and ablation had comparable lick rates during reward consumption (p = 0.1036, two-way ANOVA). All error bars: mean ± S.E.M.

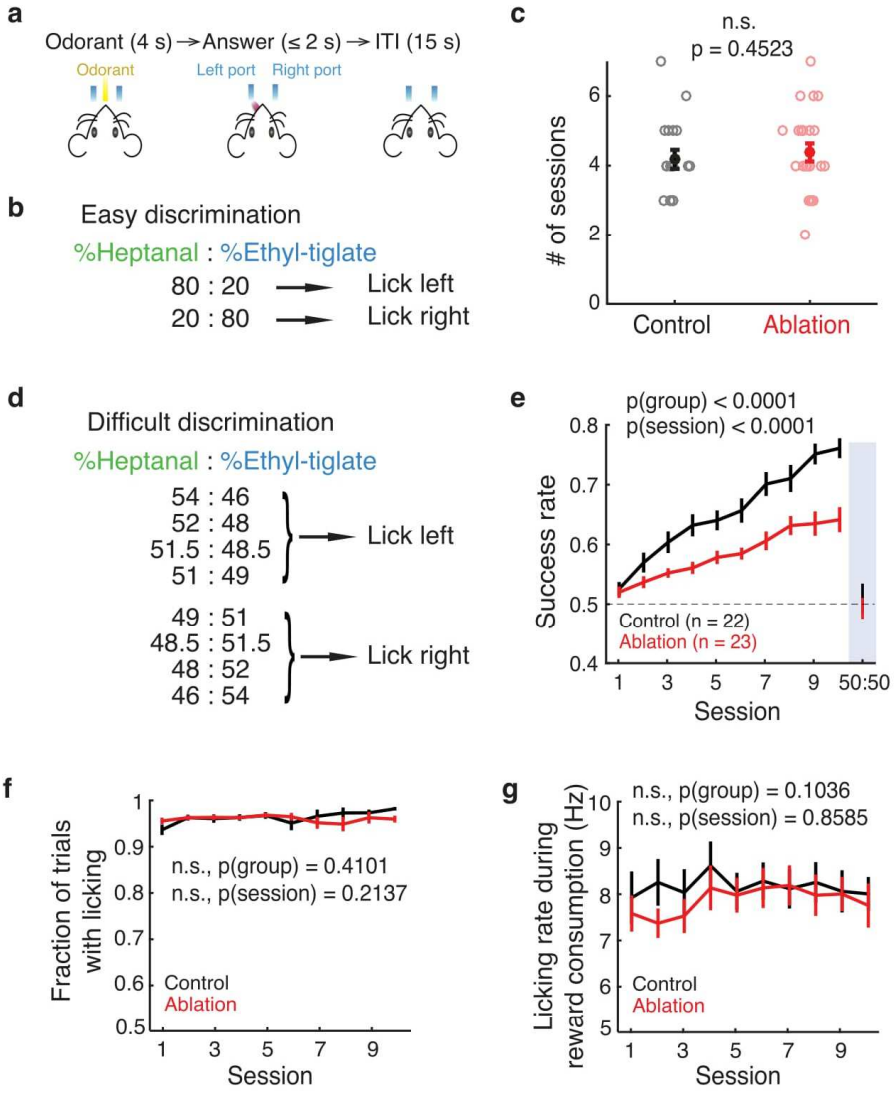
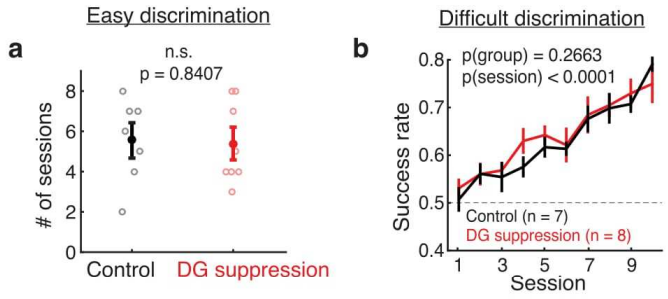

a
Odorant (4 s) → Answer (≤ 2 s) → ITI (15 s)
Odorant
Left port
Right port
b
Easy discrimination
%Heptanal : %Ethyl-tiglate
80 : 20 → Lick left
20 : 80 → Lick right
c
n.s.
p = 0.4523
# of sessions
Control
Ablation
d
Difficult discrimination
%Heptanal : %Ethyl-tiglate
54 : 46
52 : 48
51.5 : 48.5
51 : 49
→ Lick left
49 : 51
48.5 : 51.5
48 : 52
46 : 54
→ Lick right
e
p(group) < 0.0001
p(session) < 0.0001
Success rate
Control (n = 22)
Ablation (n = 23)
Session
50:50
f
Fraction of trials with licking
n.s., p(group) = 0.4101
n.s., p(session) = 0.2137
Control
Ablation
Session
g
n.s., p(group) = 0.1036
n.s., p(session) = 0.8585
Licking rate during reward consumption (Hz)
Control
Ablation
Session

Figure 2.3 Suppression of hippocampal ABNs or random ablation of GCL neurons did not cause the same behavioral deficit as GFAP-TK mice.

(**a,b**) Behavioral performance of control (black) vs. hippocampal DG ABN suppression (red) groups. (**a**) Number of sessions required to reach expertise (>80 % success rate) for the easy discrimination task. There is no significant difference between control (n = 7) and DG suppression (n = 8) groups (p = 0.8047, Wilcoxon rank sum test). (**b**) Fraction of correct trials over 10 sessions in the difficult discrimination task. There is no significant difference between control (n = 7) and DG suppression groups (n = 8; p(group) = 0.2663, two-way ANOVA). (**c,d**) Quantification of GCL neuron reduction after VGCC treatment. (**c**) Post hoc DAPI (blue) labeling shows that after ~2 months of VGCC treatment, GFAP-TK$^+$ (right) ablation group have a lower cell density in the GCL compared to GFAP-TK- (left) controls. (**d**) GCL cell density in control (n = 3, black) and ablation (n = 4, red). Asterisks represent means. On average, density (ablation) / density (control) = 89.98%, indicating that there was a 10.02% reduction in cell density. (**e,f,g**) Random cell ablation in GCL by injecting a combination of AAV2/1-CMV-Cre and AAV2/1-EF1a-FLEX-taCasp viruses caused a reduction in cell density, without changing the GCL size or affecting olfactory adult neurogenesis. (**e**) DAPI labeling 10 days after right OB unilateral injection shows a reduced cell density in GCL of the injected right OB (right) compared to the uninjected left OB (left). Blue: DAPI. (**f**) Post hoc quantification of GCL DAPI signal density 1.5 months after 300 nl (n = 4, dark blue) or 500 nl (n = 7, light blue) viral cocktail bilateral injection compared to uninjected control (n = 7) (injected vs. unjected control: p < 0.0001; Wilcoxon rank sum test). Asterisks represent means. (**g**) There is no difference in GCL width with or without viral ablation (injected vs. uninjected control: p = 0.9499; Wilcoxon rank sum test). Asterisks represent means.. (**h,i**) Random ablation method did not affect olfactory adult neurogenesis. (**h**) Doublecortin (DCX) labeling of immature neurons in uninjected (left) or injected (right) OBs 10 days after right OB unilateral injection. Green: DCX. (**i**) Average DCX signal intensity of RMS-periRMS area in the OB. There is no difference between un-injected and injected OBs (p = 0.9015, Wilcoxon rank sum test), indicating that the injections did not affect subsequent adult neurogenesis. Dark blue: 300 nl injection; light blue: 500 nl injection. (**j,k**) Behavioral performance of random GCL cell ablation group (blue, n = 11), compared to control (black) and ABN ablation (red) groups shown in **Fig. 2c,e**. (**j**) Number of sessions required to reach expertise (>80 % success rate) for the easy discrimination task. There is no significant difference between random ablation group and control or ABN ablation groups (random ablation vs. control: p = 0.4430; random ablation vs. ABN ablation: p = 0.9846; Wilcoxon rank sum test). (**k**) Fraction of correct trials in the difficult discrimination task. For all 10 sessions, random ablation group is significantly different from other two groups (random ablation vs. control, p < 0.0001; random ablation vs. ABN ablation, p < 0.0001). For sessions 1-5, random ablation is worse than control (p = 0.0124), but not different from ABN ablation (p = 0.2528); for sessions 6-10, random ablation is better than ABN ablation (p < 0.0001), but not different from control (p = 0.2575). Mean ± S.E.M., two-way ANOVA.

Hippocampal ABN suppression



GC random ablation

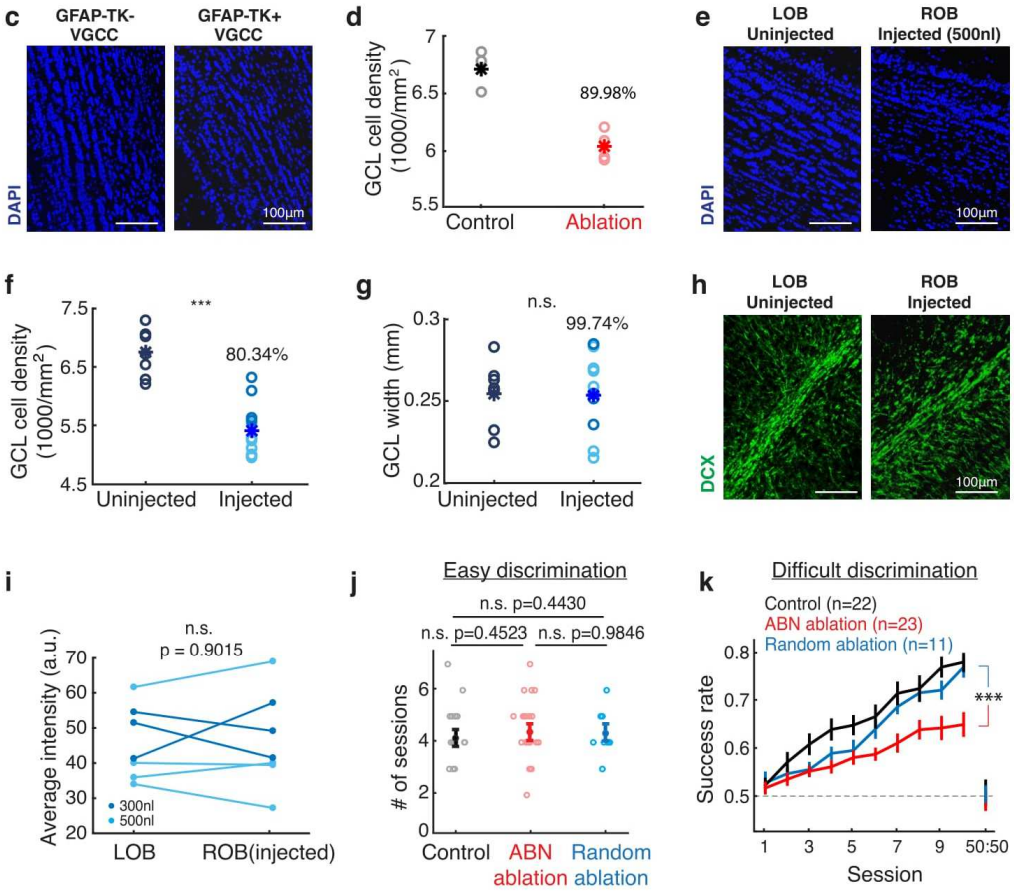


Figure 2.4 Imaging of mitral cell responses during the difficult discrimination task.

(**a**) Schematic of the olfactory bulb. AAV2/1-flex-GCaMP6f was injected into the olfactory bulb to express GCaMP6f in mitral/tufted cells in both control (GFAP-TK$^{-/-}$::Pcdh21-Cre^{+}) and ablation (GFAP-TK$^{+/-}$::Pcdh21-Cre^{+}) animals. VGCC was administered continuously to both groups, resulting in ABN ablation in the ablation group (right) but not control group (left). (**b**) Imaging timeline. After the pretraining period, mice were trained with the easy discrimination task until they reached expertise (>80 % fraction correct within a session). The mice were then trained to perform the 8-odorant difficult discrimination task for 10 sessions with two-photon imaging on sessions 1, 3, 5, 7 and 9. (**c**) A field of the same mitral cell (MC) population on first day of imaging (left) and 8 days later (right). (**d**) Odorant responses (mean ± S.E.M.) of six example MCs during the first day of the difficult discrimination task. Pink areas denote the 4-s odorant period. Red and blue dots indicate significant excitatory and suppressive responses respectively. (**e**) Population decoder accuracy during the difficult discrimination task in ablation animals (n = 10) is significantly worse than control animals (n = 12) (mean ± S.E.M., $p < 0.05$, two-way ANOVA). Black broken line indicates the chance level (0.125). (**f**) Table of contrast values between odorant pairs for the 8 odorants used in the difficult discrimination task. Different color shades in the boxes match with the binning of the contrast values in **g**. (**g**) Pairwise decoder accuracy during the difficult discrimination task plotted as a function of binned odorant pair contrasts (mean ± S.E.M.). Control is significantly better than ablation for smaller contrasts (≤3, $p < 0.001$, two-way ANOVA) but not for larger contrasts (>3, $p = 0.2530$, two-way ANOVA). (**h**) Behavioral performance in session 9 correlates with decoder accuracy of the session (mean ± S.E.M., $p < 0.05$, Pearson correlation).

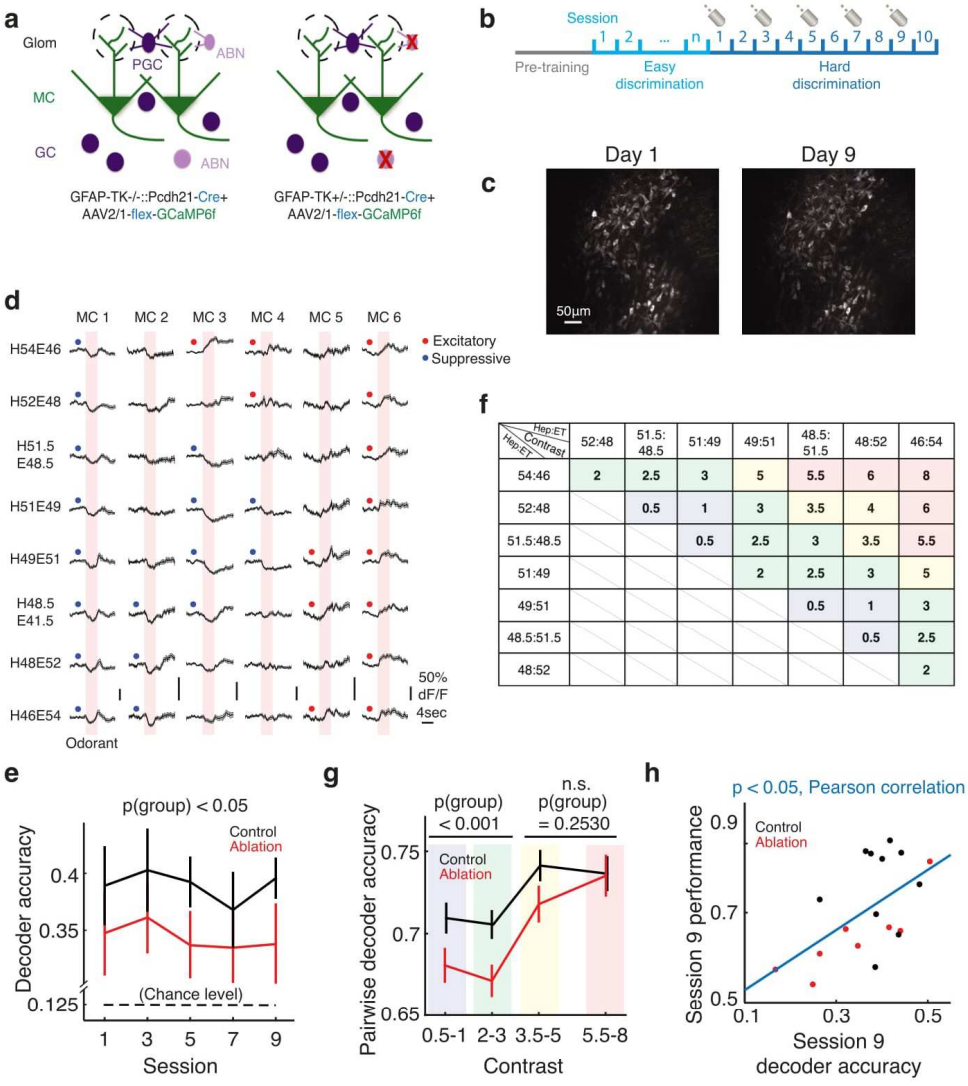

a
Glom
MC
GC
PGC
ABN
ABN
GFAP-TK-/-::Pcdh21-Cre+
AAV2/1-flex-GCaMP6f
GFAP-TK+/-::Pcdh21-Cre+
AAV2/1-flex-GCaMP6f
b
Session
1 2 ... n 1 2 3 4 5 6 7 8 9 10
Pre-training
Easy discrimination
Hard discrimination
c
Day 1
Day 9
50μm
d
MC 1 MC 2 MC 3 MC 4 MC 5 MC 6
Excitatory
Suppressive
H54E46
H52E48
H51.5 E48.5
H51E49
H49E51
H48.5 E41.5
H48E52
H46E54
50% dF/F
4sec
Odorant
f
Hep:ET Contrast / Hep:ET
52:48 | 51.5:48.5 | 51:49 | 49:51 | 48.5:51.5 | 48:52 | 46:54
54:46 | 2 | 2.5 | 3 | 5 | 5.5 | 6 | 8
52:48 | | 0.5 | 1 | 3 | 3.5 | 4 | 6
51.5:48.5 | | | 0.5 | 2.5 | 3 | 3.5 | 5.5
51:49 | | | | 2 | 2.5 | 3 | 5
49:51 | | | | | 0.5 | 1 | 3
48.5:51.5 | | | | | | 0.5 | 2.5
48:52 | | | | | | | 2
e
p(group) < 0.05
Control
Ablation
Decoder accuracy
0.4
0.35
0.125
(Chance level)
1 3 5 7 9
Session
g
p(group) < 0.001
n.s. p(group) = 0.2530
Control
Ablation
Pairwise decoder accuracy
0.75
0.7
0.65
0.5-1 2-3 3.5-5 5.5-8
Contrast
h
p < 0.05, Pearson correlation
Control
Ablation
Session 9 performance
0.9
0.7
0.5
0.1 0.3 0.5
Session 9
decoder accuracy

Figure 2.5 Fraction of responsive MCs (top) and responsive MC-odorant pairs (bottom) during the difficult discrimination task.

Control: n = 12; ablation: n = 10. Mean ± S.E.M. (**a**) MCs with either excitatory or suppressive responses. Fraction of MCs: $p < 0.001$; fraction of MC-odorant pairs: $p < 0.01$. (**b**) MCs with excitatory responses. Fraction of MCs: $p = 0.0813$; fraction of MC-odorant pairs: $p = 0.6039$. (**c**) MCs with suppressive responses. Fraction of MCs: $p < 0.0001$; fraction of MC-odorant pairs: $p < 0.0001$. All p values are for two-way ANOVA, control vs. ablation. Note that a given MC can have excitatory responses to some odorants and suppressive responses to others, and thus the total response is not necessarily a sum of excitatory and suppressive.

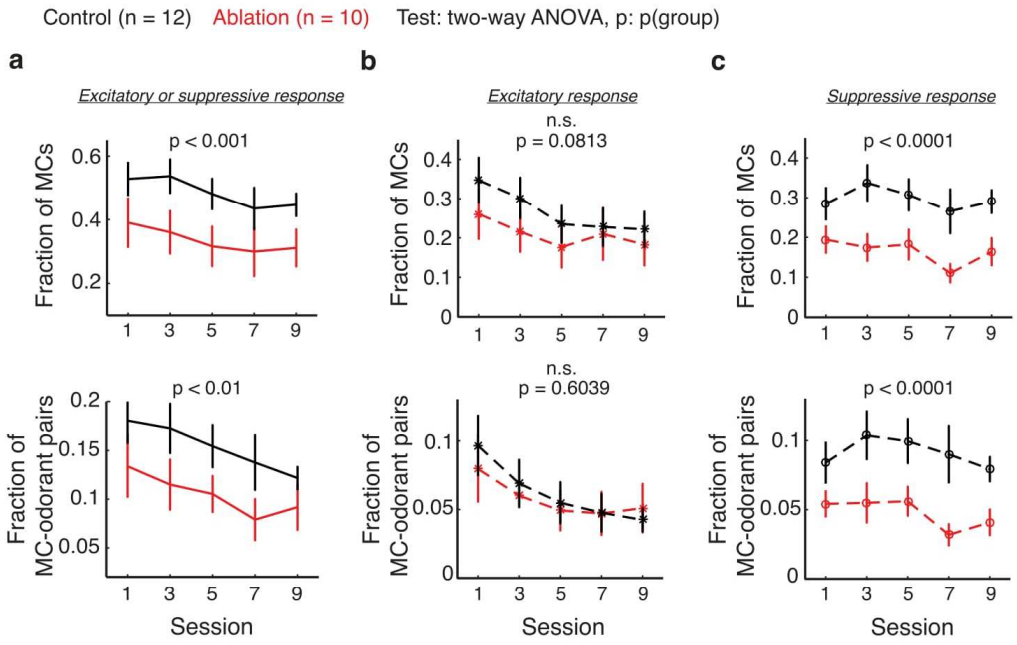

Control (n = 12) Ablation (n = 10) Test: two-way ANOVA, p: p(group)
a
Excitatory or suppressive response
p < 0.001
Fraction of MCs
p < 0.01
Fraction of MC-odorant pairs
Session
b
Excitatory response
n.s.
p = 0.0813
n.s.
p = 0.6039
c
Suppressive response
p < 0.0001
p < 0.0001

Figure 2.6 Relationship of responsive MC fractions with MC ensemble discriminability and behavioral performance.

(**a**) Session 9 decoder accuracy correlates with fraction of responsive MCs (left, $p < 0.01$, Pearson correlation) and MC-odorant pairs (right, $p < 0.01$, Pearson correlation). (**b**) Session 9 behavioral performance correlates with fraction of responsive MCs (left, $p < 0.01$, Pearson correlation) and MC-odorant pairs (right, $p < 0.05$, Pearson correlation). (**c**) Session 9 decoder accuracy correlates with suppressive but not excitatory MC responses. Top left: excitatory MCs, $p = 0.2089$; bottom left: excitatory MC-odorant pairs, $p = 0.1272$; top right: suppressive MCs, $p < 0.01$; bottom right: suppressive MC-odorant pairs, $p < 0.01$, Pearson correlation. (**d**) Session 9 behavioral performance correlates with MC suppressive responses. Top left: excitatory MCs, $p < 0.05$; bottom left: excitatory MC-odorant pairs, $p = 0.1459$; top right: suppressive MCs, $p < 0.05$; bottom right: suppressive MC-odorant pairs, $p < 0.05$, Pearson correlation.

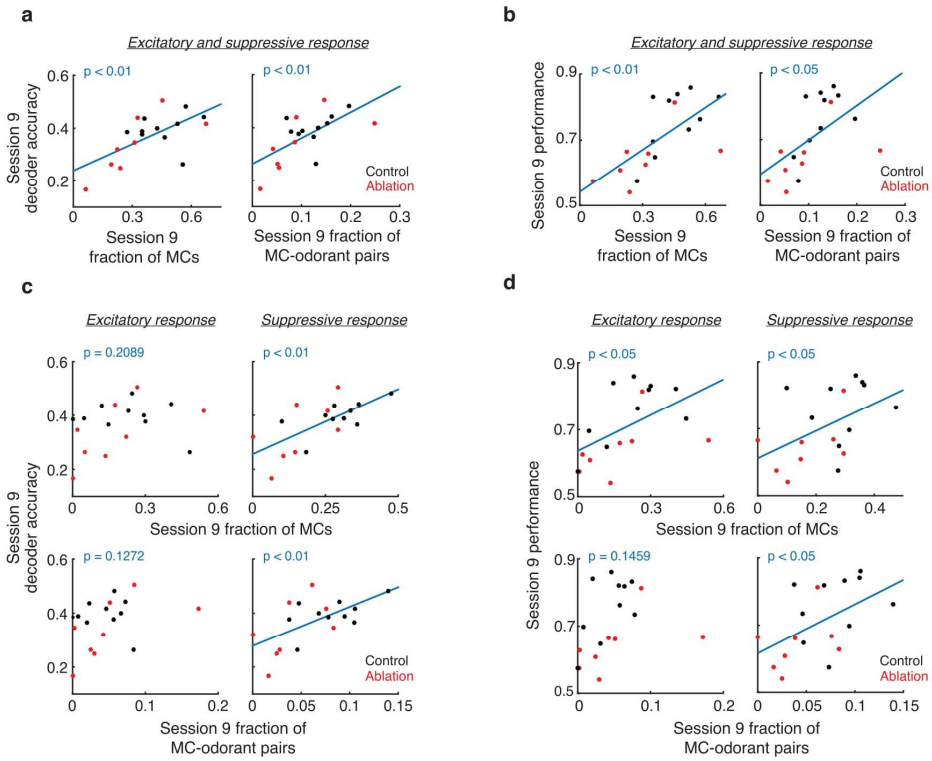

a
Excitatory and suppressive response
p < 0.01
p < 0.01
Session 9 decoder accuracy
Session 9 fraction of MCs
Session 9 fraction of MC-odorant pairs
Control
Ablation
b
Excitatory and suppressive response
p < 0.01
p < 0.05
Session 9 performance
Session 9 fraction of MCs
Session 9 fraction of MC-odorant pairs
Control
Ablation
c
Excitatory response
Suppressive response
p = 0.2089
p < 0.01
p = 0.1272
p < 0.01
Session 9 decoder accuracy
Session 9 fraction of MCs
Session 9 fraction of MC-odorant pairs
Control
Ablation
d
Excitatory response
Suppressive response
p < 0.05
p < 0.05
p = 0.1459
p < 0.05
Session 9 performance
Session 9 fraction of MCs
Session 9 fraction of MC-odorant pairs
Control
Ablation

Figure 2.7 Task engagement enhances MC suppressive responses through ABNs.

(**a-c**) Fraction of responsive MCs (top) and responsive MC-odorant pairs (bottom) during passive exposure. Control passive: n = 10; ablation passive: n = 7. Mean ± S.E.M. (**a**) MCs with either excitatory or suppressive responses. Fraction of MCs: p = 0.6162; fraction of MC-odorant pairs: p = 0.2043. (**b**) MCs with excitatory responses. Fraction of MCs: p = 0.7625; fraction of MC-odorant pairs: p = 0.8691. (**c**) MCs with suppressive responses. Fraction of MCs: p = 0.2620; fraction of MC-odorant pairs: p = 0.0625. All p values are for two-way ANOVA, control passive vs. ablation passive.

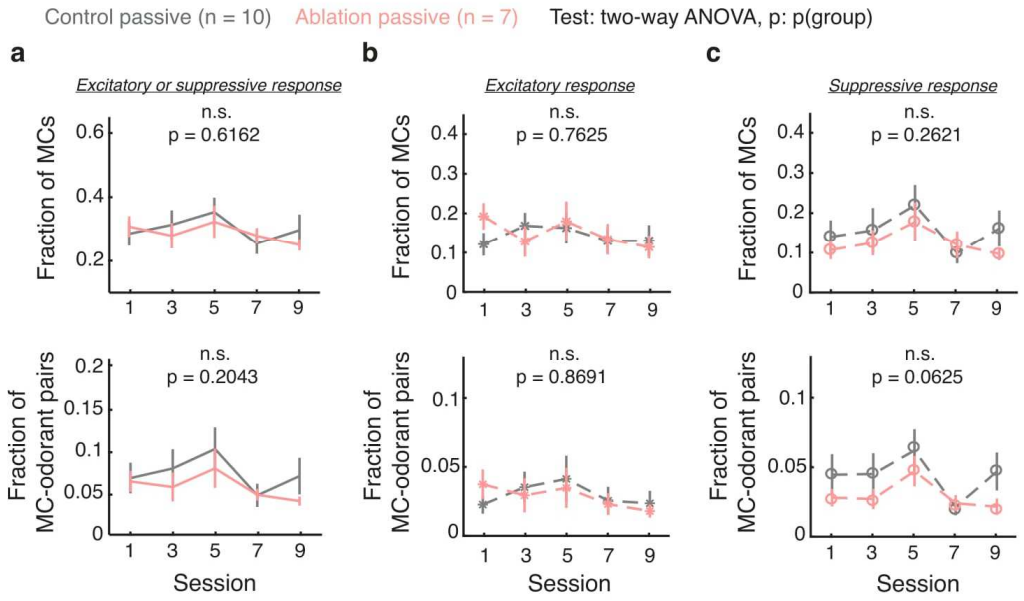
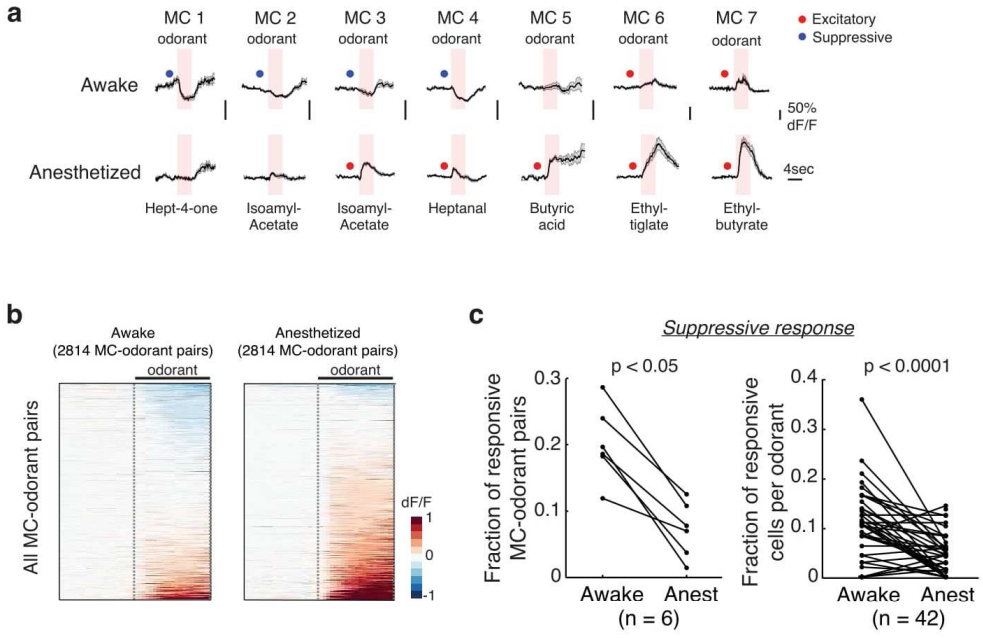

Control passive (n = 10)
Ablation passive (n = 7)
Test: two-way ANOVA, p: p(group)
a
Excitatory or suppressive response
n.s.
p = 0.6162
Fraction of MCs
n.s.
p = 0.2043
Fraction of MC-odorant pairs
Session
b
Excitatory response
n.s.
p = 0.7625
Fraction of MCs
n.s.
p = 0.8691
Fraction of MC-odorant pairs
Session
c
Suppressive response
n.s.
p = 0.2621
Fraction of MCs
n.s.
p = 0.0625
Fraction of MC-odorant pairs
Session

Figure 2.8 Anesthesia further reduces MC suppressive responses.

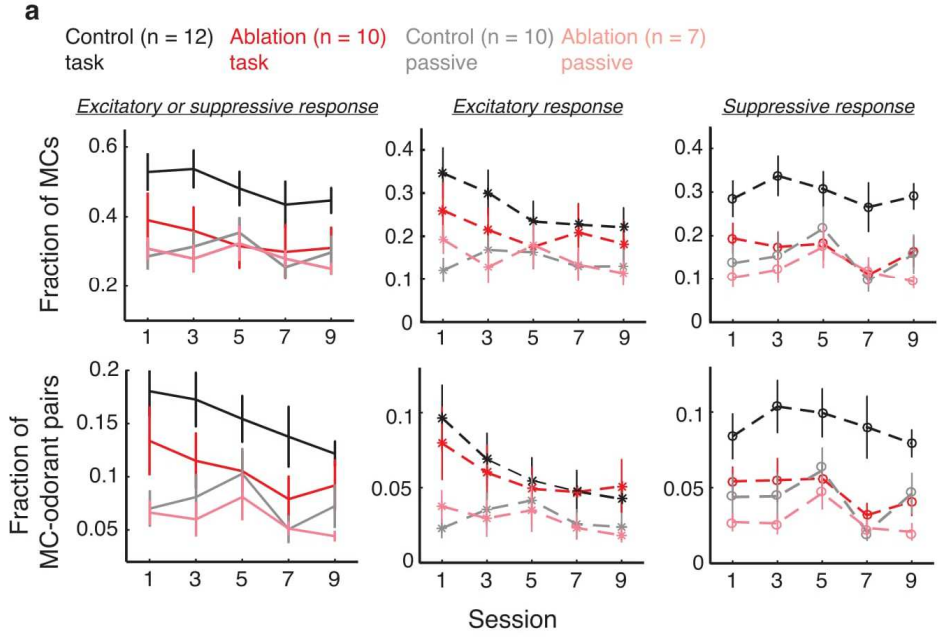
Re-analysis of data from Kato et al., 2012. (**a**) Odorant responses (mean ± S.E.M. from 7 trials) from seven example MCs in both awake (top) and anesthetized (bottom) states. Pink areas denote the 4-s odorant period. Red and blue dots denote excitatory and suppressive responses respectively. (**b**) Activity of all MC-odorant pairs in awake (left) and anesthetized (right) states. Each row represents one MC-odorant pair, and black horizontal bars denote the 4-s odorant period. Rows are sorted based on the mean dF/F of the odorant period. (**c**) Anesthesia reduces MC suppressive responses. Left: fraction of suppressive MC-odorant pairs ($n = 6$ animals, $p < 0.05$, Wilcoxon signed-rank test). Right: fraction of suppressive MCs for each odorant-animal pair ($n = 42$ odorant-animal pairs, $p < 0.0001$, Wilcoxon signed-rank test).


a
MC 1
odorant
MC 2
odorant
MC 3
odorant
MC 4
odorant
MC 5
odorant
MC 6
odorant
MC 7
odorant
Excitatory
Suppressive
Awake
Anesthetized
50%
dF/F
4sec
Hept-4-one
Isoamyl-
Acetate
Isoamyl-
Acetate
Heptanal
Butyric
acid
Ethyl-
tiglate
Ethyl-
butyrate
b
Awake
(2814 MC-odorant pairs)
odorant
Anesthetized
(2814 MC-odorant pairs)
odorant
All MC-odorant pairs
dF/F
1
0
-1
c
Suppressive response
p < 0.05
Fraction of responsive
MC-odorant pairs
0.3
0.2
0.1
0
Awake
Anest
(n = 6)
p < 0.0001
Fraction of responsive
cells per odorant
0.4
0.3
0.2
0.1
0
Awake
Anest
(n = 42)

Extended Figure 2.1 Fraction of responsive MCs during difficult discrimination task and passive exposure.

Control behavior: n = 12; ablation behavior: n = 10; Control passive: n = 10; ablation passive: n = 7. (**a**) Overlay of **Fig. 5a-c** and **Fig. 7a-c**. Mean ± S.E.M. (**b**) Summary of comparisons between each pair of groups for total, excitatory and suppressive response fractions. P values: two-way ANOVA, group effect. Green: p values indicate statistical significance.

a



b

Excitatory or suppressive response

Fraction of MCs

2-ANOVA p(group)	Ablation	Control passive	Ablation passive
Control	0.0002	<0.0001	<0.0001
Ablation		0.2592	0.1519
Control passive			0.9703

Fraction of MC-odorant pairs

2-ANOVA p(group)	Ablation	Control passive	Ablation passive
Control	0.0027	<0.0001	<0.0001
Ablation		0.1286	0.0145
Control passive			0.7273

Excitatory response

Fraction of MCs

2-ANOVA p(group)	Ablation	Control passive	Ablation passive
Control	0.1596	<0.0001	0.0008
Ablation		0.1345	0.2915
Control passive			0.9963

Fraction of MC-odorant pairs

2-ANOVA p(group)	Ablation	Control passive	Ablation passive
Control	0.9331	0.0019	0.0044
Ablation		0.0281	0.0408
Control passive			0.9995

Suppressive response

Fraction of MCs

2-ANOVA p(group)	Ablation	Control passive	Ablation passive
Control	<0.0001	<0.0001	<0.0001
Ablation		0.9705	0.4718
Control passive			0.7066

Fraction of MC-odorant pairs

2-ANOVA p(group)	Ablation	Control passive	Ablation passive
Control	<0.0001	<0.0001	<0.0001
Ablation		0.9730	0.2262
Control passive			0.4015

Chapter 2 is a reprint of the material as it appears in Li WL, Chu MW, Wu A, Suzuki Y, Imayoshi I, Komiyama T (2017). Adult-born neurons facilitate olfactory bulb pattern separation during task engagement. In submission. The dissertation author was the primary investigator and author of this paper.

References

Abney, E. R., P. P. Bartlett and M. C. Raff (1981). "Astrocytes, ependymal cells, and oligodendrocytes develop on schedule in dissociated cell cultures of embryonic rat brain." Developmental Biology **83**(2): 301-310.

Abraham, N. M., V. Egger, D. R. Shimshek, R. Renden, I. Fukunaga, R. Sprengel, P. H. Seeburg, M. Klugmann, T. W. Margrie, A. T. Schaefer and T. Kuner (2010). "Synaptic Inhibition in the Olfactory Bulb Accelerates Odor Discrimination in Mice." Neuron **65**(3): 399-411.

Abraham, N. M., H. Spors, A. Carleton, T. W. Margrie, T. Kuner and A. T. Schaefer (2004). "Maintaining accuracy at the expense of speed: Stimulus similarity defines odor discrimination time in mice." Neuron **44**: 865-876.

Aimone, James B., W. Deng and Fred H. Gage (2011). "Resolving New Memories: A Critical Look at the Dentate Gyrus, Adult Neurogenesis, and Pattern Separation." Neuron **70**(4): 589-596.

Alonso, M., G. Lepousez, W. Sebastien, C. Bardy, M. M. Gabellec, N. Torquet and P. M. Lledo (2012). "Activation of adult-born neurons facilitates learning and memory." Nat Neurosci **15**(6): 897-904.

Alonso, M., C. Viollet, M. M. Gabellec, V. Meas-Yedid, J. C. Olivo-Marin and P. M. Lledo (2006). "Olfactory discrimination learning increases the survival of adult-born neurons in the olfactory bulb." J Neurosci **26**: 10508-10513.

Altman, J. (1962). "Are New Neurons Formed in the Brains of Adult Mammals?" Science **135**(3509): 1127-1128.

Altman, J. (1963). "Autoradiographic investigation of cell proliferation in the brains of rats and cats." Anat Rec **145**(4): 573-591.

Altman, J. and G. D. Das (1965). "Autoradiographic and histological avidence of postnatal neurogenesis in rats." J Comp Neurol **124**(3): 319-335.

Altman, J. and G. D. Das (1965). "Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats." J Comp Neurol **124**(3): 319-335.

Alvarado, A. S. (2000). "Regeneration in the metazoans: why does it happen?" BioEssays **22**(6): 578-590.

Aroniadou-Anderjaska, V., F.-M. Zhou, C. A. Priest, M. Ennis and M. T. Shipley (2000). "Tonic and Synaptically Evoked Presynaptic Inhibition of Sensory Input to the Rat Olfactory Bulb Via GABA_BHeteroreceptors." Journal of Neurophysiology **84**(3): 1194.

Bakker, A., C. B. Kirwan, M. Miller and C. E. L. Stark (2008). "Pattern Separation in the Human Hippocampal CA3 and Dentate Gyrus." Science **319**(5870): 1640-1642.

Balu, R., R. T. Pressler and B. W. Strowbridge (2007). "Multiple Modes of Synaptic Excitation of Olfactory Bulb Granule Cells." J Neurosci **27**(21): 5621-5632.

Bardy, C., M. Alonso, W. Bouthour and P.-M. Lledo (2010). "How, When, and Where New Inhibitory Neurons Release Neurotransmitters in the Adult Olfactory Bulb." The Journal of Neuroscience **30**(50): 17023.

Bath, K. G., N. Mandairon, D. Jing, R. Rajagopal, R. Kapoor, Z. Y. Chen, T. Khan, C. C. Proenca, R. Kraemer, T. A. Cleland, B. L. Hempstead, M. V. Chao and F. S. Lee (2008). "Variant Brain-Derived Neurotrophic Factor (Val66Met) Alters Adult Olfactory Bulb Neurogenesis and Spontaneous Olfactory Discrimination." J Neurosci **28**(10): 2383-2393.

Bergmann, O., J. Liebl, S. Bernard, K. Alkass, Maggie S. Y. Yeung, P. Steier, W. Kutschera, L. Johnson, M. Landén, H. Druid, Kirsty L. Spalding and J. Frisén (2012). "The Age of Olfactory Bulb Neurons in Humans." Neuron **74**(4): 634-639.

Bergmann, O., K. L. Spalding and J. Frisén (2015). "Adult Neurogenesis in Humans." Cold Spring Harbor Perspectives in Biology **7**(7).

Berkowicz, D. A. and P. Q. Trombley (2000). "Dopaminergic modulation at the olfactory nerve synapse." Brain Research **855**(1): 90-99.

Bhardwaj, R. D., M. A. Curtis, K. L. Spalding, B. A. Buchholz, D. Fink, T. Björk-Eriksson, C. Nordborg, F. H. Gage, H. Druid, P. S. Eriksson and J. Frisén (2006). "Neocortical neurogenesis in humans is restricted to development." Proceedings of the National Academy of Sciences **103**(33): 12564-12568.

Blomqvist, E. H., A. Bramerson, P. Stjarne and S. Nordin (2004). "Consequences of olfactory loss and adopted coping strategies." Rhinol Intl **42**(4): 189-194.

Bolteus, A. J. and A. Bordey (2004). "GABA Release and Uptake Regulate Neuronal Precursor Migration in the Postnatal Subventricular Zone." The Journal of Neuroscience **24**(35): 7623.

Bovetti, S., A. Veyrac, P. Peretto, A. Fasolo and S. de Marchis (2009). "Olfactory enrichment influences adult neurogenesis modulating GAD67 and plasticity-related molecules expression in newborn cells of the olfactory bulb." PLoS ONE **4**.

Boyd, A. M., H. K. Kato, T. Komiyama and J. S. Isaacson (2015). "Broadcasting of Cortical Activity to the Olfactory Bulb." Cell Rep **10**(7): 1032-1039.

Boyd, A. M., J. F. Sturgill, C. Poo and J. S. Isaacson (2012). "Cortical feedback control of olfactory bulb circuits." Neuron **76**(6): 1161-1174.

Brennan, P. A. and E. B. Keverne (2004). "Something in the Air? New Insights into Mammalian Pheromones." Current Biology **14**(2): R81-R89.

Breton-Provencher, V., M. Lemasson, M. R. Peralta and A. Saghatelyan (2009). "Interneurons produced in adulthood are required for the normal functioning of the olfactory bulb network and for the execution of selected olfactory behaviors." J Neurosci **29**(48): 15245-15257.

Buck, L. and R. Axel (1991). "A novel multigene family may encode odorant receptors: A molecular basis for odor recognition." Cell **65**(1): 175-187.

Burd, G. D. and F. Nottebohm (1985). "Ultrastructural characterization of synaptic terminals formed on newly generated neurons in a song control nucleus of the adult canary forebrain." J Comp Neurol **240**(2): 143-152.

Carlson, B. (2007). "Principles of regenerative biology " Elsevier.

Castillo, P. E., A. Carleton, J. D. Vincent and P. M. Lledo (1999). "Multiple and opposing roles of cholinergic transmission in the main olfactory bulb." J Neurosci **19**(21): 9180-9191.

Cecchi, G. A., L. T. Petreanu, A. Alvarez-Buylla and M. O. Magnasco (2008). "Unsupervised learning and adaptation in a model of adult neurogenesis." J. Comput. Neurosci. **11**(2): 175-182.

Chapuis, J., Y. Cohen, X. He, Z. Zhang, S. Jin, F. Xu and D. A. Wilson (2013). "Lateral Entorhinal Modulation of Piriform Cortical Activity and Fine Odor Discrimination." J Neurosci **33**(33): 13449-13459.

Chaudhury, D., O. Escanilla and C. Linster (2009). "Bulbar Acetylcholine Enhances Neural and Perceptual Odor Discrimination." J Neurosci **29**(1): 52-60.

Chu, M. W., W. L. Li and T. Komiyama (2016). "Balancing the Robustness and Efficiency of Odor Representations during Learning." Neuron **92**(1): 174-186.

Chu, Monica W., Wankun L. Li and T. Komiyama (2016). "Balancing the Robustness and Efficiency of Odor Representations during Learning." Neuron **92**(1): 174-186.

Chu, M. W., W. L. Li and T. Komiyama (2017). "Lack of Pattern Separation in Sensory Inputs to the Olfactory Bulb during Perceptual Learning." eneuro.

Clark, G. A. (1995). "Emotional Learning: Fear and loathing in the amygdala." Current Biology **5**(3): 246-248.

Clelland, C. D., M. Choi, C. Romberg, G. D. Clemenson, A. Fragniere, P. Tyers, S. Jessberger, L. M. Saksida, R. A. Barker, F. H. Gage and T. J. Bussey (2009). "A Functional Role for Adult Hippocampal Neurogenesis in Spatial Pattern Separation." Science **325**(5937): 210-213.

Colgin, L. L., E. I. Moser and M.-B. Moser (2008). "Understanding memory through hippocampal remapping." Trends in Neurosciences **31**(9): 469-477.

Cong, L., F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. D. Hsu, X. Wu, W. Jiang, L. A. Marraffini and F. Zhang (2013). "Multiplex Genome Engineering Using CRISPR/Cas Systems." Science **339**(6121): 819-823.

Corotto, F. S., J. R. Henegar and J. A. Maruniak (1994). "Odor deprivation leads to reduced neurogenesis and reduced neuronal survival in the olfactory bulb of the adult mouse." Neuroscience **61**(4): 739-744.

Croy, I., S. Negoias, L. Novakova, B. N. Landis and T. Hummel (2012). "Learning about the Functions of the Olfactory System from People without a Sense of Smell." PLOS ONE **7**(3): e33365.

Cummings, D. M., J. S. Snyder, M. Brewer, H. A. Cameron and L. Belluscio (2014). "Adult neurogenesis is necessary to refine and maintain circuit specificity." J Neurosci **34**(41): 13801-13810.

Curtis, M. A., M. Kam, U. Nannmark, M. F. Anderson, M. Z. Axell, C. Wikkelso, S. Holtås, W. M. C. van Roon-Mom, T. Björk-Eriksson, C. Nordborg, J. Frisén, M. Dragunow, R. L. M. Faull and P. S. Eriksson (2007). "Human Neuroblasts Migrate to the Olfactory Bulb via a Lateral Ventricular Extension." Science **315**(5816): 1243-1249.

Davis, M. and P. J. Whalen (2000). "The amygdala: vigilance and emotion." Molecular Psychiatry **6**: 13.

de Castro, F. (2009). "Wiring Olfaction: The Cellular and Molecular Mechanisms that Guide the Development of Synaptic Connections from the Nose to the Cortex." Frontiers in Neuroscience **3**: 52.

Deems, D. A., R. L. Doty, R. Settle and et al. (1991). "Smell and taste disorders, a study of 750 patients from the university of pennsylvania smell and taste center." Archives of Otolaryngology–Head & Neck Surgery **117**(5): 519-528.

Derer, P., V. S. Caviness and R. L. Sidman (1977). "Early cortical histogenesis in the primary olfactory cortex of the mouse." Brain Research **123**(1): 27-40.

Devor, M. (1976). "Fiber trajectories of olfactory bulb efferents in the hamster." J Comp Neurol **166**(1): 31-47.

Devore, S. and C. Linster (2012). "Noradrenergic and cholinergic modulation of olfactory bulb sensory processing." Front Behav Neurosci. **6**(52).

Doucette, W. and D. Restrepo (2008). "Profound context-dependent plasticity of mitral cell responses in olfactory bulb." PLoS Biol **6**(10): e258.

Dunnett, S., A. Björklund, F. H. Gage and U. Stenevi (1985). Transplantation of mesencephalic dopamine neurons to the striatum of adult rats.

Ennis, M., F.-M. Zhou, K. J. Ciombor, V. Aroniadou-Anderjaska, A. Hayar, E. Borrelli, L. A. Zimmer, F. Margolis and M. T. Shipley (2001). "Dopamine D2 Receptor–Mediated

Presynaptic Inhibition of Olfactory Nerve Terminals." Journal of Neurophysiology **86**(6): 2986.

Enwere, E., T. Shingo, C. Gregg, H. Fujikawa, S. Ohta and S. Weiss (2004). "Aging Results in Reduced Epidermal Growth Factor Receptor Signaling, Diminished Olfactory Neurogenesis, and Deficits in Fine Olfactory Discrimination." J Neurosci **24**(38): 8354-8365.

Eriksson, P. S., E. Perfilieva, T. Björk-Eriksson, A.-M. Alborn, C. Nordborg, D. A. Peterson and F. H. Gage (1998). "Neurogenesis in the adult human hippocampus." Nature Medicine **4**: 1313.

Ernst, A., K. Alkass, S. Bernard, M. Salehpour, S. Perl, J. Tisdale, G. Possnert, H. Druid and J. Frisén (2014). "Neurogenesis in the Striatum of the Adult Human Brain." Cell **156**(5): 1072-1083.

Escanilla, O., A. Arrellanos, A. Karnow, M. Ennis and C. Linster (2010). "Noradrenergic modulation of behavioral odor detection and discrimination thresholds in the olfactory bulb." Eur J Neurosci **32**(3): 458-468.

Fuentes, R. A., M. I. Aguilar, M. L. Aylwin and P. E. Maldonado (2008). "Neuronal activity of mitral-tufted cells in awake rats during passive and active odorant stimulation." J Neurophysiol **100**(1): 422-430.

Fukunaga, I., J. T. Herb, M. Kollo, E. S. Boyden and A. T. Schaefer (2014). "Independent control of gamma and theta activity by distinct interneuron networks in the olfactory bulb." Nat Neurosci **17**(9): 1208-1216.

Gabor, M. H. and R. D. Hotchkiss (1979). "Parameters governing bacterial regeneration and genetic recombination after fusion of *Bacillus subtilis* protoplasts." J Bacteriol **137**(3): 1346-1353.

Gage, F. H. (2002). "Neurogenesis in the Adult Brain." The Journal of Neuroscience **22**(3): 612-613.

Gage, F. H., P. W. Coates, T. D. Palmer, H. G. Kuhn, L. J. Fisher, J. O. Suhonen, D. A. Peterson, S. T. Suhr and J. Ray (1995). "Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain." Proceedings of the National Academy of Sciences of the United States of America **92**(25): 11879-11883.

Garcia, A. D., N. B. Doan, T. Imura, T. G. Bush and M. V. Sofroniew (2004). "GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain." Nat Neurosci **7**(1): 1233-1241.

Gheusi, G., H. Cremer, H. McLean, G. Chazal, J. D. Vincent and P. M. Lledo (2000). "Importance of newly generated neurons in the adult olfactory bulb for odor discrimination." Proc. Natl. Acad. Sci. U. S. A. **97**(4): 1823-1828.

Gilbert, C. D. and M. Sigman (2007). "Brain States: Top-Down Influences in Sensory Processing." Neuron **54**(5): 677-696.

Gilbert, P. E., R. P. Kesner and I. Lee (2001). "Dissociating hippocampal subregions: A double dissociation between dentate gyrus and CA1." Hippocampus **11**(6): 626-636.

Giovanni, G. C. and M. Randolf (2000). "Odour perception in honeybees: coding information in glomerular patterns." Current Opinion in Neurobiology **10**(4): 504-510.

Gonçalves, J. T., S. T. Schafer and F. H. Gage (2016). "Adult Neurogenesis in the Hippocampus: From Stem Cells to Behavior." Cell **167**(4): 897-914.

Gould, E. (2007). "How widespread is adult neurogenesis in mammals?" Nat Rev Neurosci **8**: 481-488.

Graeff, F. G., F. S. Guimarães, T. G. C. S. De Andrade and J. F. W. Deakin (1996). "Role of 5-HT in stress, anxiety, and depression." Pharmacology Biochemistry and Behavior **54**(1): 129-141.

Greer, C. A. (1991). "Structural organization of the olfactory system. In et al. T.V.Getchell(ed): Smell and taste in health and disease." New York: Raven Press: 65-81.

Gschwend, O., N. M. Abraham, S. Lagier, F. Begnaud, I. Rodriguez and A. Carleton (2015). "Neuronal pattern separation in the olfactory bulb improves odor discrimination learning." Nat Neurosci **18**(10): 1474-1482.

Gudziol, V., S. Wolff-Stephan, K. Aschenbrenner, P. Joraschky and T. Hummel (2009). "ORIGINAL RESEARCH—PSYCHOLOGY: Depression Resulting from Olfactory Dysfunction is Associated with Reduced Sexual Appetite—A Cross-Sectional Cohort Study." The Journal of Sexual Medicine **6**(7): 1924-1929.

Haider, B., M. Häusser and M. Carandini (2013). "Inhibition dominates sensory responses in the awake cortex." Nature **493**(7430): 97-100.

Handberg-Thorsager, M., E. Fernandez and E. Salo (2008). "Stem cells and regeneration in planarians." Front Biosci **13**: 6374-6394.

Hopfield, J. J. (1991). "Olfactory computation and object perception." Proceedings of the National Academy of Sciences **88**(15): 6462-6466.

Hsu, P. D., E. S. Lander and F. Zhang (2014). "Development and Applications of CRISPR-Cas9 for Genome Engineering." Cell **157**(6): 1262-1278.

Imayoshi, I., M. Sakamoto, T. Ohtsuka, K. Takao, T. Miyakawa, M. Yamaguchi, K. Mori, T. Ikeda, S. Itohara and R. Kageyama (2008). "Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain." Nat Neurosci **11**(10): 1153-1161.

Isaacson, J. S. (1999). "Glutamate Spillover Mediates Excitatory Transmission in the Rat Olfactory Bulb." Neuron **23**(2): 377-384.

Isaacson, J. S. and B. W. Strowbridge (1998). "Olfactory reciprocal synapses: dendritic signaling in the CNS." Neuron **20**(4): 749-761.

Jun, J. J., N. A. Steinmetz, J. H. Siegle, D. J. Denman, M. Bauza, B. Barbarits, A. K. Lee, C. A. Anastassiou, A. Andrei, Ç. Aydın, M. Barbic, T. J. Blanche, V. Bonin, J. Couto, B. Dutta, S. L. Gratiy, D. A. Gutnisky, M. Häusser, B. Karsh, P. Ledochowitsch, C. M. Lopez, C. Mitelut, S. Musa, M. Okun, M. Pachitariu, J. Putzeys, P. D. Rich, C. Rossant, W.-l. Sun, K. Svoboda, M. Carandini, K. D. Harris, C. Koch, J. O'Keefe and T. D. Harris (2017). "Fully integrated silicon probes for high-density recording of neural activity." Nature **551**: 232.

Kano, M. and K. Hashimoto (2009). "Synapse elimination in the central nervous system." Current Opinion in Neurobiology **19**(2): 154-161.

Kapoor, V., A. C. Provost, P. Agarwal and V. N. Murthy (2016). "Activation of raphe nuclei triggers rapid and distinct effects on parallel olfactory bulb output channels." Nat Neurosci **19**(2): 271-282.

Katagiri, H., M. Pallotto, A. Nissant, K. Murray, M. Sassoè-Pognetto and P.-M. Lledo (2011). "Dynamic development of the first synapse impinging on adult-born neurons in the olfactory bulb circuit." Neural Systems & Circuits **1**(1): 6.

Kato, H. K., M. W. Chu, J. S. Isaacson and T. Komiyama (2012). "Dynamic Sensory Representations in the Olfactory Bulb: Modulation by Wakefulness and Experience." Neuron **76**(5): 962-975.

Kauer, J. S. and J. White (2001). "Imaging and Coding in the Olfactory System." Annual Review of Neuroscience **24**(1): 963-979.

Keller, A. and D. Malaspina (2013). "Hidden consequences of olfactory dysfunction: a patient report series." BMC Ear, Nose, and Throat Disorders **13**: 8-8.

Kelsch, W., C.-W. Lin and C. Lois (2008). "Sequential development of synapses in dendritic domains during adult neurogenesis." Proceedings of the National Academy of Sciences **105**(43): 16803-16808.

Kelsch, W., C. W. Lin and C. Lois (2008). "Sequential development of synapses in dendritic domains during adult neurogenesis." Proc. Natl. Acad. Sci. U. S. A. **105**(43): 16803-16808.

Kelsch, W., C. W. Lin, C. P. Mosley and C. Lois (2009). "A Critical Period for Activity-Dependent Synaptic Development during Olfactory Bulb Adult Neurogenesis." J Neurosci **29**(38): 11852-11858.

Keverne, E. B. (2004). "Importance of olfactory and vomeronasal systems for male sexual function." Physiology & Behavior **83**(2): 177-187.

Kiselycznyk, C. L., S. Zhang and C. Linster (2006). "Role of centrifugal projections to the olfactory bulb in olfactory processing." Learn Mem. **13**: 575-579.

Klingler, E. (2017). "Development and Organization of the Evolutionarily Conserved Three-Layered Olfactory Cortex." eneuro **4**(1).

Kole, A. J., R. P. Annis and M. Deshmukh (2013). "Mature neurons: equipped for survival." Cell Death &Amp; Disease **4**: e689.

Kornack, D. R. and P. Rakic (1999). "Continuation of neurogenesis in the hippocampus of the adult macaque monkey." Proc. Natl. Acad. Sci. U. S. A. **96**(10): 5768-5773.

Kornack, D. R. and P. Rakic (2001). "Cell Proliferation Without Neurogenesis in Adult Primate Neocortex." Science **294**(5549): 2127-2130.

Koulakov, A. A. and D. Rinberg (2011). "Sparse Incomplete Representations: A Potential Role of Olfactory Granule Cells." Neuron **72**: 124-136.

Kuchibhotla, K. V., J. V. Gill, G. W. Lindsay, E. S. Papadoyannis, R. E. Field, T. A. Sten, K. D. Miller and R. C. Froemke (2017). "Parallel processing by cortical inhibition enables context-dependent behavior." Nat Neurosci **10**(1): 62-71.

Kuhn, H. G., H. Dickinson-Anson and F. H. Gage (1996). "Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation." The Journal of Neuroscience **16**(6): 2027.

Lazarini, F. and P.-M. Lledo (2011). "Is adult neurogenesis essential for olfaction?" Trends in Neurosciences **34**(1): 20-30.

Lazarini, F., M. A. Mouthon, G. Gheusi, F. de Chaumont, J. C. Olivo-Marin, S. Lamarque, D. N. Abrous, F. D. Boussin and P. M. Lledo (2009). "Cellular and behavioral effects of cranial irradiation of the subventricular zone in adult mice." PLoS One **4**(9): e7017.

Le Vay, S., T. N. Wiesel and D. H. Hubel (1980). "The development of ocular dominance columns in normal and visually deprived monkeys." J Comp Neurol **191**(1): 1-51.

LeDoux, J. (2003). "The emotional brain, fear, and the amygdala." Cell Mol Neurobiol **23**(4-5): 727-738.

Lee, C. C. and J. C. Middlebrooks (2011). "Auditory cortex spatial sensitivity sharpens during task performance." Nat Neurosci **14**(1): 108-114.

Lemasson, M., A. Saghatelyan, J. C. Olivo-Marin and P. M. Lledo (2005). "Neonatal and Adult Neurogenesis Provide Two Distinct Populations of Newborn Neurons to the Mouse Olfactory Bulb." J Neurosci **25**(29): 6816-6825.

Lepousez, G., A. Nissant, A. K. Bryant, G. Gheusi, C. A. Greer and P. M. Lledo (2014). "Olfactory learning promotes input-specific synaptic plasticity in adult-born neurons." Proc. Natl. Acad. Sci. U. S. A. **111**: 13984-13989.

Leutgeb, J. K., S. Leutgeb, M.-B. Moser and E. I. Moser (2007). "Pattern Separation in the Dentate Gyrus and CA3 of the Hippocampus." Science **315**(5814): 961-966.

Li, A., D. H. Gire and D. Restrepo (2015). "ϒ Spike-Field Coherence in a Population of Olfactory Bulb Neurons Differentiates between Odors Irrespective of Associated Outcome." The Journal of Neuroscience **35**(14): 5808.

Lin, C.-W., S. Sim, A. Ainsworth, M. Okada, W. Kelsch and C. Lois (2010). "Genetically Increased Cell-Intrinsic Excitability Enhances Neuronal Integration into Adult Brain Circuits." Neuron **65**(1): 32-39.

Linster, C., P. A. Garcia, M. E. Hasselmo and M. G. Baxter (2001). "Selective loss of cholinergic neurons projecting to the olfactory system increases perceptual generalization between similar, but not dissimilar, odorants. ." Behav Neurosci **115**(4): 826-833.

Liu, H.-L., S.-F. Liu, Y.-J. Ge, J. Liu, X.-Y. Wang, L.-P. Xie, R.-Q. Zhang and Z. Wang (2007). "Identification and Characterization of a Biomineralization Related Gene PFMG1 Highly Expressed in the Mantle of Pinctada fucata." Biochemistry **46**(3): 844-851.

Liu, X., Q. Wang, T. F. Haydar and A. Bordey (2005). "Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors." Nature Neuroscience **8**: 1179.

Livneh, Y., Y. Adam and A. Mizrahi (2014). "Odor processing by adult-born neurons." Neuron **81**(5): 1097-1110.

Lledo, P. M., M. Alonso and M. S. Grubb (2006). "Adult neurogenesis and functional plasticity in neuronal circuits." Nat Rev Neurosci **7**(3): 179-193.

Lois, C. and A. Alvarez-Buylla (1994). "Long-distance neuronal migration in the adult mammalian brain." Science **264**(5162): 1145-1148.

Lois, C. and A. Alvarez-Buylla (1994). "Long-distance neuronal migration in the adult mammalian brain." Science **264**(5162): 1145-1148.

Lois, C., J.-M. García-Verdugo and A. Alvarez-Buylla (1996). "Chain Migration of Neuronal Precursors." Science **271**(5251): 978-981.

Luo, M. and L. C. Katz (2001). "Response Correlation Maps of Neurons in the Mammalian Olfactory Bulb." Neuron **32**(6): 1165-1179.

Luo, S. X. and E. J. Huang (2016). "Dopaminergic Neurons and Brain Reward Pathways: From Neurogenesis to Circuit Assembly." The American Journal of Pathology **186**(3): 478-488.

Ma, M. and M. Luo (2012). "Optogenetic activation of basal forebrain cholinergic neurons modulates neuronal excitability and sensory responses in the main olfactory bulb." J Neurosci **32**(30): 10105-10116.

Malnic, B., J. Hirono, T. Sato and L. B. Buck (1999). "Combinatorial Receptor Codes for Odors." Cell **96**(5): 713-723.

Mandairon, N. and C. Linster (2009). "Odor Perception and Olfactory Bulb Plasticity in Adult Mammals." Journal of Neurophysiology **101**(5): 2204.

Mandairon, N., C. Stack, C. Kiselycznyk and C. Linster (2006). "Broad activation of the olfactory bulb produces long-lasting changes in odor perception." Proceedings of the National Academy of Sciences **103**(36): 13543-13548.

Mandairon, N., C. Stack and C. Linster (2006). "Olfactory enrichment improves the recognition of individual components in mixtures." Physiology & Behavior **89**(3): 379-384.

Markopoulos, F., D. Rokni, D. H. Gire and V. N. Murthy (2012). "Functional Properties of Cortical Feedback Projections to the Olfactory Bulb." Neuron **76**(6): 1175-1188.

Martin, C., R. Gervais, P. Chabaud, B. Messaoudi and N. Ravel (2004). "Learning-induced modulation of oscillatory activities in the mammalian olfactory system: The role of the centrifugal fibres." Journal of Physiology-Paris **98**(4): 467-478.

Martinez, M. C., J. Blanco, B. M. M and A. F. J (1987). "Structure of the piriform cortex of the adult rat. A Golgi study." J Himforsch **28**(3): 341-348.

Michalopoulos, G. K. and M. C. DeFrances (1997). "Liver Regeneration." Science **276**(5309): 60-66.

Ming, G. L. and H. Song (2011). "Adult neurogenesis in the mammalian brain: significant answers and significant questions." Neuron **70**(4): 687-702.

Mizrahi, A. (2007). "Dendritic development and plasticity of adult-born neurons in the mouse olfactory bulb." Nat Neurosci **10**: 444-452.

Mladinic, M., K. J. Muller and J. G. Nicholls (2009). "Central nervous system regeneration: from leech to opossum." J Physiol **587**(12): 2775-2782.

Mombaerts, P., F. Wang, C. Dulac, S. K. Chao, A. Nemes, M. Mendelsohn, J. Edmondson and R. Axel (1996). "Visualizing an Olfactory Sensory Map." Cell **87**(4): 675-686.

Moreno, M. M., K. Bath, N. Kuczewski, J. Sacquet, A. Didier and N. Mandairon (2012). "Action of the Noradrenergic System on Adult-Born Cells Is Required for Olfactory Learning in Mice." J Neurosci **32**(11): 3748-3758.

Moreno, M. M., C. Linster, O. Escanilla, J. Sacquet, A. Didier and N. Mandairon (2009). "Olfactory perceptual learning requires adult neurogenesis." Proc. Natl. Acad. Sci. U. S. A. **106**(42): 17980-17985.

Morris, R. G. M. (1999). "D.O. Hebb: The Organization of Behavior, Wiley: New York; 1949." Brain Research Bulletin **50**(5): 437.

Mouret, A., G. Gheusi, M. M. Gabellec, F. de Chaumont, J. C. Olivo-Marin and P. M. Lledo (2008). "Learning and survival of newly generated neurons: when time matters." J Neurosci **28**(45): 11511-11516.

Nguyen, L., B. Malgrange, I. Breuskin, L. Bettendorff, G. Moonen, S. Belachew and J.-M. Rigo (2003). "Autocrine/Paracrine Activation of the GABA_A Receptor Inhibits the Proliferation of Neurogenic Polysialylated Neural Cell Adhesion Molecule-Positive (PSA-NCAM⁺) Precursor Cells from Postnatal Striatum." The Journal of Neuroscience **23**(8): 3278.

Nissant, A., C. Bardy, H. Katagiri, K. Murray and P. M. Lledo (2009). "Adult neurogenesis promotes synaptic plasticity in the olfactory bulb." Nat Neurosci **12**(6): 728-730.

Nissant, A. and M. Pallotto (2011). "Integration and maturation of newborn neurons in the adult olfactory bulb - from synapses to function." Euro J Neuro **33**(6): 1069-1077.

Nordin, S., E. Hedén Blomqvist, P. Olsson, P. Stjärne and A. Ehnhage (2011). "Effects of smell loss on daily life and adopted coping strategies in patients with nasal polyposis with asthma." Acta Oto-Laryngologica **131**(8): 826-832.

Nunez-Parra, A., R. K. Maurer, K. Krahe, R. S. Smith and R. C. Araneda (2013). "Disruption of centrifugal inhibition to olfactory bulb granule cells impairs olfactory discrimination." Proc. Natl. Acad. Sci. U. S. A. **110**(36): 14777-14782.

Odelberg, S. J. (2004). "Unraveling the Molecular Basis for Regenerative Cellular Plasticity." PLOS Biology **2**(8): e232.

Otazu, G. H., H. Chae, M. B. Davis and D. F. Albeanu (2015). "Cortical Feedback Decorrelates Olfactory Bulb Output in Awake Mice." Neuron **86**(6): 1461-1477.

Otazu, G. H., L. H. Tai, Y. Yang and A. M. Zador (2009). "Engaging in an auditory task suppresses responses in auditory cortex." Nat Neurosci **12**(5): 646-654.

Palmer, T. D., J. Takahashi and F. H. Gage (1997). "The Adult Rat Hippocampus Contains Primordial Neural Stem Cells." Molecular and Cellular Neuroscience **8**(6): 389-404.

Panzanelli, P., C. Bardy, A. Nissant, M. Pallotto, M. Sassoè-Pognetto, P.-M. Lledo and J.-M. Fritschy (2009). "Early Synapse Formation in Developing Interneurons of the Adult Olfactory Bulb." The Journal of Neuroscience **29**(48): 15039.

Paridaen, J. T. M. L. and W. B. Huttner (2014). "Neurogenesis during development of the vertebrate central nervous system." EMBO Reports **15**(4): 351-364.

Paton, J. and F. Nottebohm (1984). "Neurons generated in the adult brain are recruited into functional circuits." Science **225**(4666): 1046-1048.

Peters, A. J., J. Lee, N. G. Hedrick, K. O'Neil and T. Komiyama (2017). "Reorganization of corticospinal output during motor learning." Nature Neuroscience **20**: 1133.

Petreanu, L. and A. Alvarez-Buylla (2002). "Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction." J Neurosci **22**(14): 6106-6113.

Platel, J.-C., K. A. Dave, V. Gordon, B. Lacar, M. E. Rubio and A. Bordey (2010). "NMDA Receptors Activated by Subventricular Zone Astrocytic Glutamate Are Critical for Neuroblast Survival Prior to Entering a Synaptic Network." Neuron **65**(6): 859-872.

Platel, J.-C., S. Stamboulian, I. Nguyen and A. Bordey (2010). "Neurotransmitter signaling in postnatal neurogenesis: The first leg." Brain Research Reviews **63**(1): 60-71.

Platel, J. C., T. Heintz, S. Young, V. Gordon and A. Bordey (2008). "Tonic activation of GLUK5 kainate receptors decreases neuroblast migration in whole-mounts of the subventricular zone." J Physiol **586**: 3783-3793.

Platel, J. C., B. Lacar and A. Bordey (2007). "GABA and glutamate signaling: homeostatic control of adult forebrain neurogenesis." J Mol Histol **38**(6): 602-610.

Price, J. L. and T. P. S. Powell (1970). "The Synaptology of the Granule Cells of the Olfactory Bulb." Journal of Cell Science **7**(1): 125.

Quast, K. B., K. Ung, E. Froudarakis, L. Huang, I. Herman, A. P. Addison, J. Ortiz-Guzman, K. Cordiner, P. Saggau, A. S. Tolias and B. R. Arenkiel (2016). "Developmental broadening of inhibitory sensory maps." Nat Neurosci **20**: 189-189.

Rall, W., G. M. Shepherd, T. S. Reese and M. W. Brightman (1966). "Dendro-dendritic synaptic pathway for inhibition in the olfactory bulb." Experimental Neurology **14**(1): 44-56.

Rall, W., G. M. Shepherd, T. S. Reese and M. W. Brightman (1966). "Dendro-dendritic synaptic pathway for inhibition in the olfactory bulb." Exp Neurol **14**(1): 44-56.

Ramon y Cajal, S. (1890). "Origen y Terminación de las Fibras Nerviosas Olfatorias." Bacelona:GacSan: 1-21.

Reddien, P. W. and A. S. Alvarado (2004). "Fundamentals of planarian regeneration." Annu Rev Cell Dev Biol **20**: 725-757.

Restrepo, D., J. Arellano, A. M. Oliva, M. L. Schaefer and W. Lin (2004). "Emerging views on the distinct but related roles of the main and accessory olfactory systems in responsiveness to chemosensory signals in mice." Hormones and Behavior **46**(3): 247-256.

Resulaj, A. and D. Rinberg (2015). "Novel Behavioral Paradigm Reveals Lower Temporal Limits on Mouse Olfactory Decisions." J Neurosci **35**(33): 11667-11673.

Reynolds, B. and S. Weiss (1992). "Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system." Science **255**(5052): 1707-1710.

Richards, L. J., T. J. Kilpatrick and P. F. Bartlett (1992). "De novo generation of neuronal cells from the adult mouse brain." Proceedings of the National Academy of Sciences of the United States of America **89**(18): 8591-8595.

Rinberg, D., A. Koulakov and A. Gelperin (2006). "Speed-Accuracy Tradeoff in Olfaction." Neuron **51**: 351-358.

Rochefort, C., G. Gheusi, J. D. Vincent and P. M. Lledo (2002). "Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory." J Neurosci **22**(7): 2679-2689.

Rothermel, M., R. M. Carey, A. Puche, M. T. Shipley and M. Wachowiak (2014). "Cholinergic inputs from Basal forebrain add an excitatory bias to odor coding in the olfactory bulb." J Neurosci **34**(13): 4654-4664.

Rothermel, M. and M. Wachowiak (2014). "Functional imaging of cortical feedback projections to the olfactory bulb." Front Neural Circuits **8**(73).

Sachse, S. and C. G. Galizia (2002). "Role of Inhibition for Temporal and Spatial Odor Representation in Olfactory Output Neurons: A Calcium Imaging Study." Journal of Neurophysiology **87**(2): 1106.

Sahay, A., Donald A. Wilson and R. Hen (2011). "Pattern Separation: A Common Function for New Neurons in Hippocampus and Olfactory Bulb." Neuron **70**(4): 582-588.

Sailor, K. A., M. T. Valley, M. T. Wiechert, H. Riecke, G. J. Sun, W. Adams, J. C. Dennis, S. Sharafi, G. l. Ming, H. Song and P. M. Lledo (2016). "Persistent Structural Plasticity Optimizes Sensory Information Processing in the Olfactory Bulb." Neuron **91**: 384-396.

Sakamoto, M., N. Leki, G. Miyoshi, D. Mochimaru, H. Miyachi, T. Imura, M. Yamaguchi, G. Fishell, K. Mori, R. Kageyama and I. Imayoshi (2014). "Continuous postnatal neurogenesis contributes to formation of the olfactory bulb neural circuits and flexible olfactory associative learning." J Neurosci **34**(17): 5788-5799.

Sanai, N., M. S. Berger, J. M. Garcia-Verdugo and A. Alvarez-Buylla (2007). "Comment on "Human Neuroblasts Migrate to the Olfactory Bulb via a Lateral Ventricular Extension"." Science **318**(5849): 393-393.

Sanai, N., T. Nguyen, R. A. Ihrie, Z. Mirzadeh, H.-H. Tsai, M. Wong, N. Gupta, M. S. Berger, E. Huang, J.-M. Garcia-Verdugo, D. H. Rowitch and A. Alvarez-Buylla (2011). "Corridors of Migrating Neurons in Human Brain and Their Decline during Infancy." Nature **478**(7369): 382-386.

Sanai, N., A. D. Tramontin, A. Quiñones-Hinojosa, N. M. Barbaro, N. Gupta, S. Kunwar, M. T. Lawton, M. W. McDermott, A. T. Parsa, J. Manuel-García Verdugo, M. S. Berger and A. Alvarez-Buylla (2004). "Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration." Nature **427**: 740.

Schoppa, N. E. and N. N. Urban (2003). "Dendritic processing within olfactory bulb circuits." Trends in Neurosciences **26**(9): 501-506.

Schultz, E. F. and J. T. Tapp (1973). "Olfactory control of behavior in rodents." Psychological Bulletin **79**(1): 21-44.

Schultz, W. (2015). "Neuronal Reward and Decision Signals: From Theories to Data." Physiological Reviews **95**(3): 853-951.

Schwob, J. E. and J. L. Price (1984). "The development of axonal connections in the central olfactory system of rats." J Comp Neurol **223**(2): 177-202.

Scott, J. W., R. L. McBride and S. P. Schneider (1980). "The organization of projections from the olfactory bulb to the piriform cortex and olfactory tubercle in the rat." J Comp Neurol **194**(3): 519-534.

Shepherd, G. M. (1963). "Responses of mitral cells to olfactory nerve volleys in the rabbit." J Physiol **168**(1): 101-117.

Shepherd, G. M. (2004). "The synaptic organization of the brain." Oxford University Press, New York.

Shepherd, G. M. (2005). "Perception without a Thalamus: How Does Olfaction Do It?" Neuron **46**(2): 166-168.

Shepherd, G. M., W. R. Chen and G. C. A (2004). "Olfactory bulb. In the synaptic organization of the brain, 5th edn. Shepherd, G.M." Oxford University Press, New York **165-216**.

Shipley, M. T., F. J. Halloran and J. de la Torre (1985). "Surprisingly rich projection from locus coeruleus to the olfactory bulb in the rat." Brain Res **329**(1-2): 294-299.

Snyder, J. S., A. Soumier, M. Brewer, J. Pickel and H. A. Cameron (2011). "Adult hippocampal neurogenesis buffers stress responses and depressive behaviour." Nature **476**(7361): 458-461.

Sofroniew, N. J., D. Flickinger, J. King and K. Svoboda (2016). "A large field of view two-photon mesoscope with subcellular resolution for in vivo imaging." eLife **5**: e14472.

Temmel, A. P., C. Quint, B. Schickinger-Fischer, L. Klimek, E. Stoller and T. Hummel (2002). "Characteristics of olfactory disorders in relation to major causes of olfactory loss." Archives of Otolaryngology–Head & Neck Surgery **128**(6): 635-641.

Uchida, N. and Z. F. Mainen (2003). "Speed and accuracy of olfactory discrimination in the rat." Nat Neurosci **6**: 1224-1229.

Urban, N. N. and B. Sakmann (2002). "Reciprocal intraglomerular excitation and intra- and interglomerular lateral inhibition between mouse olfactory bulb mitral cells." J Physiol **542**(2): 355-367.

Wachowiak, M. (2011). "All in a Sniff: Olfaction as a Model for Active Sensing." Neuron **71**: 962-973.

Wachowiak, M. and L. B. Cohen (1999). "Presynaptic Inhibition of Primary Olfactory Afferents Mediated by Different Mechanisms in Lobster and Turtle." The Journal of Neuroscience **19**(20): 8808.

Wachowiak, M., M. N. Economo, M. Díaz-Quesada, D. Brunert, D. W. Wesson, J. A. White and M. Rothermel (2013). "Optical dissection of odor information processing in vivo using GCaMPs expressed in specified cell types of the olfactory bulb." J Neurosci **33**(12): 5285-5300.

Wang, D. D., D. D. Krueger and A. Bordey (2003). "GABA depolarizes neuronal progenitors of the postnatal subventricular zone via GABAa receptor activation." J Physiol **550**(3): 785-800.

Wellis, D. P. and J. W. Scott (1990). "Intracellular responses of identified rat olfactory bulb interneurons to electrical and odor stimulation." Journal of Neurophysiology **64**(3): 932.

Whitman, M. C. and C. A. Greer (2007). "Synaptic Integration of Adult-Generated Olfactory Bulb Granule Cells: Basal Axodendritic Centrifugal Input Precedes Apical Dendro-dendritic Local Circuits." The Journal of Neuroscience **27**(37): 9951.

Whitman, M. C. and C. A. Greer (2007). "Synaptic integration of adult-generated olfactory bulb granule cells: basal axodendritic centrifugal input precedes apical dendro-dendritic local circuits." J. Neurosci. **27**(37): 9951-9961.

Wilson, D. A. (2009). "Pattern Separation and Completion in Olfaction." Annals of the New York Academy of Sciences **1170**: 306-312.

Wilson, D. A. and R. J. Stevenson (2003). "Olfactory perceptual learning: the critical role of memory in odor discrimination." Neuroscience & Biobehavioral Reviews **27**(4): 307-328.

Wilson, D. A. and R. M. Sullivan (1995). "The D2 antagonist spiperone mimics the effects of olfactory deprivation on mitral/tufted cell odor response patterns." The Journal of Neuroscience **15**(8): 5574.

Winner, B., C. M. Cooper-Kuhn, R. Aigner, J. Winkler and H. G. Kuhn (2002). "Long-term survival and cell death of newly generated neurons in the adult rat olfactory bulb." European Journal of Neuroscience **16**(9): 1681-1689.

Xu, F., C. A. Greer and G. M. Shepherd (2000). "Odor maps in the olfactory bulb." J Comp Neurol **422**(4): 489-495.

Yamada, Y., K. Bhaukaurally, T. J. Madarász, A. Pouget, I. Rodriguez and A. Carleton (2017). "Context- and Output Layer-Dependent Long-Term Ensemble Plasticity in a Sensory Circuit." Neuron: 1-15.

Yamaguchi, M. and K. Mori (2005). "Critical period for sensory experience-dependent survival of newly generated granule cells in the adult mouse olfactory bulb." Proc. Natl. Acad. Sci. U. S. A. **102**(27): 9697-9702.

Yang, C. F., M. C. Chiang, D. C. Gray, M. Prabhakaran, M. Alvarado, S. A. Juntti, E. K. Unger, J. A. Wells and N. M. Shah (2013). "Sexually dimorphic neurons in the ventromedial hypothalamus govern mating in both sexes and aggression in males." Cell **153**(4): 896-909.

Yohn, C. N., M. M. Gergues and B. A. Samuels (2017). "The role of 5-HT receptors in depression." Molecular Brain **10**(1): 28.

Yokoi, M., K. Mori and S. Nakanishi (1995). "Refinement of odor molecule tuning by dendro-dendritic synaptic inhibition in the olfactory bulb." Proc. Natl. Acad. Sci. U. S. A. **92**(8): 3371-3375.

Yu, Y., M. Migliore, M. L. Hines and G. M. Shepherd (2014). "Sparse Coding and Lateral Inhibition Arising from Balanced and Unbalanced Dendro-dendritic Excitation and Inhibition." J Neurosci **34**(41): 13701-13713.

Zariwala, H. A., A. Kepecs, N. Uchida, J. Hirokawa and Z. F. Mainen (2013). "The limits of deliberation in a perceptual decision task." Neuron **78**(2): 339-351.

Zhang, X. and S. Firestein (2002). "The olfactory receptor gene superfamily of the mouse." Nature Neuroscience **5**: 124.

Zhao, C., W. Deng and F. H. Gage (2008). "Mechanisms and Functional Implications of Adult Neurogenesis." Cell **132**(4): 645-660.

Zucker, R. S. and W. G. Regehr (2002). "Short-Term Synaptic Plasticity." Annual Review of Physiology **64**(1): 355-405.

Chapter 3. What is next?

"The more I learn, the more I realize how much I don't know" (Albert Einstein). The findings of my study in Chapter 2 reveal the special importance of ABNs in difficult discrimination, as well as the modulation of MC populations mainly through inhibitory inputs of these cells in a behavior state dependent manner. However, these lead to more questions yet to be answered, especially the detailed mechanisms of circuitry regulation and state dependency.

Olfactory perception

In this study, we used odor mixtures of heptanal and ethyl-tiglate for both easy and difficult discrimination tasks. Analogously, purple is a mix of blue and red; but when we perceive only purple, we see a new color and cannot parse it out into the two components unless we are told so. When exposed to a mixture of several smells, we may perceive something completely different from any of the components. Is this phenomenon reflected in the odor representation in the OB for mice? How dissimilar is it for mixtures from each pure component? Further, does whether or not young ABNs exist bring a difference? For instance, chemical A can activate a certain group of glomeruli, and then the corresponding MCs. Now another chemical B is mixed in, and two possible scenarios can happen: either B-evoked MCs are non-overlapping with A-evoked ones (linear summation), or B takes up some MC activity resources from A (non-linear transformation). With the two possibilities, the question regarding the role of ABNs would become: would ABNs facilitate perception of mixtures towards recognizing individual components

(perceptual stability), or towards forming a new stimulus for which the representation is highly distinguishable from linear summation of separate components (perceptual discrimination)? In the recent years, studies on related topics, such as olfactory pattern separation vs. completion, have been done (Wilson 2009, Chapuis and Wilson 2011), but research specifically on the impact of adult neurogenesis is still lacking.

The role of ABNs on MC pattern separation

As mentioned in Chapter 1 Introduction, the only known type of outputs from ABNs is GABAergic inhibition onto TC/MCs through dendro-dendritic contacts. Our study showed that, with the presence of young ABNs, MCs experienced more suppression during difficult discrimination, which is in accordance with the property of ABNs being inhibitory neurons. With the population quantification done, in-depth single-cell analysis could provide us more insights on how ABNs work in this regulation-through-inhibition process. If the directionality (excitation or suppression) of MCs is largely stable, that implies new inhibitory synapse formation and/or strengthening of existing inputs from young ABNs quickly occur when the task is switched from easy to difficult discrimination; otherwise, the inhibitory regulation of ABNs is rather dynamic and global instead of TC/MC-selective. Biphasic cells are interesting too. A small fraction of MCs would switch response directionality during odor delivery period. These neurons can give us some information on the temporal characteristics of the function of ABNs. Also, what gain does the system have from

these neurons that send mixed signals? Would it be possible that the switching was caused by some kind of real-time feedback from olfactory cortical area?

During the study, we saw the value of acute ablation, or manipulation in general, of young ABNs. This direction has led to some recent tempts (Alonso, Lepousez et al. 2012). Acute ablation can help explain some findings that might be caused by compensatory mechanisms. For example, we were surprised to find that the amount of MC excitatory responses did not change in ABN ablation animals although inhibitory responses went down. Is it because the genetic ablation was chronic (within the span of ~ 2 months), and as a result, the circuitry compensates for the loss and brings the excitability back to normal? If we acutely silence young ABNs, would the fraction of MC excitation responses go up, as we intuitively expect? More importantly, with the use of chronic genetic ablation method, we had to prove that the effect was ABN-specific by removing comparable percentage of GCs (Figure 2.3). With acute and reversible manipulation, ABNs are still alive and integrated into the circuit, and their role can be observed in a more controlled and temporally accurate manner.

The state dependency of ABN functions

This part is rather complicated, multi-facet and our study on it has quite a component of postulation. There are four major reasons that cause the difficulty to study: 1. "Brain state" itself is a poorly-defined concept, and in reality, the brain state of an animal can fluctuate from moment to moment, even during the so-called "awake-ness"; 2. With the assumption that brain states can be represented by the

overall brain activity level to some degree, the ABNs receive centrifugal inputs from multiple brain areas (see Chapter 1 and Chapter 2 introduction), and, without knowing the extent of redundancy or compensation among these areas, it is difficult to capture the mechanism of this "brain state dependency" once and for all; 3. The centrifugal projection is not only of multi-area, but also onto a variety of receptor types, such as GABAergic, glutamatergic, cholinergic, norepinephrinergic, and so on (see Chapter 1 and Chapter 2 introduction); 4. In order to make any claim ABN-specific, the study has to ensure a high specificity AND high efficiency of labeling regarding young ABNs, which is technically challenging, whether it is retrograde labeling of projection neurons, or tagging of certain types of receptors.

Therefore, within the scope of our study, we only had the opportunity to compare two behavioral states - active engagement and passive exposure, and through the end result of the difference in MC population activity pattern, postulate the influence of brain states onto the role of adult neurogenesis. Eventually, it would be important to follow up on exactly what brain areas and which input types are the major contributors for the enhanced MC suppressive responses during active engagement. Despite the multitude of obstacles described above, efforts can still be made to manipulate certain brain areas or certain types of receptors and compare the results. There have been studies regarding OB inhibitory interneurons in general (Choi, Stettler et al. 2011, Boyd, Sturgill et al. 2012, Root, Denny et al. 2014, Otazu, Chae et al. 2015), but not so much for ABNs specifically.

Direct observation of ABNs

Ever since the discovery of functional properties and potential uniqueness/importance of ABNs, it has been a dream experiment to record (ideally, a population of) ABN activities in vivo. It is a "dream" because the difficulty to put to reality. First of all, tools for *in vivo* population recording, such as multi-electrode or multi-photon recording, have not existed for long. Considering GCs with an age within the critical period consists ~15% of all GCs (Imayoshi, Sakamoto et al. 2008), the efficiency of blind multi-electrode recording is rather low. Multi-photon recording combined with viral or genetic labeling seems to be the better option, however, since young ABNs reside deeper in the GCL, the visibility of ABN soma under multi-photon microscope is an issue. Secondly, to record a population of ABNs simultaneously using microscope, the efficiency of labeling has to be high. Efficiency is even more demanded when it comes to manipulation, since the effect of a small fraction of total ABNs may be small and buried inside the noise. Despite the challenges, it is exciting to see that the field has been making an active effort to make *in vivo* population recording happen (Livneh, Adam et al. 2014, Quast, Ung et al. 2016).

Single cell recording has revealed physiological and morphological characteristics of young ABNs (see Chapter 1). Once we achieve satisfactory density and specificity of ABN population labeling for imaging, we would want to focus more on population characteristics: How diverse are their spontaneous responses, as well as odor-evoked responses? If they are diverse, can we categorize them? What are the trends of changes in response patterns as the ABNs age? Ultimately, the "dream experiment" would be labeling and recording MC and

ABN ensembles, or centrifugal fibers and ABNs, within the same circuitry. And then we can record both populations, or manipulate one while recording the other in a temporally sensitive and reversible fashion. That way we can know how exactly ABNs interact and influence other players in the circuitry. Studies have claimed that rodents only need a few hundreds of milliseconds to make a decision in an olfactory discrimination task (Uchida and Mainen 2003, Rinberg, Koulakov et al. 2006, Wachowiak 2011, Zariwala, Kepecs et al. 2013), would enhancing or decreasing ABN activity in the initial period of odor delivery sufficient to improve or impair MC ensemble decoding accuracy? I tend to assume that ABNs bring an impact when they migrate shallower in the OB and are morphologically and physiologically more mature (4-8 weeks post birth), but do they have a role in the circuitry at the early stage of migration and development too?

Clinical application

Since we are studying ABNs derived from stem cells millimeters apart in the SVZ at the final stages of their differentiation, it is not intuitive to associate with the focus with clinical applications. However, it is important to know the normal morphology and behavior of stem cell products in their destined environment, and our technique would allow the possibility of setting such a standard. With this standard, researchers would be able to look into the effect of different factors on adult neurogenesis, such as drugs, learning, etc., which would ultimately be beneficial for stem cell therapy.

References

Alonso, M., G. Lepousez, S. Wagner, C. Bardy, M.-M. Gabellec, N. Torquet and P.-M. Lledo (2012). "Activation of adult-born neurons facilitates learning and memory." Nature Neuroscience **15**: 897.

Boyd, Alison M., James F. Sturgill, C. Poo and Jeffry S. Isaacson (2012). "Cortical Feedback Control of Olfactory Bulb Circuits." Neuron **76**(6): 1161-1174.

Chapuis, J. and D. A. Wilson (2011). "Bidirectional plasticity of cortical pattern recognition and behavioral sensory acuity." Nature Neuroscience **15**: 155.

Choi, G. B., D. D. Stettler, B. R. Kallman, S. T. Bhaskar, A. Fleischmann and R. Axel (2011). "Driving Opposing Behaviors with Ensembles of Piriform Neurons." Cell **146**(6): 1004-1015.

Imayoshi, I., M. Sakamoto, T. Ohtsuka, K. Takao, T. Miyakawa, M. Yamaguchi, K. Mori, T. Ikeda, S. Itohara and R. Kageyama (2008). Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain.

Livneh, Y., Y. Adam and A. Mizrahi (2014). "Odor Processing by Adult-Born Neurons." Neuron **81**(5): 1097-1110.

Otazu, Gonzalo H., H. Chae, Martin B. Davis and Dinu F. Albeanu (2015). "Cortical Feedback Decorrelates Olfactory Bulb Output in Awake Mice." Neuron **86**(6): 1461-1477.

Quast, K. B., K. Ung, E. Froudarakis, L. Huang, I. Herman, A. P. Addison, J. Ortiz-Guzman, K. Cordiner, P. Saggau, A. S. Tolias and B. R. Arenkiel (2016). "Developmental broadening of inhibitory sensory maps." Nature Neuroscience **20**: 189.

Rinberg, D., A. Koulakov and A. Gelperin (2006). "Speed-Accuracy Tradeoff in Olfaction." Neuron **51**(3): 351-358.

Root, C. M., C. A. Denny, R. Hen and R. Axel (2014). "The participation of cortical amygdala in innate, odour-driven behaviour." Nature **515**: 269.

Uchida, N. and Z. F. Mainen (2003). "Speed and accuracy of olfactory discrimination in the rat." Nature Neuroscience **6**: 1224.

Wachowiak, M. (2011). "All in a Sniff: Olfaction as a Model for Active Sensing." Neuron **71**(6): 962-973.

Wilson, D. A. (2009). "Pattern Separation and Completion in Olfaction." Annals of the New York Academy of Sciences **1170**: 306-312.

Zariwala, H. A., A. Kepecs, N. Uchida, J. Hirokawa and Z. F. Mainen (2013). "The Limits of Deliberation in a Perceptual Decision Task." Neuron **78**(2): 339-351.