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REVIEW

The influence of olfactory experience on the birthrates of olfactory sensory neurons with specific odorant receptor identities

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Summary

Olfactory sensory neurons (OSNs) are one of a few neuron types that are generated continuously throughout life in mammals. The persistence of olfactory sensory neurogenesis beyond early development has long been thought to function simply to replace neurons that are lost or damaged through exposure to environmental insults. The possibility that olfactory sensory neurogenesis may also serve an adaptive function has received relatively little consideration, largely due to the assumption that the generation of new OSNs is stochastic with respect to OSN subtype, as defined by the single odorant receptor gene that each neural precursor stochastically chooses for expression out of hundreds of possibilities. Accordingly, the relative birthrates of different OSN subtypes are predicted to be constant and impervious to olfactory experience. This assumption has been called into question, however, by evidence that the birthrates of specific OSN subtypes can be selectively altered by manipulating olfactory experience through olfactory deprivation, enrichment, and conditioning paradigms. Moreover, studies of recovery of the OSN population following injury provide further evidence that olfactory sensory neurogenesis may not be strictly stochastic with respect to subtype. Here we review this evidence and consider mechanistic and functional implications of the prospect that specific olfactory experiences can regulate olfactory sensory neurogenesis rates in a subtype-selective manner.

KEYWORDS
conditioning, deprivation, enrichment, injury, neurogenesis, olfactory, sensory

1 | INTRODUCTION

Mammals possess hundreds of distinct subtypes of olfactory sensory neurons (OSNs), each of which expresses a distinct odorant receptor (OR) and thereby detects a specific set of odorant molecules (Imai, 2022). ORs that define OSN subtypes within the mammalian main olfactory epithelium include the canonical odorant receptors, the trace amino acid receptors, and the membrane spanning, 4-pass A receptors (Bear et al., 2016). OSNs are one of only a few types of neurons within the mammalian nervous system that are generated throughout life (Brann & Firestein, 2014; Schwob et al., 2017; Yu & Wu, 2017). The other major types of adult-generated neurons, which integrate into the hippocampus and olfactory bulb, play important roles in learning and memory (Lledo & Valley, 2016; Ming & Song, 2011; Opendak & Gould, 2015). By contrast, adult-born OSNs have long been thought to function solely to replace neurons that are lost or damaged due to their direct exposure to the environment. The possibility that persistent olfactory sensory neurogenesis also serves an adaptive function has received relatively little consideration, likely due to the assumption that the generation of new OSNs is strictly

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stochastic with respect to subtype identity, as it entails the evidently stochastic process of OR choice (McClintock, 2015; Monahan & Lomvardas, 2015).

As reviewed below, studies in both rodents and humans have found both indirect and direct evidence that the representations of specific OSN subtypes within the olfactory epithelium (OE) can be altered through multiple olfactory experience manipulation paradigms under non-injury conditions. These paradigms include olfactory deprivation via unilateral naris occlusion (UNO), in which one nostril is occluded via surgery or a removable plug; olfactory enrichment, in which an individual is exposed to a single odorant or an odor mixture; and olfactory conditioning, in which an individual learns the association of either an aversive or appetitive outcome with a specific odorant. Experience-dependent changes in OSN subtype representations have long been hypothesized to be mediated solely by subtype-selective changes in OSN lifespan. However, recent studies provide evidence in support of the alternative hypothesis that some of the observed changes in subtype representations are mediated by selective alterations in the neurogenesis rates of these subtypes. Perhaps relatedly, studies in both humans and rodents provide evidence that neurogenesis that takes place during recovery from injury to OSNs may also occur in a manner that is selective with respect to OR identity. These findings appear to conflict with the established model of olfactory sensory neurogenesis, wherein each post-mitotic OSN precursor stochastically chooses for expression a single OR gene that defines the identity and function of the mature OSN (McClintock, 2015; Monahan & Lomvardas, 2015). Accordingly, olfactory sensory neurogenesis is expected to be stochastic with respect to subtype identity and unaffected by experience. Findings to the contrary suggest that this model may be incomplete. As discussed below, these findings may have important implications for understanding how olfactory sensory neurogenesis is regulated and what functions it serves.

2 | EVIDENCE THAT OLFACTORY DEPRIVATION SELECTIVELY REDUCES THE NEUROGENESIS RATES OF A FRACTION OF OSN SUBTYPES

Experiments involving the manipulation of olfactory experience through broad reductions in the level of olfactory stimulation received by the OSN population have contributed important insights into the effects of olfactory experience on olfactory sensory neurogenesis. In rodents, the effects of olfactory deprivation have been studied primarily via two approaches: genetic silencing of OSNs through the inactivation of genes encoding critical components of the signal transduction pathway [e.g., Bennett et al., 2010; Fischl et al., 2014] and the physical closure of one nostril via UNO [reviewed in Coppola, 2012; Coppola & Reisert, 2023]. In this section, we will focus on studies that have employed UNO, which has become a method of choice for investigating the effects of olfactory stimulation on dynamics within the OSN population because it is simple to perform and requires no genetic manipulations [reviewed in Coppola, 2012]. UNO is commonly performed via electrocautery, an irreversible procedure that is limited primarily to neonatal animals (Barber & Coppola, 2015; Coppola & Waggener, 2012; Santoro & Dulac, 2012; van der Linden et al., 2018; Waguespack et al., 2005). Alternatively, UNO can be implemented using nose plug insertion, a reversible procedure that can be performed on both young and adult animals (Cheetham et al., 2016; Cummings et al., 1997; Cummings & Brunjes, 1994; Kass et al., 2013). Collectively, findings from UNO-based studies indicate that the age, method, and duration of UNO treatment can affect experimental outcomes. Regardless of these parameters, UNO is thought to cause broad reductions in olfactory stimulation of the OSN population on one side of the OE, an effect that can be assessed by analyzing the levels of known activity-dependent transcripts such as *S100a5* (Fischl et al., 2014; van der Linden et al., 2020).

It is important to note that despite its common use in olfactory deprivation studies, UNO causes effects beyond a simple reduction in the stimulation of OSNs on the closed side of the OE by odors (Coppola, 2012). For example, because the technique alters airflow on both sides of the OE, it is predicted to reduce both odorous and mechanical (Grosmaitre et al., 2007) stimulation on the closed side, while causing reciprocal changes on the open side. Hence, while many UNO-based studies have employed the open side of the OE as a within-subject control for assessing olfactory deprivation-induced changes, it is conceivable that observed differences between the open and closed sides of the OE could reflect, in part, increased airflow on the open side. Many of the UNO-based studies cited in this review have addressed this possibility by including non-occluded animals as a primary or secondary control. In one such study, changes in gene expression due to UNO (via cautery) were assessed by comparing transcript profiles between the whole OEs of non-occluded mice, the open-side OEs of UNO treated mice, and the closed-side OEs of UNO-treated mice (Coppola & Waggener, 2012). Notably, while the gene expression profiles of non-occluded and open-side samples were found to cluster separately from the closed-side samples, as expected, the non-occluded and open-side samples showed no such separation. These observations led the investigators to infer that the open-side and non-occluded samples had similar transcript profiles (Coppola & Waggener, 2012). Likewise, comparisons between the open-side OEs from mice that were UNO-treated via cautery or plug insertion and the OEs of non-occluded mice have revealed only subtle differences in OSN electrical activity levels (Barber & Coppola, 2015), OSN axon refinement rates (Zou et al., 2004), OSN synaptic remodeling rates (Cheetham et al., 2016), representations of specific OSN subtypes (Molinas et al., 2016; van der Linden et al., 2020), neurogenesis rates of specific subtypes (van der Linden et al., 2020), and levels olfactory marker protein (Waguespack et al., 2005), which has been found to play roles in OSN signal transduction and axon segregation (Albeanu et al., 2018; Buiakova et al., 1996; Lee et al., 2011). On the other hand, prolonged (6 weeks or more) naris occlusion of adult mice that were UNO-treated via a combination of cautery and suture was found to cause a reduction in OSN quantities within the rostral region of the open side of the OE (Maruniak et al., 1989). Moreover, the

UNO-treatment of adult mice via plug insertion has been found to produce unexpected changes in the processing of axon signals from OSNs on both the open and closed sides of the OE compared to sham-treated controls (Kass et al., 2013). These findings underscore the importance of interpreting observed differences between the open and closed sides of the OEs of UNO-treated animals with care and, when possible, in comparison to OEs from non-occluded animals.

Previous studies have observed that UNO-induced olfactory deprivation alters the representations of specific OSN subtypes on the closed side of the OE relative to the open. Gene expression analyses of mice that were UNO-treated via electrocautery between postnatal day 0 and 14 (P0–P14), for example, have revealed bidirectional changes in the transcript levels of a fraction of OR-encoding genes, with some OR transcripts reduced and others elevated on the closed side of the OE relative to open-side and non-occluded controls (Coppola & Waggener, 2012; Fischl et al., 2014; Santoro & Dulac, 2012). In one such study, approximately 4% of the OR genes analyzed exhibited significant reductions in transcript levels on the closed side of the OE relative to the open, while approximately 8% showed significant increases in transcript levels on the closed side relative to the open (Santoro & Dulac, 2012). Subsequent analyses of these OR transcripts via RNA fluorescent in situ hybridization (RNA-FISH) revealed that UNO-altered OR transcript levels reflect changes in the representations of OSNs that express the altered ORs, indicating that olfactory deprivation can cause bidirectional changes in the representations of a fraction of OSN subtypes (Santoro & Dulac, 2012; van der Linden et al., 2020). These findings are in agreement with a separate study that observed, also using RNA-FISH, that UNO-treatment of mice at P3 via cautery has complex effects on the representations of different OSN subtypes, with some reduced, some increased, and some unaffected in their representations on the closed side relative to the open (Zhao et al., 2013).

Both reductions and elevations in the representations of individual OSN subtypes observed following olfactory deprivation were originally proposed to be mediated entirely by subtype-selective changes in OSN lifespan, reflecting shortened and lengthened lifespans, respectively (Santoro & Dulac, 2012; Zhao et al., 2013). According to this model, olfactory deprivation selectively lengthens the lifespans of OSNs that normally receive excessive odor stimulation by protecting them from over-stimulation, while selectively shortening the lifespans of subtypes that normally receive low levels of odor stimulation by reducing their activity to insufficient levels. In principle, however, altered rates of neurogenesis of specific subtypes could contribute to observed changes in the representations of specific subtypes following olfactory deprivation. However, this latter potential mechanism was disfavored based on the assumption that olfactory sensory neurogenesis is stochastic with respect to OSN subtype, since it is based on the evidently stochastic process of OR choice [reviewed in McClintock, 2015; Monahan & Lomvardas, 2015]. As such, the relative birthrates of distinct OSN subtypes would not be expected to be affected by olfactory experience.

Despite predictions to the contrary, early UNO-based studies offered hints that altered neurogenesis rates might contribute to deprivation-induced changes in the representations of OSN subtypes. Perhaps most notably, UNO treatment of neonatal rats via cautery had been found to substantially reduce the overall rate of olfactory sensory neurogenesis on the closed side of the OE relative to non-occluded controls (Farbman et al., 1988), an effect that has been replicated in multiple subsequent studies of both rats and mice that were UNO-treated via both cautery and plug insertion (Cummings & Brunjes, 1994; Mirich & Brunjes, 2001; van der Linden et al., 2020). Notably, the effect of olfactory deprivation via plug insertion on overall OSN neurogenesis rates in adult mice remains less clear, as one such study observed reductions (Suh et al., 2006) while another did not (Cheetham et al., 2016), possibly due to study-specific experimental differences. Interestingly, deprivation-induced reductions in olfactory sensory neurogenesis rates have been reported to occur with a rapid onset (~70% reduced olfactory sensory neurogenesis 3 days post-UNO via plug insertion in adult mice (Suh et al., 2006)) and persist for long periods following UNO (e.g., ~40% reduced neurogenesis 30 days post-UNO via cautery of P1 rats (Farbman et al., 1988)). Based on early studies, deprivation-induced reductions in neurogenesis were proposed to reflect a lessened need for replacement of OSNs that would normally have been damaged by exposure to environmental insults, but were protected by naris closure (Cummings & Brunjes, 1994; Farbman et al., 1988; Mirich & Brunjes, 2001). A shortcoming of this explanation, however, is that it appeared inconsistent with findings that naris closure causes no difference (Brunjes & Shurling, 2003) or even a slight increase (Suh et al., 2006) in the overall rate of OSN apoptosis following UNO of P1 rats via cautery or adult mice via plug insertion, respectively. Thus, the cause of deprivation-induced reductions in olfactory sensory neurogenesis rates remained unclear.

Observations that olfactory deprivation both reduces overall rates of olfactory sensory neurogenesis and bidirectionally alters the representations of specific OSN subtypes led to a recent study to investigate whether these two phenomena might be connected (van der Linden et al., 2020). Specifically, the investigators sought to test whether olfactory deprivation selectively decreases the neurogenesis rates of the OSN subtypes that exhibit reduced overall representations on the closed side of the OE after UNO. To do so, an approach combining OR-specific RNA-FISH and EdU-birthdating was developed to identify and quantify newborn OSNs of specific subtypes on the open and closed sides of the OEs of mice that were UNO-treated at P14 via cautery, EdU-injected at P28, and dissected at ~P35 (Hossain et al., 2023; van der Linden et al., 2020). This approach was used to assess the effects of olfactory deprivation on the quantities of newborn OSNs of each of 15 different subtypes, including five with increased overall representations, seven with decreased representations, and three with unchanged representations on the closed side of the OE relative to the open following UNO (Santoro & Dulac, 2012; van der Linden et al., 2020). Remarkably, all seven OSN subtypes that had shown decreased overall representations following olfactory deprivation also exhibited substantially (up to six-fold) reduced quantities of newborn OSNs on the closed side of the OE relative to the open. Moreover, the extent to which deprivation reduced the quantities of

newborn OSNs of each of these subtypes was found to vary between postnatal days 14 and 28, which are timepoints corresponding to nursing and weaning periods, respectively, suggesting the possibility that the effects of olfactory deprivation on newborn OSN quantities may depend on an animal's age and/or olfactory environment. By contrast, the eight OSN subtypes with increased or unchanged overall representations following olfactory deprivation showed no significant differences in newborn OSN quantities between the closed and open sides of the OE. To more globally assess the effects of olfactory deprivation on quantities of newborn OSNs across subtypes, single-cell RNA-sequencing (scRNA-seq) and translating ribosome affinity purification RNA sequencing (TRAP-seq) approaches were employed (van der Linden et al., 2020). Consistent with the findings obtained via EdU-birthdating and RNA-FISH, these analyses revealed that OSN subtypes with reduced overall representations following olfactory deprivation (as assessed by OR transcript levels) (Santoro & Dulac, 2012) also exhibited, on average, three-fold fewer newborn OSNs on the closed side of the OE compared to the open (van der Linden et al., 2020). By contrast, OSN subtypes with increased or unchanged representations following deprivation exhibited no differences in newborn OSN quantities. Collectively, these findings suggested that olfactory deprivation selectively reduces the quantities of newborn OSNs of specific subtypes and that only a fraction of subtypes are affected (Figure 1a).

In principle, reductions in the quantities of newborn OSNs of specific subtypes observed following olfactory deprivation (van der Linden et al., 2020) could be caused by either selective reductions in neurogenesis of these subtypes or, alternatively, selective reductions in the survival of newborn OSNs of these subtypes. In the case of the latter mechanism, olfactory deprivation would be expected to cause:

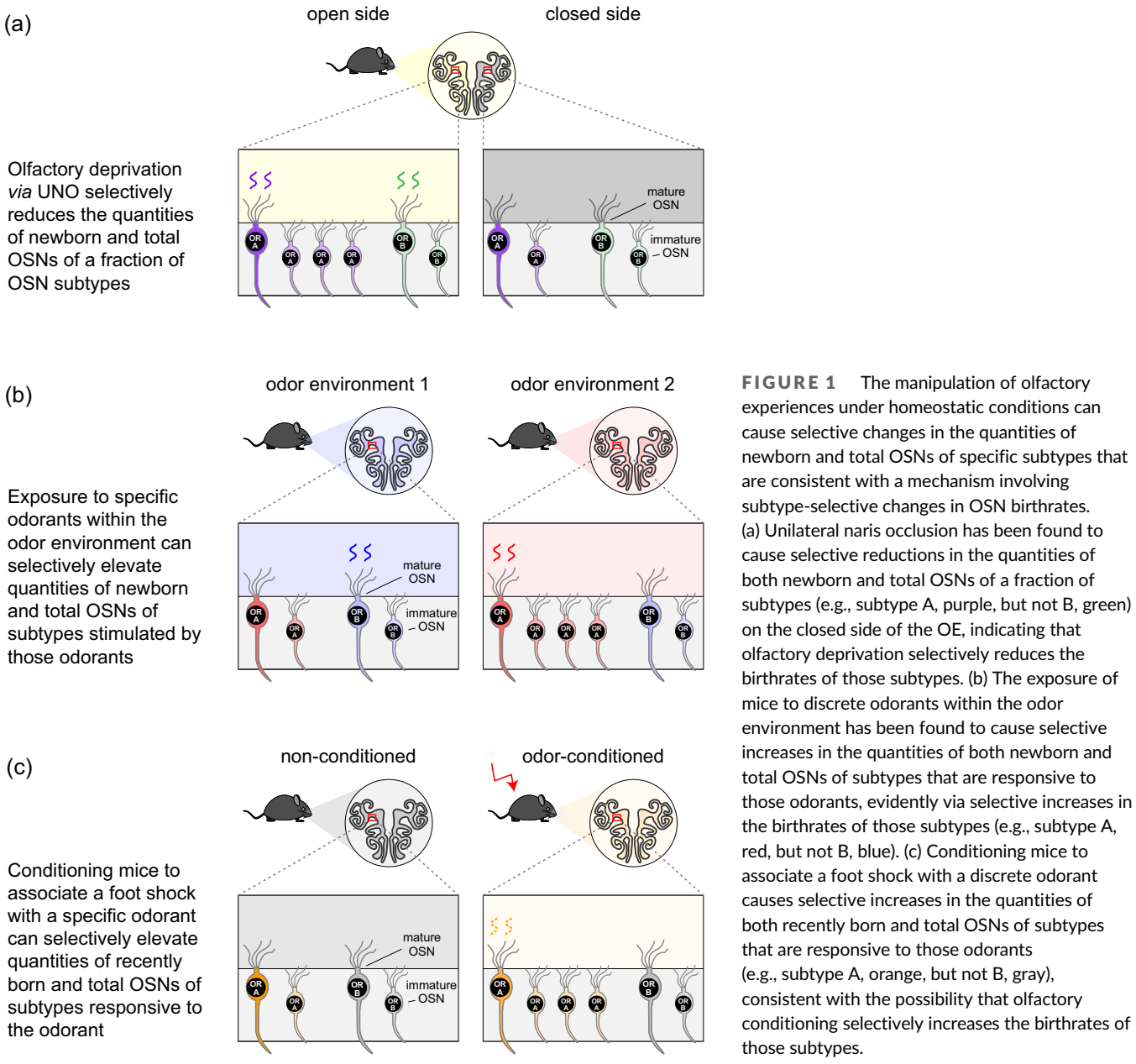

FIGURE 1 The manipulation of olfactory experiences under homeostatic conditions can cause selective changes in the quantities of newborn and total OSNs of specific subtypes that are consistent with a mechanism involving subtype-selective changes in OSN birthrates. (a) Unilateral naris occlusion has been found to cause selective reductions in the quantities of both newborn and total OSNs of a fraction of subtypes (e.g., subtype A, purple, but not B, green) on the closed side of the OE, indicating that olfactory deprivation selectively reduces the birthrates of those subtypes. (b) The exposure of mice to discrete odorants within the odor environment has been found to cause selective increases in the quantities of both newborn and total OSNs of subtypes that are responsive to those odorants, evidently via selective increases in the birthrates of those subtypes (e.g., subtype A, red, but not B, blue). (c) Conditioning mice to associate a foot shock with a discrete odorant causes selective increases in the quantities of both recently born and total OSNs of subtypes that are responsive to those odorants (e.g., subtype A, orange, but not B, gray), consistent with the possibility that olfactory conditioning selectively increases the birthrates of those subtypes.

(1) a higher rate of apoptosis among immature OSNs, and (2) a gradual reduction in the quantities of newborn OSNs of specific subtypes over time following EdU labeling. However, analyses of apoptosis rates among OSN precursors and immature OSNs in UNO-treated animals revealed no increase following olfactory deprivation (van der Linden et al., 2020). Moreover, near-maximal differences in quantities of newborn OSNs on the closed versus open sides of the OE were observed 4 days post-EdU labeling, the earliest timepoint that robust OR expression can be detected in newborn OSNs, and did not increase over time. Collectively, these findings suggested that olfactory deprivation-induced reductions in the overall representations of a fraction of OSN subtypes are caused by selective decreases in the birthrates of these subtypes and, thus, support the hypothesis that the neurogenesis rates of specific subtypes depend on olfactory stimulation (van der Linden et al., 2020).

Among the important questions raised by these findings is what distinguishes the fraction of OSN subtypes whose birthrates depend on olfactory stimulation. Importantly, these subtypes do not appear to be distinguished by the amount of odor stimulation that they receive, based on findings that their levels of *S100a5* transcript, a marker of OSN activity (Bennett et al., 2010; Fischl et al., 2014; McClintock et al., 2014; Serizawa et al., 2003), were near average relative to the OSN population as a whole (van der Linden et al., 2020). Likewise, ORs expressed by these subtypes are not encoded on specific chromosomes, nor do they exhibit an obvious phylogenetic relationship. These subtypes also do not appear to be specified by their location within the OE, as they were found to be interspersed with subtypes whose birthrates are not stimulation dependent. Moreover, these subtypes were found within all four canonical zones of the OE (Miyamichi et al., 2005; Norlin et al., 2001; Ressler et al., 1993; Vassar et al., 1993), though they appeared concentrated in zones two and three (van der Linden et al., 2020). Notably, zones two and three were also observed to exhibit the greatest deprivation-induced reductions in overall olfactory sensory neurogenesis (van der Linden et al., 2020), suggesting a causal link. As discussed below, a recent study has found evidence in support of the hypothesis that these subtypes are distinguished by the salience of odorants that they detect (Hossain et al., 2024). However, broad testing of this hypothesis will require deorphanizing additional ORs that define these subtypes. In addition to determining what distinguishes the fraction of OSN subtypes whose birthrates are altered by stimulation, future studies will be needed to elucidate the mechanism by which this process occurs.

In contrast to OSN subtypes that showed reduced representations following olfactory deprivation, subtypes that showed deprivation-increased representations were found to receive above-average levels of stimulation by environmental odors, based on their expression of *S100a5* (van der Linden et al., 2020). These findings, combined with observations that the birthrates of these latter subtypes are not affected by olfactory deprivation, indicate that olfactory deprivation-induced increases in the representations of specific subtypes are caused by their enhanced survival, possibly due to protection from over-stimulation (van der Linden et al., 2020), as previously hypothesized (Santoro & Dulac, 2012; Zhao et al., 2013).

3 | EVIDENCE THAT OLFACTORY ENRICHMENT CAN INCREASE THE NEUROGENESIS RATES OF SPECIFIC OSN SUBTYPES

As described above, findings from previous studies suggest that olfactory deprivation decreases the representations of a fraction of OSN subtypes by reducing their neurogenesis rates. These observations suggest the possibility that some OSN subtypes have a special capacity to undergo accelerated neurogenesis in the presence of olfactory stimulation (van der Linden et al., 2020). However, olfactory deprivation via UNO diminishes an individual's exposure to likely hundreds or thousands of odors, as well as mechanical stimuli, and thus lowers the level of olfactory stimulation received by a large fraction of the OSN population, raising questions about the nature of the stimuli that selectively affect the neurogenesis rates of specific OSN subtypes. Specifically, are these stimuli discrete odorants that selectively stimulate specific OSN subtypes or, rather, generic odors or even mechanical stimuli that activate large numbers of subtypes non-selectively? Moreover, is there a relationship between the stimuli that promote the neurogenesis of specific subtypes and the receptor identities of the subtypes whose birthrates are promoted? Recent studies have begun to address these questions using olfactory enrichment paradigms, in which discrete odorants or odor mixtures are added to the normal odor environment and thereby selectively increase the level of stimulation received by OSN subtypes that are responsive to the added odors. This type of manipulation has enabled targeted investigations into the effects of odor stimulation of specific OSN subtypes on neurogenesis rates within the OE. Here, we will focus on studies that have found evidence of increased representations of specific OSN subtypes following olfactory enrichment, as these are expected, based on findings from UNO experiments, to potentially reflect odor stimulation-accelerated OSN birthrates.

Early findings that olfactory enrichment can increase the representations of specific OSN subtypes were obtained from a study in which RNA-seq was used to identify ORs with altered mRNA transcript levels within the OEs of mice exposed to discrete odorants for 24 weeks (starting from P0) compared to control mice that were not exposed to the odorants (Ibarra-Soria et al., 2017). In this study, the odorants (acetophenone, eugenol, heptanol, and (*R*)-carvone) were introduced as a mixture into the drinking water, such that mice were exposed to the highest odor concentrations while drinking. This approach resulted in the identification of 16 ORs with significantly increased transcript levels and 20 with significantly reduced levels within the OEs of odor-exposed mice compared to controls. Notably, a time course analysis revealed that transcriptional changes for some ORs were evident after as few as 4 weeks of olfactory enrichment. Moreover, the investigators found via RNA-FISH that enrichment-mediated changes in OR transcript levels reflected altered representations of corresponding OSN subtypes. Based on these findings, the investigators speculated that the observed changes in OSN representations, both increases and decreases, are mediated by altered OSN survival (Ibarra-Soria et al., 2017). However, recent findings that the

birthrates of a fraction of OSN subtypes appear to depend on olfactory stimulation (Santoro & Dulac, 2012; van der Linden et al., 2020), raise the question of whether some of the observed changes in OSN representations reflect subtype-selective changes in OSN birthrates. Specifically, the 16 OSN subtypes that were increased in abundance following odor enrichment appear analogous to subtypes found in UNO experiments to have higher representations and birthrates on the open side of the OE compared to the closed. Accordingly, at least some of these subtypes might be predicted to show higher rates of neurogenesis in the presence of the odor mixture compared to the absence. By contrast, the 20 OSN subtypes that were decreased in abundance following odor enrichment appear analogous to subtypes found in UNO experiments to have equivalent birthrates but lower overall representations on the open side of the OE compared to the closed, presumably due to reduced survival as a result of overstimulation. Additional studies will be required to test these predictions. Intriguingly, the method by which mice were exposed to odors was found to strongly affect the results. Presentation of the odorant mixture continuously in the animals' cages over the same time course failed to significantly alter the transcript levels of any of the ORs that had previously been found to be differentially expressed following odor presentation in drinking water, indicating that the mode and/or discontinuous nature of odorant exposure is important (Ibarra-Soria et al., 2017). In this regard, it is notable that the introduction of odorants into drinking water might conceivably cause mice to associate these odors with the quenching of thirst, which can be satiating and pleasureful (Augustine et al., 2019). As will be discussed below, previous studies have found that pairing an odor with an appetitive or aversive stimulus can increase the representations of OSN subtypes responsive to the conditioned odor, raising the question of whether the changes in OSN representations observed following the introduction of odors to drinking water are due to simple olfactory enrichment, olfactory conditioning, or both.

Two subsequent studies provided additional evidence that olfactory enrichment can selectively increase the representations of OSN subtypes responsive to specific odors to which mice are exposed (van der Linden et al., 2018; Vihani et al., 2020). These studies sought to investigate whether exposure of mice to environments containing distinct sets of salient odors would cause differences in the representations of OSN subtypes responsive to odors differentially present within the two environments. Taking advantage of prior findings that the compositions of odors emitted by male and female mice differ substantially (Doyle et al., 2016; Fu et al., 2015; Kimoto et al., 2005; Lin et al., 2005; Nodari et al., 2008; Schwende et al., 1986; Stopka et al., 2016; Stopkova et al., 2017; Stopková et al., 2007), these studies tested the prediction that a comparison of the OSN populations of male and female mice housed only with members of the same sex (referred to as sex-separated) should produce differences in the representations of OSN subtypes responsive to sexually dimorphic odors. Indeed, females and males housed sex-separated from weaning until 6 months (van der Linden et al., 2018) or 10 months of age (Vihani et al., 2020) were found to exhibit extensive differences in the levels of specific OR transcripts, which were subsequently found via RNA-FISH to reflect differences in quantities of OSNs of the corresponding subtypes. Moreover, differences in OSN subtype representations were found to be largely attenuated when comparing male and female mice that had been housed sex-combined (females and males in the same cage) and thereby exposed to both female- and male-emitted odors. These findings supported the hypothesis that salient odors that are differentially present within the odor environment can promote changes in the representations of specific OSN subtypes.

Notably, the OSN subtypes that showed the greatest differences in representations between sex-separated male and female mice were also found to be responsive to odors emitted specifically by males (van der Linden et al., 2018; Vihani et al., 2020). Moreover, as was observed following odor deprivation and the introduction of odorants into drinking water, the differences appeared bidirectional, with some OSN subtypes exhibiting reduced representations following exposure to male mice, and others showing increased representations. OSN subtypes that exhibited reduced representations following male odor exposure were found to include Olfr912 *(Or8b48)*, and Olfr1295 (*Or4k45)* (van der Linden et al., 2018; Vihani et al., 2020), which selectively detect 2-sec-butyl-4,5-dihydrothiazole (SBT) and (methylthio) methanethiol (MTMT), respectively, (Vihani et al., 2020), two odors that are known components of male mouse urine (Harvey et al., 1989; Lin et al., 2005). OSN subtypes that showed increased representations were found to include Olfr235 (*Or5an11*) and Olfr1437 (*Or5an1b*), which are known to selectively detect musk odors (McClintock et al., 2014; Sato-Akuhara et al., 2016; Vihani et al., 2020), suggesting the possibility that musk-like odors are differentially present within odors emitted by male and female mice. Both categories of changes in OSN subtype representations were proposed to be mediated by altered OSN lifespan (lengthening or shortening, respectively) (van der Linden et al., 2018; Vihani et al., 2020). And although evidence was found to support this hypothesis for subtypes whose representations were reduced following exposure to male odors (e.g., Olfr912 and Olfr1295) (Vihani et al., 2020), recent findings that olfactory stimulation can accelerate the birthrates of specific subtypes (van der Linden et al., 2020) raised the question of whether accelerated neurogenesis rates could also play a role in the observed changes, specifically those whose representations were elevated following exposure to male odors (e.g., Olfr235 and Olfr1437).

Findings that exposure of mice to male-emitted odors can increase the representations of OSN subtypes that detect components of those odors led to a subsequent study to investigate whether discrete odors can accelerate the birthrates of OSN subtypes that they stimulate (Hossain et al., 2024). For this purpose, subtype Olfr235 was identified as particularly interesting due to observations that it is more highly represented in mice exposed to male odors (van der Linden et al., 2018; Vihani et al., 2020) and is selectively responsive to male-emitted (van der Linden et al., 2020) and musk odors (McClintock et al., 2014; Sato-Akuhara et al., 2016; Vihani et al., 2020). Moreover, Olfr235 belongs to a subfamily of known musk-responsive ORs (McClintock et al., 2014), many of which were found, like Olfr235, to be more highly represented in mice exposed to

male odors (Hossain et al., 2024; van der Linden et al., 2018). These observations suggested the possibility that exposure of mice to one or more components of male odors or musk-like odorants might elevate the representations of musk-responsive OSN subtypes by selectively accelerating their rates of neurogenesis. In support of this prediction, analyses of UNO-treated mice using both scRNA-seq-based and histological approaches found evidence that olfactory deprivation in juvenile males reduced the quantities of newborn OSNs of musk-responsive subtypes (Hossain et al., 2024). Additionally, the exposure of mice to male odors and/or muscone, a component of musk odors, was found to: (1) intensify deprivation-induced reductions in quantities of newborn Olfr235 OSNs in female mice, and (2) increase quantities of newborn Olfr235, Olfr1440 (*Or5an6*), and Olfr1431 (*Or5an9*) OSNs within the OEs of non-occluded females. Moreover, observations that the magnitudes of male odor and muscone exposure-induced increases in the quantities of newborn OSNs of musk/male-responsive OSN subtypes do not differ between 4 and 7 days post-EdU indicated that these changes reflect altered rates of neurogenesis, as opposed to apoptosis of newborn OSNs or OR switching. Collectively, these findings provide evidence that discrete odors can selectively increase the birthrates of OSNs of subtypes that detect them (Figure 1b).

Findings that exposing mice to certain odors can alter the birthrates of OSN subtypes responsive to those odors may be relevant to the intriguing but as-yet unexplained observations that the exposure of both mice and humans to particular odorants can selectively increase sensitivity to those odorants. This phenomenon, known as olfactory induction, was discovered serendipitously when an investigator who was studying the basis of a specific anosmia to the odorant androstenone, and was initially insensitive to it, acquired the ability to detect the odorant after months of intermittent exposure (Wysocki et al., 1989). The investigators went on to experimentally demonstrate that some individuals who were previously anosmic to androstenone, a group that comprises 40%–50% of adults, experienced selective increases in sensitivity to the odorant following systematic exposure three times daily over 6 weeks (Wysocki et al., 1989). The discovery of olfactory induction in humans prompted a series of subsequent studies in rodents aiming to elucidate the mechanism of this phenomenon. In one such study, the exposure of mice to androstenone for 16 h per day for 2 weeks was found to cause 16–100-fold increases in sensitivity to the odorant, as assessed by a Y-maze behavioral assay (Voznessenskaya et al., 1995). In a complementary study, the repeated presentation of androstenone to a strain of mice that initially showed low sensitivity to the odorant caused a selective increase in androstenone-induced OE electrical responses, as measured via electro-olfactogram (EOG) (Wang et al., 1993). A similar result was observed following exposure of mice to the unrelated odorant isovaleric acid, indicating that this phenomenon is not limited to androstenone (Wang et al., 1993). To determine if olfactory induction causes similar increases in electrical responses in the OEs of humans, a subsequent study measured androstenone-induced electrical activity within the OEs of human participants who had initially exhibited low sensitivity to the odorant, following exposure for 3 min, three times per day (Wang et al., 2004). As was observed in mice, exposure of humans to androstenone was found to selectively increase EOG responses and reduce the detection threshold for that odorant (Wang et al., 2004).

The extent to which olfactory induction is a general phenomenon or, rather, limited to specific odors, remains unknown. Notably, one study observed that human females of reproductive age showed dramatic increases in sensitivity to three out of the four odors to which they were exposed, while men and women outside of childbearing age showed no such changes (Dalton et al., 2002). These findings suggested that the capacity for olfactory induction might vary depending on the sex and age of a subject, as well as the odors to which they are exposed. Also unknown is the mechanism by which olfactory induction occurs. The authors of these studies proposed a mechanism involving increases in the quantity and/or sensitivity of OSNs that are responsive to the odorant to which an individual is exposed (Wang et al., 1993, 2004; Wysocki et al., 1989). Recent observations in mice that the birthrates of specific OSN subtypes can be accelerated by olfactory stimulation (Hossain et al., 2024; van der Linden et al., 2020) provide a rationale to test for the involvement of this phenomenon in olfactory induction.

4 | EVIDENCE THAT OLFACTORY CONDITIONING CAN INCREASE THE NEUROGENESIS RATES OF OSNs RESPONSIVE TO CONDITIONED ODORS

Olfactory conditioning, also known as associative olfactory learning, is a third type of olfactory experience paradigm that has been implicated in selectively affecting the neurogenesis rates of specific OSN subtypes. Olfactory conditioning enables the learned association of odor cues with specific contexts. In nature, olfactory conditioning is thought to facilitate behavioral adaptation to environmental conditions, efficient navigation within the environment, and the connection of specific odor experiences with favorable or unfavorable outcomes (reviewed in Reinert & Fukunaga, 2022; Ross & Fletcher, 2018). Olfactory conditioning, which can be recapitulated in a laboratory setting, causes both physiological and structural changes within various parts of the olfactory pathway, including the OE, OB, olfactory cortex, and other brain regions. In the laboratory, olfactory conditioning typically involves the pairing of a previously neutral odorant with either an aversive or appetitive outcome, thereby causing a behavioral response to the odorant once learning has taken place. This type of learning has also been found to cause selective increases in the representations of OSN subtypes responsive to the conditioned odor. While the mechanism by which these increases occur remains under investigation, one hypothesis is that olfactory conditioning selectively increases the lifespan of mature OSNs and/or the survival of newborn OSNs of subtypes responsive to the conditioned odor, thereby increasing their representations over time within the OE. Here, we consider the alternative hypothesis that observed increases in the representations of specific OSN subtypes following olfactory conditioning are mediated

by selective increases in the neurogenesis rates of these subtypes (Figure 1c).

Multiple studies have found direct and/or indirect evidence that conditioning adult mice to associate acetophenone, an odorant that is detected selectively by M71 (Olfr151; *OR8a1*) OSNs, with an aversive or appetitive outcome results in increased quantities of M71 OSNs within the OEs of conditioned mice relative to mice that did not experience olfactory conditioning with the same odor (Aoued et al., 2020; Dias & Ressler, 2014; Jones et al., 2008; Liff et al., 2023; Morrison et al., 2015). In the first of these studies, mice subjected to conditioning (two training sessions per week for 3 weeks) to associate acetophenone with either a foot shock or cocaine treatment were found to have elevated quantities of M71 OSNs within their OE and larger M71 glomeruli compared to unconditioned mice (Jones et al., 2008), with the latter change likely causally connected to the former (Bressel et al., 2016). In the same study, three consecutive days of aversive olfactory conditioning (10 training sessions/day) was found to be sufficient to observe similar changes 3 weeks after the onset of training. These findings were replicated in a follow-up study from the same group, in which mice trained for 3 days exhibited greater M71 OSN quantities and larger glomerular sizes compared to unconditioned mice, both 3 and 6 weeks after training (Morrison et al., 2015). Moreover, extinction training, in which olfactory conditioned mice received 90 acetophenone-alone presentations over 3 days, was found to attenuate the changes associated with conditioning, indicating that learning-associated increases in the representations of OSN subtypes responsive to conditioned odors are reversible (Morrison et al., 2015). In a subsequent study by a separate group (findings from which were available as a preprint at the time of publication of this review), whole-OE tissue clearing, imaging, and automated counting were used to precisely quantify fluorescently labeled OSNs of specific subtypes in olfactory conditioned mice and controls (Liff et al., 2023). Using this approach, the investigators observed a 33% increase in the quantity of M71 OSNs and a 39% increase in the quantity of MOR23 (Olfr16; *Or10j5*) OSNs compared to controls 3 weeks after the onset of a 3-day aversive olfactory conditioning paradigm with the cognate odorants acetophenone or lyral, respectively (Liff et al., 2023). Notably, the representations of these subtypes were found to remain significantly elevated in conditioned animals for at least 60 days after training. Moreover, the observed increases in OSN quantities were found to be accompanied by robust behavioral responses toward the conditioned odors. Collectively, these studies contribute to a compelling body of evidence that associative olfactory conditioning can increase the representations of OSN subtypes responsive to the conditioned odors.

The mechanism underlying increases in the representations of OSN subtypes responsive to conditioned odors observed following olfactory conditioning has long remained mysterious. The authors of early studies proposed that these changes might reflect learning-dependent increases in either the neurogenesis rates or lifespans of mature OSNs of those subtypes (Jones et al., 2008; Morrison et al., 2015). A recent study sought to test these alternative hypotheses by using an EdU birthdating approach to investigate the effects of olfactory conditioning on the quantities of newborn and mature OSNs of subtypes responsive to conditioned odors (Liff et al., 2023). In an experiment designed to test the hypothesis of conditioning-altered neurogenesis, conditioned and control mice were EdU-injected on each of the 3 days of conditioning and the 2 days post-conditioning to enable identification and quantification of M71 and MOR23 OSNs that were born during and shortly after the conditioning period. Using whole-OE analyses of EdU-labeled OSNs from mice dissected 3 weeks after the onset of training (16 days after the last EdU injection), the investigators observed a six-fold greater quantity of EdU-labeled M71 OSNs and a four-fold greater quantity of EdU-labeled MOR23 OSNs in conditioned mice compared to controls. The authors attributed these differences to selective increases in the neurogenesis rates of M71 and MOR23 OSNs in the trained animals. In a second experiment designed to exclude the increased lifespan hypothesis, EdU was injected for each of 5 days starting 12 days prior to the onset of training, with the reasoning that all EdU-labeled neurons should be mature by the onset of olfactory training (Liff et al., 2023). The investigators observed no differences in the quantities of EdU-labeled M71 or MOR23 OSNs between trained and untrained animals, leading them to infer that selective increases in the lifespan of mature OSNs of these subtypes do not contribute to increases in their representations following associative olfactory learning. A caveat of these experiments, however, is that neither appears to exclude the possibility that observed increases in the representations of M71 and MOR23 OSNs are due to selective increases in the rates of integration and survival of newly generated OSNs of these subtypes. In this regard, previous studies have found that a majority of OSNs born in postnatal mice fail to incorporate and survive, with only a small fraction surviving by 14 days post-BrdU labeling (Kondo et al., 2010). Thus, if olfactory learning were able to increase the fraction of newborn M71 and MOR23 OSNs that integrate and survive, the chase period of 16–21 days employed in the first EdU-birthdating experiment would be expected to provide sufficient time for differences in the quantity of EdU-labeled OSNs of these subtypes to become evident. Notably, it has been proposed that a learning-dependent increase in release of BDNF, a neurotrophic factor that plays roles in neuron survival, accompanied by selective upregulation of BDNF receptors on immature neurons specific to the conditioned odorant may contribute to their increased survival (Morrison et al., 2015; Ross & Fletcher, 2018). This, in turn, could conceivably explain the increased representations of M71 and MOR23 OSNs observed following olfactory conditioning. In the second EdU-birthdating experiment, EdU was administered 12 days prior to the onset of olfactory training, a timepoint which precludes assessment of olfactory learning on the integration and survival of OSNs born around the time of conditioning. Thus, although results of the second experiment indicate that olfactory conditioning does not increase the lifespans of mature M71 and MOR23 OSNs, they do not exclude a mechanism involving the increased integration and survival of newborn OSNs of these subtypes following olfactory learning. Hence, based on findings from this study, selective increases in the rates of both neurogenesis and survival of newborn neurons appear to remain plausible hypotheses to

explain the elevated representations of specific OSN subtypes observed following associative olfactory learning. Additional experiments will be needed to distinguish between these two potential mechanisms.

In addition to findings that olfactory conditioning selectively increases the representations of OSN subtypes responsive to the conditioned odors, multiple studies have found that conditioning-associated increases in the representations of those subtypes can be transferred to subsequent generations of offspring that experienced no conditioning (Aoued et al., 2020; Dias & Ressler, 2014; Liff et al., 2023). It has been hypothesized that these transgenerational changes reflect increased rates of olfactory sensory neurogenesis of the conditioned odor-responsive subtypes during the development of the offspring of trained animals (Aoued et al., 2020; Dias & Ressler, 2014; Liff et al., 2023). However, conclusive evidence for such a mechanism has yet to be demonstrated. Additional studies will be needed to test this and other potential hypotheses.

5 | EVIDENCE THAT RECOVERY OF THE OSN POPULATION FROM INJURY ENTAILS NEUROGENESIS THAT IS NON-STOCHASTIC WITH RESPECT TO SUBTYPE

To this point, we have reviewed evidence based on experience manipulation that is consistent with the possibility that, under non-injury conditions, neurogenesis within the OE is not strictly stochastic with respect to OSN subtype identity. Here, we turn our attention to studies that provide hints that, under conditions of injury in both rodents and humans, neurogenesis is also not entirely stochastic with respect to OSN subtype.

Rodent olfactory injury models are useful for investigating the natural recovery process of the OE following injury. However, injury to the OE is likely to cause cell death with little or no selectivity with respect to OSN subtype identity, which complicates the assessment of whether regenerative neurogenesis is stochastic with respect to subtype. This limitation may be overcome, however, through the use of a genetically engineered mouse model in which a single OSN subtype can be conditionally ablated. This approach was employed in a study in which P2 (Olfr17; *Or10a4*) OSNs were selectively and synchronously ablated through the inducible and selective expression of diphtheria toxin by P2 OSNs within adult mice upon administration of doxycycline for 3 weeks (Gogos et al., 2000). Examination of the OEs of doxycycline-treated mice 8 weeks after treatment revealed that the P2 OSN population had nearly completely recovered, including axonal projections to the same glomerular location as age-matched non-doxycycline-treated control mice. As discussed in a subsequent review, the rapidity and completeness with which this recovery occurred appears inconsistent with a neurogenesis mechanism that is strictly stochastic with respect to OSN subtype (Yu & Wu, 2017). While a stochastic mechanism would be expected to permit near-complete regeneration of OSNs of a given OSN subtype on a timescale of 8 weeks following widespread ablation of the OSN population, recovery following ablation of only P2 OSNs, which comprise just ~0.1% of the OSN population, would be expected to require a much longer recovery period. These results led to the proposal that neurogenesis may occur in a subtype-selective manner during recovery by way of an unknown feedback mechanism that impacts OR choice depending on the subtypes of OSNs lost (Yu & Wu, 2017).

Insights into the stochasticity of olfactory sensory neurogenesis with respect to subtype identity during recovery from injury have also been obtained by examining whether the exposure of mice to specific odors following injury causes selective improvements in sensitivity to those odors. One such study investigated the effects of exposing mice to either amyl acetate or androstenone daily for 10 days following bilateral olfactory nerve transection surgery, which triggers widespread OSN apoptosis (Yee & Wysocki, 2001). Six weeks after surgery, mice were found to exhibit selective increases in sensitivity to the odorant to which they had been exposed during recovery. These findings support the possibility that odor stimulation after injury can facilitate recovery in a manner that is selective for the OSN subtypes that were stimulated. To explain these findings, the authors hypothesized that exposing mice to odors during the regeneration period might selectively increase the intrinsic sensitivity of OSNs in a subtype-selective manner. A conceivable alternative possibility, however, is that odorant stimulation selectively increases the representations of OSN subtypes responsive to the odors to which mice were exposed. Consistent with this latter possibility, a separate study found that mice exposed to four odorants three times daily over 3 weeks following 3-methylindol-induced injury showed an upregulation of genes associated with neurogenesis compared to control mice that were not odor-exposed (Kim et al., 2019). These gene expression differences in the odor-exposed animals, which were accompanied by more rapid improvements in olfactory ability, led the authors to hypothesize that odor stimulation can speed recovery from injury via altered neurogenesis. Collectively, these findings appear consistent with the possibility that recovery of the OE from injury can be facilitated by odor stimulation via a mechanism involving accelerated olfactory sensory neurogenesis in a manner that may be non-stochastic with respect to subtype. Further experiments will be needed, however, to test this and alternative hypotheses.

Findings from the animal studies described above may also provide mechanistic insights into observations that exposing humans to odors following olfactory injury can accelerate recovery. Olfactory dysfunction, which can result from injury due to infection, inflammation, or head trauma, affects around 5% of the general population and is associated with reduced quality of life and negative health outcomes [reviewed in Hummel et al., 2017; Whitcroft et al., 2023]. A subset of these cases appears to be due to a loss of sensory neurons. One of the only options currently available for the treatment of olfactory dysfunction from a variety of causes is olfactory training, which involves the repeated "systematic sniffing" of various odorants over weeks or months with the aim of improving overall olfactory ability. Although apparently effective for some individuals, the mechanism by which olfactory training works remains poorly understood [reviewed in Pieniak et al., 2022; Turner, 2020].

Several studies have demonstrated the efficacy of olfactory training for treating olfactory dysfunction of various etiologies in humans (Huang et al., 2021; Sorokowska et al., 2017). For example, one such study found that olfactory training improves olfactory ability in patients with dysfunction due to either upper respiratory tract infection (URTI) or craniofacial trauma, which are frequent causes of olfactory disorders (Konstantinidis et al., 2013). In this study, general olfactory ability was assessed by evaluating subjects' odor threshold, discrimination, and identification (TDI) ability for 16 common odors, with higher TDI scores reflecting superior olfactory ability. In post-URTI patients who completed olfactory training with four odors over 16 weeks, two thirds experienced a significant increase in TDI score, compared to one-third of non-training post-URTI patients. Among post-traumatic patients who completed the same training, one-third showed an increase in TDI score, compared to only 13.3% of non-training post-traumatic patients. These findings indicate that olfactory training can facilitate OE recovery and the improvement of overall olfactory ability after injury from URTI or craniofacial trauma. A similar study showed that, among hyposmic subjects who were exposed to four odors twice daily over the course of 12 weeks, 28% saw an increase in TDI score compared to 6% of subjects who did not undergo olfactory training (Hummel et al., 2009). Moreover, relative to untrained control subjects, subjects who underwent training demonstrated improved odor threshold scores for three of the four specific odorants used in training. Based on these findings, the authors suggested that the improvements might reflect increases in the quantities of OSNs (Hummel et al., 2009). Future studies will be needed to test this and alternative possibilities.

OSNs are unique among neurons within the mammalian nervous system in their ability to recover after injury. This process enables olfactory loss to return via a still-poorly-understood mechanism that can be facilitated by exposure to odors in some cases. While olfactory training remains one of the only treatments available for olfactory dysfunction, it proves difficult for many patients due to the long duration of treatment and limited efficacy (Pieniak et al., 2022; Turner, 2020). If enhanced olfactory sensory neurogenesis is found to explain some of the improvement observed with olfactory training, understanding its mechanism might enable the identification of molecular pathways that could be modulated to produce treatment options that are more effective in improving the many areas of human life that are directly or indirectly impacted by olfactory dysfunction.

6 | OPEN QUESTIONS AND FUTURE DIRECTIONS

6.1 | Mechanistic implications of experience-regulated neurogenesis of specific OSN subtypes

The studies discussed above provide evidence that some olfactory experiences can selectively alter the birthrates of specific OSN subtypes in mammals. These findings appear to conflict with the established model that olfactory sensory neurogenesis is stochastic with respect to OSN subtype identity, suggesting that this model may be incomplete. These findings thus raise intriguing questions regarding how experience might selectively alter the neurogenesis rates of specific OSN subtypes. One mechanism that has been proposed by multiple investigators is that olfactory experiences can selectively alter OR gene choices. In this scenario, olfactory experiences might trigger, perhaps through an unknown signaling pathway originating from mature OSNs, OSN precursor cells to favor the choices of specific OR genes at the expense of others. Below, after briefly outlining the basic aspects of how OR gene choice is thought to occur in mammals, we review the limited evidence in support of this hypothesis.

The expression of a single OR allele, out of the hundreds contained within a typical mammalian genome, is a key event of mammalian OSN differentiation [reviewed in McClintock, 2015; Monahan & Lomvardas, 2015]. The chosen OR defines the subtype identity and functional properties of the resulting mature OSN. The process of OR choice has been found to involve the transcriptional de-repression of a small number of OR alleles via the histone demethylase LSD1 (Lyons et al., 2013). Following OR de-repression, the transcription of only one OR allele is evidently facilitated by the formation of a transcription factor-mediated complex between the promoter of a single OR allele and multiple OR enhancers located throughout the genome (Monahan et al., 2017, 2019). Formation of this OR-enhancer hub complex leads to high-level transcription of the OR allele, resulting in a feedback signal that blocks the activation of additional ORs (Dalton et al., 2013; Lewcock & Reed, 2004; Lyons et al., 2013; Pourmorady et al., 2024; Serizawa et al., 2003; Shykind et al., 2004). Notably, a given OSN precursor is restricted in the repertoire of OR genes from which it can choose based on its location within the OE, which is broadly divided into five to nine zones along the dorsoventral axis (Miyamichi et al., 2005; Ressler et al., 1993; Vassar et al., 1993; Zapiec & Mombaerts, 2020). Interestingly, a recent study has found evidence that zones within the OE are established through a combination of stochastic polygenic OR transcription and step-wise heterochromatic silencing of ORs whose expression is restricted within a specific zone (Bashkirova et al., 2023). Also of note, lineage-tracing studies have observed that nascent OSNs can switch their OR choice at a rate of ~10%, or higher if the initially-chosen OR is a pseudogene (Shykind et al., 2004).

Importantly, OR genes available for choice by an OSN precursor are not selected with equal probability, but rather are determined, in part, by their genomic context. This aspect of the mechanism was initially demonstrated by studies involving manipulation of the "H element", a cis-acting OR enhancer located adjacent to a cluster of OR-encoding genes that is completely or partially required for selection of ORs within the cluster (Fuss et al., 2007; Nishizumi et al., 2007; Serizawa et al., 2003). Reducing the genomic distance between the H element and the OR cluster was found to cause a substantial increase in the choice frequency of MOR28 (Olfr1507; *Or4e5*) the OR gene located in closest proximity to H, while simultaneously reducing the choice frequencies of ORs located more distally from H (Serizawa et al., 2003). These findings, along with those obtained from studies of other OR enhancer elements that are located throughout the

mouse genome (Khan et al., 2011; Markenscoff-Papadimitriou et al., 2014; Vassalli et al., 2011), provide evidence that OR genes are chosen stochastically, with distinct probabilities that depend in part on their genomic position relative to specific enhancer elements and other OR genes. These differences appear to be largely responsible for the large distribution in observed representations of distinct OSN subtypes (Ibarra-Soria et al., 2017).

Considering that a given OR gene's genomic context is fixed, how could its choice probability be increased as a consequence of altered olfactory experience? Conceivably, such changes could be enabled by selective increases in the accessibility of specific OR promoters through experience-dependent epigenetic modifications [for reviews, see, e.g. Fujita et al., 2022; Klemm et al., 2019; Pudelko & Cabianca, 2024]. In this scenario, OR-selective epigenetic changes could be triggered by unknown signals from mature OSNs of specific subtypes. Indeed, selective increases in OR choice probabilities have been hypothesized to explain changes in the quantities of OSNs of specific subtypes observed following olfactory conditioning (Liff et al., 2023), possibly via epigenetic changes (Morrison et al., 2015). Whether and how selective epigenetic modifications to specific OR alleles could be achieved within OSN progenitors in response to olfactory conditioning, however, remain unclear. Interestingly, selective increases in quantities of OSNs of specific subtypes have also been observed in the offspring (F1 and F2) of olfactory-conditioned male mice (F0), suggesting that the mechanisms driving changes in OSN quantities can be inherited by subsequent generations (Aoued et al., 2020; Dias & Ressler, 2014; Liff et al., 2023). How the effects of olfactory conditioning experiences are transferred across generations is an open question. Notably, one recent study found evidence that such information may be carried in sperm RNA (Aoued et al., 2020). Moreover, bisulfite sequencing of sperm DNA from conditioned F0 males and F1 naive offspring revealed reduced methylation of CpG sites within the gene locus of an OR responsive to a conditioned odor (Aoued et al., 2019; Dias & Ressler, 2014). Determining whether and how these changes relate to inherited increases in the representations of specific OSN subtypes will require additional study.

A prediction of the hypothesis that experience-dependent changes in the birthrates of specific OSN subtypes are driven by altered OR choice frequencies (including, potentially, directed OR switching within nascent OSNs) is that increases in the birthrates of specific subtypes should be reciprocated by decreases in the birthrates of others. Thus, this mechanism predicts no net change in the overall rate of olfactory sensory neurogenesis following olfactory experience manipulation. In the case of changes in the neurogenesis rates of specific OSN subtypes following olfactory deprivation via UNO, this prediction was tested experimentally by examining the directions of changes in the birthrates of subtypes that showed stimulation dependent neurogenesis (van der Linden et al., 2020). Notably, olfactory deprivation was found to cause only selective reductions in the birthrates of specific OSN subtypes, with no corresponding increases observed (van der Linden et al., 2020). Accordingly, previous studies have found that olfactory deprivation reduces the overall rate of olfactory sensory neurogenesis (Cummings & Brunjes, 1994; Farbman et al., 1988; Mirich & Brunjes, 2001; Suh et al., 2006; van der Linden et al., 2020), and that this reduction is concentrated in canonical zones 2 and 3, where the majority of subtypes found to undergo stimulation-dependent neurogenesis reside (van der Linden et al., 2020). These observations do not appear to support a mechanism in which altered OR choice frequencies mediate olfactory deprivation-induced changes in the birthrates of specific OSN subtypes, highlighting a need to formulate and test alternative hypotheses. However, it is conceivable that putative increases in the birthrates of specific OSN subtypes following other types of experience manipulations, such as olfactory conditioning or injury, could be mediated by changes in the probabilities of specific OR choices. Future studies will be required to test this possibility.

Another important question concerns the molecular and developmental mechanisms that impart specific OSN subtypes with a capacity to undergo accelerated neurogenesis in response to specific olfactory experiences. As discussed above, only a fraction of OSN subtypes have been found to have this capacity in naïve mice (Hossain et al., 2024; van der Linden et al., 2020). Interestingly, changes consistent with increased OSN birthrates have been observed for each of the two OSN subtypes that have been analyzed following olfactory fear conditioning using cognate odors (Liff et al., 2023). These findings suggest the possibility that while some OSN subtypes might have an innate capacity for experience-dependent neurogenesis, other subtypes may be able to acquire such a capacity through olfactory learning. Might these distinct capacities be related, for example, to differences in the developmental timing with which OSNs of individual subtypes have been found to arise (Rodriguez-Gil et al., 2010) and project to defined glomeruli within the olfactory bulb (Bailey et al., 1999; Porter & Winberg, 1999; Royal & Key, 1999)? Relatedly, what, if any, are the relative contributions of the coding regions and loci (Feinstein & Mombaerts, 2004) of the OR genes that define subtypes with capacities for experience-regulated neurogenesis? Finally, what molecular pathways enable signaling from the odor sensing OSNs of specific subtypes to promote the birth of new neurons of the same subtype? These and related questions remain to be addressed by future studies.

6.2 | Functional implications of experience-regulated neurogenesis of specific OSN subtypes

The assumption that olfactory sensory neurogenesis is stochastic with respect to OSN subtype identity has long supported the theory that, unlike other regions of the nervous system where adult neurogenesis is known to play important adaptive roles (Lledo & Valley, 2016; Ming & Song, 2011; Opendak & Gould, 2015), persistent neurogenesis within the OE serves the sole function of replacing neurons lost to turnover and injury. Studies reviewed above, however, provide evidence that neurogenesis may not be entirely stochastic with respect to subtype, but rather that the birthrates of a fraction of OSN subtypes can be selectively and directionally regulated by odor experiences. These findings suggest that persistent neurogenesis within the

OE may serve, in part, an unknown adaptive function in addition to the known reparative one. It is conceivable, for example, that odor-accelerated birthrates of specific subtypes could enhance sensitivity to certain odors with special salience (Apfelbach et al., 1991; D'Hulst et al., 2016; Meisami, 1989). Moreover, considering that the positions of glomeruli are determined, in part, by the levels of stimulation received by the OSNs that innervate them [reviewed in (Sakano, 2020; Zou et al., 2009)], and that chronic odor exposure has been found to cause the formation of supernumerary glomeruli corresponding to subtypes responsive to those odors (Valle-Leija et al., 2012), it is conceivable that odor-dependent neurogenesis of specific OSN subtypes could facilitate the formation of additional glomeruli corresponding to these subtypes. If so, stimulation-dependent neurogenesis might conceivably facilitate the formation of new connections with projection neurons, whose odor representations have been found to reorganize in an activity-dependent manner (Yamada et al., 2017). Future studies will be needed to investigate these possibilities.

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