Neuron
Article

Odor Processing by Adult-Born Neurons

Yoav Livneh,[1] Yoav Adam,[1] and Adi Mizrahi[1,*]
[1]Department of Neurobiology, Institute for Life Sciences and The Edmond and Lily Safra Center for Brain Sciences, The Hebrew University of Jerusalem, Edmond J. Safra Campus, Givat Ram, 91904 Jerusalem, Israel
*Correspondence: mizrahi.adi@mail.huji.ac.il
http://dx.doi.org/10.1016/j.neuron.2014.01.007

SUMMARY

The adult mammalian brain is continuously supplied with adult-born neurons in the olfactory bulb (OB) and hippocampus, where they are thought to be important for circuit coding and plasticity. However, direct evidence for the actual involvement of these neurons in neural processing is still lacking. We recorded the spiking activity of adult-born periglomerular neurons in the mouse OB in vivo using two-photon-targeted patch recordings. We show that odor responsiveness reaches a peak during neuronal development and then recedes at maturity. Sensory enrichment during development enhances the selectivity of adult-born neurons after maturation, without affecting neighboring resident neurons. Thus, in the OB circuit, adult-born neurons functionally integrate into the circuit, where they acquire distinct response profiles in an experience-dependent manner. The constant flow of these sensitive neurons into the circuit provides it with a mechanism of long-term plasticity, wherein new neurons mature to process odor information based on past demands.

INTRODUCTION

The adult mammalian brain is continuously supplied with adult-born neurons throughout life, primarily in two regions—the hippocampus and olfactory bulb (OB; Altman, 1969; Lois and Alvarez-Buylla, 1994). In the OB, it is particularly the interneurons that are continuously replaced (Adam and Mizrahi, 2010; Lledo et al., 2006). Adult-born OB interneurons have been shown to play an important role in olfactory-driven behaviors (Lazarini and Lledo, 2011). However, the mechanisms by which adult-born neurons exert their function in the brain are still unknown. In fact, we still do not know whether and how adult-born neurons process odor information. Moreover, whether adult-born neurons serve only as a neuronal replacement mechanism (due to wear of preexisting neurons) is still debated. Do adult-born neurons mature to become like any other resident neuron? Alternatively, do they mature to form unique subpopulations with distinct functions?

Adult-born neurons are generated in the subventricular zone, from which they migrate through the rostral migratory stream (RMS) to reach the OB (Lois and Alvarez-Buylla, 1994). Upon arrival to the OB, they begin a process of morphological and physiological maturation, which lasts ~4 weeks (Lledo et al., 2006). During this time, synaptogenesis is at its peak (Kelsch et al., 2010). The young neurons go through a coordinated process in which they first receive synaptic input and then gradually form synaptic output (Bardy et al., 2010; Carleton et al., 2003; Grubb et al., 2008; Kelsch et al., 2008; Livneh et al., 2009; Whitman and Greer, 2007b). During this period of enhanced synaptogenesis, adult-born neurons also exhibit heightened long-term potentiation and concomitantly undergo a period of competitive survival (Nissant et al., 2009; Petreanu and Alvarez-Buylla, 2002; Yamaguchi and Mori, 2005). The heightened capacity for plasticity has been shown to serve their competitive survival and may also be important for sensory coding (Lepousez et al., 2013).

The involvement of adult-born neurons in OB odor processing and plasticity has been implicated from a variety of methods and the evidence remains contradictory to some extent (Lazarini and Lledo, 2011). Manipulations of adult-born neurons include irradiation, genetic ablation, and optogenetic activation, while using odor-guided behaviors as a readout (Alonso et al., 2012; Breton-Provencher et al., 2009; Imayoshi et al., 2008; Lazarini et al., 2009; Moreno et al., 2009; Sultan et al., 2010; Valley et al., 2009). Most of these approaches still manipulate the neurons as one without regarding the possibly more intricate physiological contribution of specific neuronal ages and sensory response profiles to odor processing. Indeed, we argue that a major missing link in the field of adult neurogenesis is a direct demonstration of the involvement of adult-born neurons in sensory processing.

Here, we directly recorded from adult-born neurons in vivo. We used two-photon-targeted cell-attached recordings to describe how odor response profiles of adult-born neurons change during development and after maturation. We demonstrate a developmental period of reduced selectivity, akin to a critical period. Moreover, sensory enrichment during this period increased the odor selectivity of adult-born neurons. We suggest a mechanism whereby adult-born neurons do not have a specific predetermined function in olfaction. Rather, their "physiological fate" and future contribution to olfactory processing is determined by experience-dependent plasticity.

RESULTS

In Vivo Odor Responses of Adult-Born Neurons

Since their discovery over 50 years ago, adult-born neurons of any kind have never been recorded from in vivo. We thus started by developing a preparation to record from adult-born neurons and explore the basic properties of their responses to sensory stimuli. To record from adult-born neurons, we combined viral

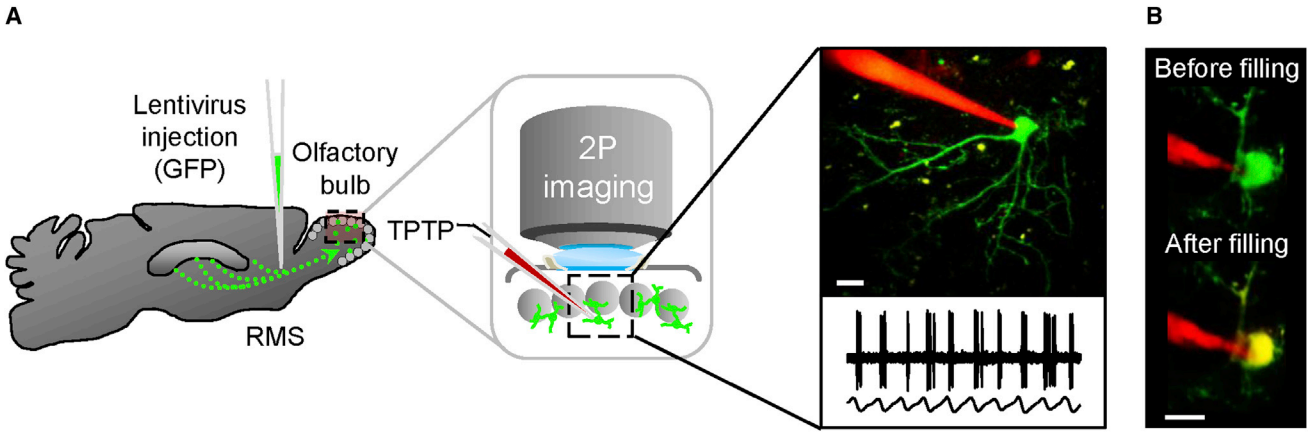

Figure 1. Two-Photon-Targeted Recordings of Adult-Born Neurons, In Vivo

(A) Scheme of the experimental setup. Left: mice were injected with lentivirus into the RMS to label adult-born neurons. Middle: 2–9 weeks later, labeled adult-born PGNs were imaged in the OB and recorded from using two-photon-targeted patch (TPTP). Right: example of a recorded neuron (top), spike trace, and respiration trace (bottom; respiration cycle ~300 ms). Scale bar, 10 μm. See also Figure S1.

(B) Example of verification of correct targeting by postrecording filling of a recorded neuron (green) with the electrode dye (red). Note that the neuron's color changed from green to yellow after filling. Scale bar, 20 μm.

injections and two-photon-targeted cell-attached recordings (Komai et al., 2006). We labeled adult-born neurons by injecting a lentivirus encoding GFP into the RMS, resulting in labeled adult-born neurons in the OB (Figure 1A). In anesthetized mice and under two-photon visual guidance, we targeted a dye-filled patch pipette (red dye, Alexa Fluor 568) to record the spiking output of GFP-labeled adult-born periglomerular neurons (PGNs; Figure 1A). At the end of each recording, we verified the identity of the neuron by filling it with the pipette red fluorescent dye and also verified that only one neuron was recorded by spike waveform analysis (Figure 1B; Figure S1 available online).

Due to the small soma size of PGNs (<15 μm) and their superficial location, achieving stable recordings was technically challenging and restricted in duration. We therefore limited our stimulus panel to seven different monomolecular odors that were chosen based on intrinsic signal imaging (ISI) of the glomerular response to 25 odors (see Experimental Procedures; Fantana et al., 2008; Meister and Bonhoeffer, 2001; Soucy et al., 2009; Uchida and Mainen, 2003; Wachowiak and Cohen, 2003). The odor panel (ethyl-acetate, butanal, pentanal, ethyl-tiglate, propanal, methyl-propionate, and ethyl-butyrate) was chosen in a biased manner to collectively achieve broad activation of glomeruli in the dorsal surface of the OB, thereby increasing the probability that each recorded neuron would be responsive (Figure 2A). Using this odor panel, adult-born neurons clearly responded to odors (Figure 2B). We divided odor responses to three basic types: increases in spike rate ("excitatory"), decreases in spike rate ("inhibitory"), and changes in respiration phase tuning ("phase tuning"). We observed all three basic types of odor responses (Figures 2B–2E). These examples demonstrate that adult-born neurons do indeed respond to odors in vivo, verifying that they are an integral part of the odor-processing circuitry. Furthermore, these examples demonstrate that a given adult-born neuron can receive a dynamic balance of diverse inputs, yielding different types of responses to different stimuli. We next characterized the odor response profiles of adult-born neurons at different developmental stages and under different experimental conditions.

Young Adult-Born Neurons Are Highly Responsive to Sensory Stimuli

To investigate whether and how response characteristics of adult-born neurons change during their development, we recorded their odor responses at three different developmental stages (Figure 3). Specifically, we targeted adult-born neurons at the beginning of the period of synaptic integration (2 weeks postinjection [wpi]), at the final stages of maturation (4 wpi), and long after complete maturation (8–9 wpi; Figure 3A; Lledo et al., 2006). These successive developmental stages were also evident in the evolution of dendritic morphology (see below), and in the distribution of the putative presynapses of adult-born neurons (Figures S2A and S2B), consistent with our previous work (Livneh and Mizrahi, 2011). For comparing the different age groups to preexisting neurons, we also recorded in the same mice from neighboring GFP$^-$ resident neurons (see below). In total, we recorded from 87 neurons in the different experimental groups (2 wpi: n = 19 neurons from 6 mice; 4 wpi: n = 22 neurons from 11 mice; 8–9 wpi: n = 24 neurons from 9 mice; resident: n = 22 neurons from 16 mice).

Sensory response profiles of adult-born neurons changed during development. Specifically, odor responses were evident from the earliest developmental stage that we recorded from (2 wpi), in which 84% (16/19) of neurons responded to at least one of the seven odors (Figures 3A and 3B). Responsiveness increased and peaked at 4 wpi such that almost all neurons of this age group (95%; 21/22) responded to at least one odor (Figures 3A and 3B). This high rate of responsiveness did not last indefinitely. Rather, 8–9 wpi neurons receded to lower levels of responsiveness so that at maturity only ~55% responded to at least one odor (13/24; Figures 3A and 3B).

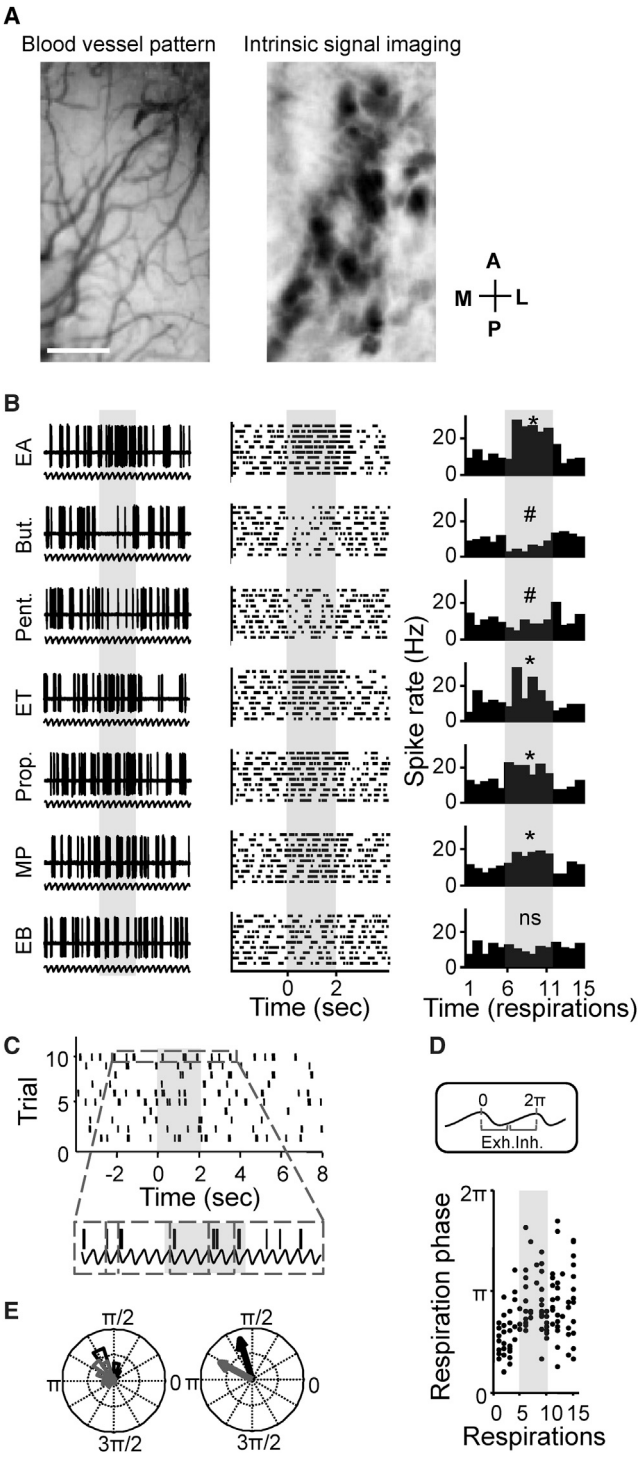

Figure 2. Odor Responses of Adult-Born Neurons

(A) Left: blood vessel pattern of the dorsal OB. Right: ISI of the glomerular activation pattern for all seven odors. The image is a composite sum of the seven individual ISI maps. M, medial; L, lateral; A, anterior; P, posterior. Scale bar, 0.5 mm.

(B) Example of spike rate responses of one neuron for all seven odors (gray: 2 s odor stimulation). Left: spike and respiration traces. Middle: raster plots. Right: PSTHs, binned by respirations. Each bin is a single respiration lasting ~300 ms. *, significant excitatory response; #, significant inhibitory response; ns, nonsignificant response, Mann-Whitney test.

(C–E) Example of a respiration phase-tuning response.

(C) Raster plot (gray: 2 s odor stimulation). Note that there is no significant change in spike rate during odor stimulation.

(D) Raster plot of spike phase in the respiration cycle from all trials. Note that there is a significant change in spike respiration phase tuning during odor stimulation. Inh., inhalation; Exh., exhalation.

(E) Analysis of spike respiration phase (black, before odor stimulation; gray, during stimulation). Left: binned spike phases; right: average vectors, showing a significant change in respiration phase tuning.

Next, we assessed odor selectivity by examining the number of odors to which a significant response was evident (1/7, highest selectivity; 7/7, lowest selectivity). Young adult-born neurons showed moderate levels of selectivity such that ~50% of responsive neurons (10/21) responded to three or more odors (Figure 3B; 2 wpi: orange, average 2/7 odors; 4 wpi: red, average 2.5/7 odors). By 8–9 wpi, neurons became significantly more selective such that only ~30% of responsive neurons (4/13) responded to three or more odors (Figure 3B, brown, average 1/7 odors). Therefore, the peak responsiveness at 4 wpi was also accompanied by lower selectivity, reflecting that these neurons are somewhat more promiscuous with relatively broader odor receptive fields during this age (Figure 3B). In addition, spike rate responses to odor stimuli were significantly stronger in 4 wpi neurons as compared to 8–9 wpi and resident neurons (Figure 3C). These data suggest that 4 wpi neurons are, on average, more easily recruited by odor stimulation.

We next analyzed the type of odor responses neurons exhibited during development and after maturation. Spike rate responses of all groups were dominated by excitatory responses, with lower rates of inhibitory responses (Figure 3D). This suggests that adult-born neurons at all these developmental stages receive both inhibitory and excitatory synaptic inputs, consistent with previous slice electrophysiology work (Grubb et al., 2008). Interestingly, the differences between young (4 wpi) and mature (8–9 wpi) adult-born neurons were specific to odor-evoked activity, as both groups had relatively similar spontaneous spike rate and spike rate variability (Figures S2C and S2D). On the other hand, 2 wpi neurons had lower spontaneous firing rates, but those were highly variable (Figures S2C and S2D). Accordingly, spontaneous spike rate did not predict odor selectivity ($R = 0.09$, $p = 0.34$, Pearson Correlation).

Seeing as PGNs are molecularly and morphologically heterogeneous, the differences in odor response profiles that we found could potentially be a result of differential representation and, therefore, sampling of consistently distinct adult-born PGN subtypes at different neuronal ages. To address this possibility, we performed immunohistochemical analyses of the main PGN subtypes (Kosaka and Kosaka, 2007; Parrish-Aungst et al., 2007) at 4 wpi and 8–9 wpi. Consistent with previous findings (Bagley et al., 2007; Whitman and Greer, 2007a), different subtypes were nonuniformly but similarly represented at both age groups (Figures S2E and S2F). Additionally, the dendritic morphology of both 4 wpi and 8–9 wpi groups were similarly heterogeneous (dendritic length range, 4 wpi: 623–1,655 μm, 8–9 wpi: 526–1,917 μm; dendritic length mean ± STD, 4 wpi: 1,028 ± 330,

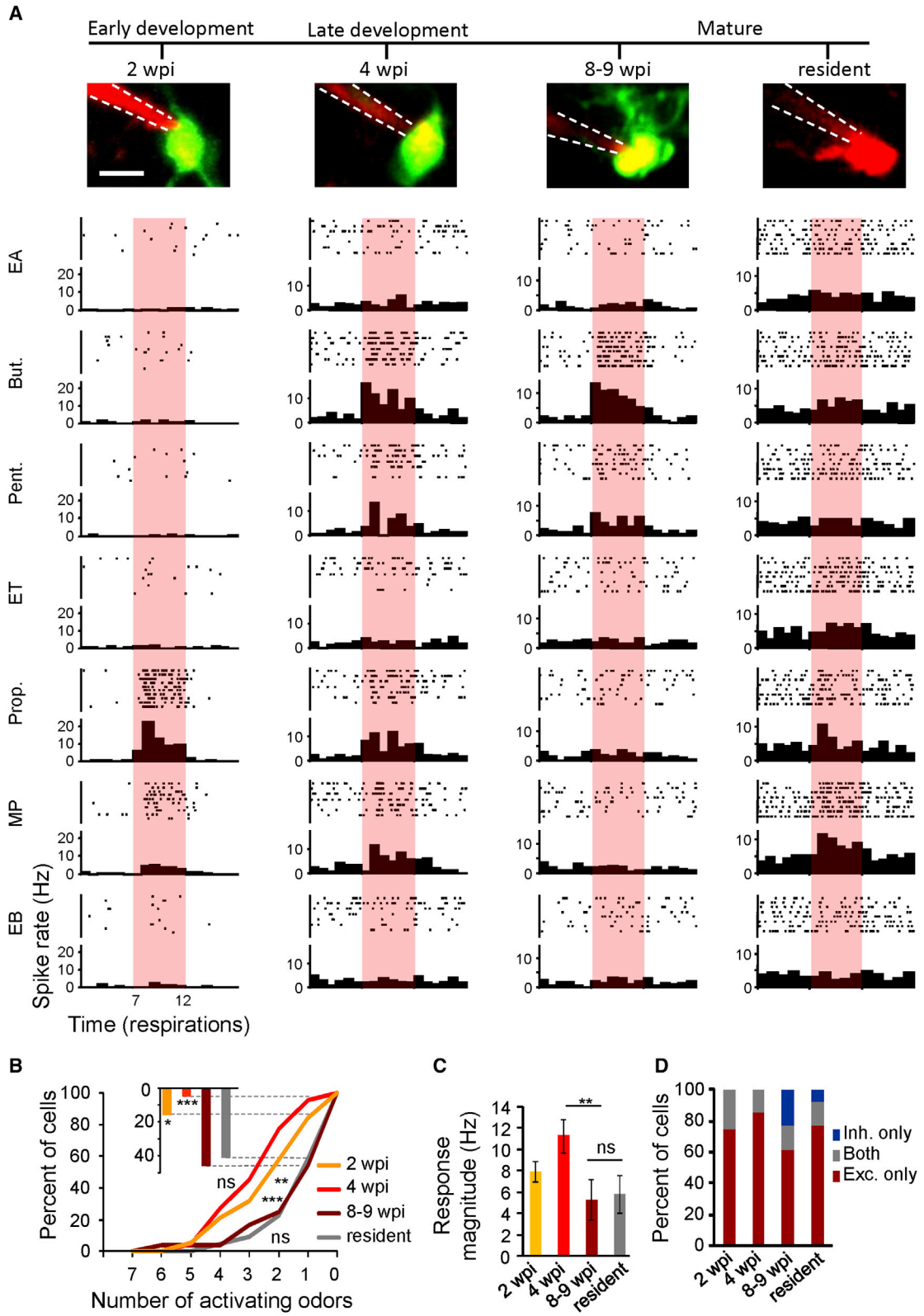

Figure 3. Dynamic Odor Response Profiles during Development

(A) Representative examples of TPTP recordings and odor responses from the four different experimental groups. Top: two-photon micrographs of the recorded neurons. Bottom: raster plots (time in seconds) and PSTHs (time in respirations) of each of the seven odors. Scale bar, 10 μm. See also Figures S2 and S3.

(legend continued on next page)

8–9 wpi: 1,246 ± 457; see also Figure 5B). Thus, a bias toward a specific subtype cannot explain the differences in odor response profiles that we found between 4 wpi and 8–9 wpi.

The differences in odor response profiles that we found could also be potentially explained by some consistent bias of our recordings toward specific active or silent loci in the dorsal OB. To test this possibility, for each mouse that we recorded from we also acquired its glomerular odor activation maps using ISI and placed the exact location of our neuronal recordings within these maps. We then analyzed the number of odors that activated the region around each neuron at increasing radii (Figure S3). This analysis showed that there was no regional bias in our recordings across all groups (Figures S3A and S3B). Additionally, when comparing 4 wpi and 8–9 wpi neurons, we found that the number of odors that activated the glomerular region around each neuron did not predict the number of odors that activated the neuron in either group (Figure S3C; see Experimental Procedures). These results refute the possibility of functional sampling bias. Therefore, our results underscore a clear developmental signature of adult-born neurons, wherein they are highly responsive and less selective at the final stages of their maturation. The high responsiveness period does not last indefinitely and recedes at maturity.

Spontaneous and Odor-Evoked Respiration Phase Tuning

Odor information is encoded in the OB by mitral/tufted cells by changes in spike rates but also by changes in the relative timing of spikes in the respiration cycle (respiration phase tuning; e.g., Dhawale et al., 2010; Fukunaga et al., 2012; Smear et al., 2011). Indeed, most neurons in the OB show firing that is entrained to the respiration cycle (Bathellier et al., 2008; Cang and Isaacson, 2003; Fukunaga et al., 2012; Macrides and Chorover, 1972), and adult-born neurons were no exception (Figures 2C–2E). To test whether this property changes during their development, we assessed spontaneous phase tuning at each developmental stage (Figures 4A and 4B). The majority of neurons in all groups were spontaneously phase tuned, with no significant difference between the groups during development (Figure 4C). In addition, the circular variance of phase tuning, reflecting the variability of spike times in the respiration cycle, was similar between all groups (Figure 4D). Moreover, across all age groups, adult-born neurons' preferred phase was mostly restricted to the same half of the respiration cycle, that of exhalation (Figure 4E).

We next tested whether phase-tuning responses change during development, similar to the observed spike rate responses (Figure 3). Despite the fact that most neurons across all groups were spontaneously phase tuned, very few responded to odors with changes in phase tuning (Figure 4F). The fraction of phase-tuning responsive neurons was similarly low in all groups (Figure 4F). Since PGNs typically responded to odors at a constant respiration phase, they might be the source of mitral cells' odor-evoked respiration phase-tuning changes, as has been recently proposed by computational modeling (Fukunaga et al., 2012).

Functional Maturation of Adult-Born Neurons

A central question in the field of adult neurogenesis is whether new neurons eventually mature to become similar to resident neurons, or whether they mature to form functionally unique subpopulations. To address this question with regard to sensory response profiles, we compared the odor response profiles of mature adult-born neurons (8–9 wpi) to those of GFP$^-$ neurons, to which we refer as "resident" (Figure 3; e.g., Ge et al., 2007; Grubb et al., 2008; Nissant et al., 2009; Saghatelyan et al., 2005). We and others have shown that adult-born PGNs accumulate at a rate of 2%–3% per month (Mizrahi et al., 2006; Ninkovic et al., 2007). Thus, at 8–9 wpi we estimate that only ~4%–6% of the PGN population will be younger than our recorded neurons. Consequently, in our resident neuron data set (n = 22), we estimate that at least 21/22 neurons are older than 8–9 wpi neurons. Additionally, these GFP$^-$ neurons were similar to mature adult-born neurons in all the parameters that we tested in vivo (Figures 3 and 4; Figures S2C and S2D) and those previously tested using whole-cell recordings in OB slices (Grubb et al., 2008), thus arguing against cell-type bias between these groups.

At 8–9 wpi, adult-born neurons and resident neurons were similar. Similarity was evident in sensory response profiles (Figure 3B), response strength (Figure 3C), the extent of excitatory and inhibitory responses (Figure 3D), spontaneous phase tuning (Figures 4A–4E), and phase-tuning sensory responses (Figure 4F). These results show that the spontaneous firing characteristics and the basic odor response profiles of adult-born neurons eventually mature to become similar to those of resident neurons. These results build upon and seem to support earlier morphological and biophysical characteristics of adult-born neurons along development (Carleton et al., 2003; Grubb et al., 2008; Mizrahi, 2007). Thus, at least under normal conditions in which animals are not behaviorally challenged with novel sensory stimuli in their environment, adult-born neurons seem to mature to become similar to resident neurons (but see below).

Additional Glomerular Inputs, Rather Than Morphology, Are Associated with Higher Sensitivity in Young Neurons

Our results thus far reveal that adult-born neurons' responsiveness and response promiscuity reach a peak at 4 wpi and then recede. Accordingly, we sought to examine whether the developing neurons have structural characteristics that could explain

(B) Cumulative distribution of the percentage of neurons responding to odors (spike rate responses only). Inset: percentage of cells that did not respond to any odor. *$p < 0.01$ (2 wpi versus 8–9 wpi), **$p < 0.008$ (2 wpi versus 8–9 wpi), ***$p < 0.0002$ (4 wpi versus 8–9 wpi); ns, not significant, binomial proportions test and Mann-Whitney test; 2 wpi: n = 19 neurons from 6 mice; 4 wpi: n = 22 neurons from 11 mice; 8–9 wpi: n = 24 neurons from 9 mice; resident: n = 22 neurons from 16 mice.

(C) Response magnitude of odor responses. **$p < 0.005$ (4 wpi versus 8–9 wpi); ns, not significant, Mann-Whitney test. All values are represented as mean ± SEM; 2 wpi: n = 16 neurons from 6 mice; 4 wpi: n = 21 neurons from 11 mice; 8–9 wpi: n = 13 neurons from 6 mice; resident: n = 13 neurons from 12 mice.

(D) Percentage of cells in each group with either excitatory or inhibitory responses, or both; 2 wpi: n = 16 neurons from 6 mice; 4 wpi: n = 21 neurons from 11 mice; 8–9 wpi: n = 13 neurons from 6 mice; resident: n = 13 neurons from 12 mice.

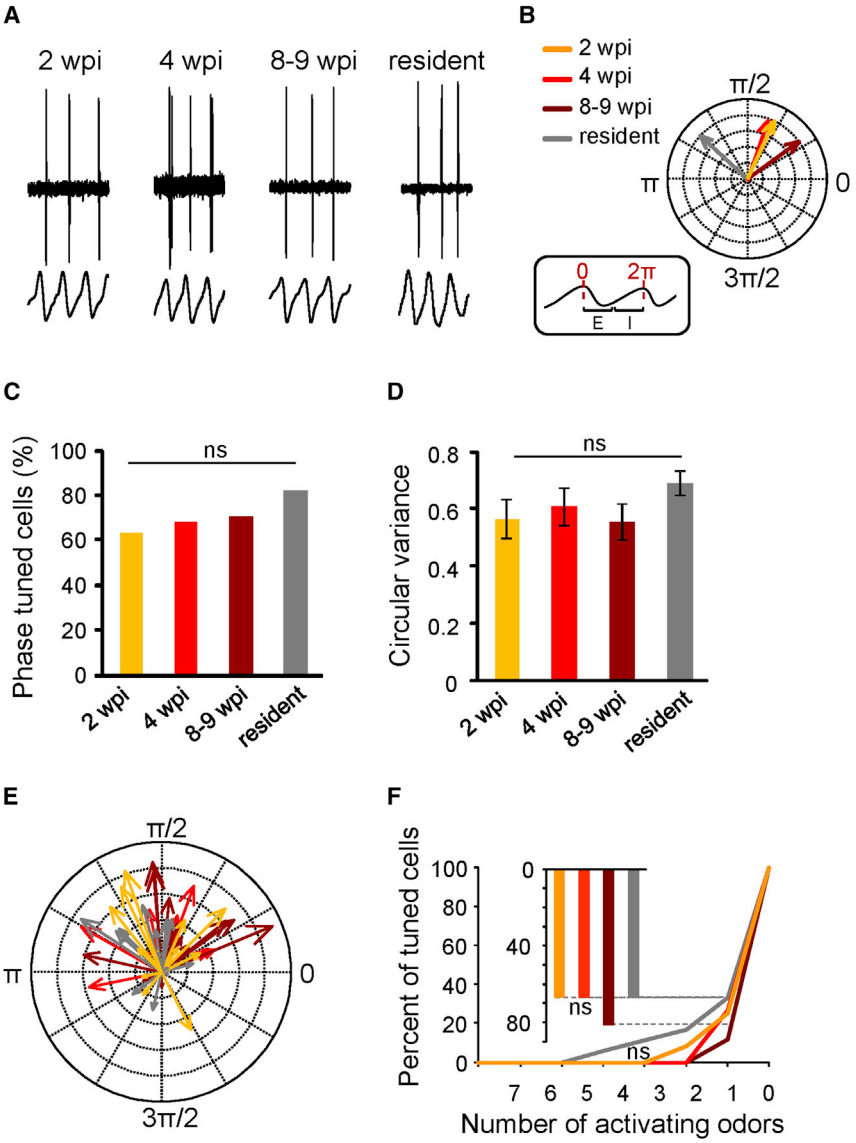

Figure 4. Spontaneous and Odor-Evoked Respiration Phase Tuning Are Stable during Development

(A) Examples of recordings from phase-tuned neurons from each experimental group. Top: raw spikes traces. Bottom: respirations. Three respirations for each neuron are shown; respiration cycle ~300 ms.

(B) Average spike respiration phase of each of the neurons in (A). E, exhalation; I, inhalation.

(C) Percentage of cells that were significantly spontaneously phase tuned in the different groups. There was no significant difference between the groups ($p > 0.08$ for all comparisons; ns, not significant, binomial proportions test); 2 wpi: n = 19 neurons from 6 mice; 4 wpi: n = 22 neurons from 11 mice; 8–9 wpi: n = 24 neurons from 9 mice; resident: n = 22 neurons from 16 mice.

(D and E) Circular variance (D) and mean spike respiration phases (E) of all the cells that were significantly phase tuned in the different groups. There was no significant difference between the groups ($p > 0.09$ for all comparisons; ns, not significant, Mann-Whitney test); 2 wpi: n = 12 neurons from 6 mice; 4 wpi: n = 15 neurons from 7 mice; 8–9 wpi: n = 17 neurons from 8 mice; resident: n = 18 neurons from 13 mice. All values in (D) are represented as mean ± SEM.

(F) Cumulative distribution of the respiration phase-tuning odor responses. Inset: percentage of cells that did not respond to any odor. There was no significant difference between the groups ($p > 0.08$ for all comparisons; ns, not significant, binomial proportions test and Mann-Whitney test); 2 wpi: n = 19 neurons from 6 mice; 4 wpi: n = 22 neurons from 11 mice; 8–9 wpi: n = 24 neurons from 9 mice; resident: n = 22 neurons from 16 mice.

their dynamic sensory response profiles. To this end, when imaging conditions allowed, we also imaged in vivo and reconstructed the complete dendritic morphology of the recorded neurons from the different age groups (Figure 5A).

Consistent with our previously published data sets (Livneh et al., 2009; Livneh and Mizrahi, 2011; Mizrahi, 2007), the total dendritic length and the total number of branch points of adult-born neurons increased from 2 wpi to 8–9 wpi (Figure 5B). Since total dendritic length is a structural correlate of input resistance, this observation is consistent with the reported decrease in input resistance during development (Grubb et al., 2008). Higher input resistance potentially increases the excitability of neurons, thus making them more sensitive to synaptic input (e.g., Kernell, 1966). Thus, both input resistance measurements and neuronal morphology cannot explain the enhanced sensory responsiveness and promiscuity of young adult-born neurons. For example, 4 wpi neurons were significantly more responsive to odors than 8–9 wpi neurons (Figure 3), yet their dendritic morphology was not significantly different (Figure 5B). Additionally, as compared to 8–9 wpi neurons, 4 wpi and 2 wpi neurons had relatively similar response profiles (Figure 3) but significantly different dendritic morphology (Figure 5B). Because of the variability of neuronal morphology and PGN subtype heterogeneity in all groups, we further explored this issue on a cell-by-cell basis. We found no significant correlation between total dendritic length and the number of odors that activated each neuron across all age groups ($R = -0.13$, $p = 0.65$, $n = 14$ neurons, Pearson Correlation). Furthermore, dendritic structure could not predict responsiveness at any specific age group (data not shown). This further strengthens the conclusion that the increased responsiveness of young adult-born neurons is not a direct consequence of their dendritic structure alone.

We next considered an alternative explanation to the increased responsiveness at young neuronal age. We hypothesized that young neurons' increased responsiveness may arise from a different pattern of synaptic connectivity as compared to that of mature neurons. One possibility is that increased responsiveness would be acquired by receiving inputs,

originating directly or indirectly, from additional glomeruli. To examine this issue, we used the ISI maps of glomerular activation and examined them in relation to single-cell activity (Fantana et al., 2008; Luo and Katz, 2001). Specifically, we calculated ISI-electrophysiology correlation maps by constructing a pixel-by-pixel correlation matrix (uncentered correlation coefficient; see Supplemental Experimental Procedures) between each responsive neuron and its own surrounding glomerular activation pattern (Figures S4A–S4C). For qualitative evaluation of the entire data set per group, we constructed an average map for each experimental group by calculating a "neuron-centric" average per pixel (Figure 5C). This type of analysis reveals the spatial origin of inputs (both direct and indirect) that possibly drive the neurons that we recorded from (Fantana et al., 2008; Luo and Katz, 2001). We reasoned that if young neurons receive input from additional glomeruli, as compared to mature neurons, they will have higher correlation values with additional glomerular loci. In support of this idea, the average correlation maps of young neurons had higher correlation values in total and also more high-correlation loci surrounding the location of the neurons (Figure 5C).

To obtain a quantitative statistical comparison between the groups, we measured the number of glomerular correlation "hot spots" per neuron at different distances, pooling together mature neurons (8–9 wpi adult-born and resident neurons; Figure 5D). As expected from the sensory response profiles (Figure 3) and from the average maps (Figure 5C), 4 wpi neurons had on average twice as many correlation hot spots at distances of approximately 2–4 glomeruli (300–450 μm). We also corroborated this finding using a different type of analysis quantifying absolute pixel correlation values only, thereby precluding any possible bias due to hot spot identification criteria (Figure 5E). Furthermore, we also used a binary similarity index as an additional ISI-electrophysiology correlation measure, which shows the same trend and thus excludes the possibility that these results are biased by neuronal responsiveness (Figure S4D). These results lead us to suggest that the increased promiscuity of young neurons potentially arises from a differential pattern of synaptic connectivity, possibly via lateral connectivity (Aungst et al., 2003; Tan et al., 2010).

Sensory Enrichment during Development Induces Distinct Response Profiles in Mature Adult-Born Neurons

Since young adult-born neurons are more likely to respond to odors (Figure 3) and are also highly plastic (Kelsch et al., 2009; Livneh et al., 2009; Nissant et al., 2009), we hypothesized that sensory experience during this critical period could have long-lasting effects on their sensory response profiles after maturation. To test this hypothesis, we enriched lentivirus-injected animals with the panel of seven odors during 2–5 wpi (the period of high responsiveness) and then recorded from these mice at 8–9 wpi (Figure 6A). Using this protocol, we recorded from two experimental groups: "enriched adult-born neurons" (8–9 wpi, n = 18 from 8 mice) and "enriched resident neurons" (GFP^- neurons in the same mice; n = 18 from 10 mice).

The odor responsiveness of enriched 8–9 wpi neurons decreased significantly as compared to that of 8–9 wpi neurons from naive mice (Figures 6B and 6C). However, enriched resident neurons were not affected by odor enrichment, as their response profiles were similar to those of resident neurons from naive mice (Figures 6B and 6C). These data show that enrichment affected the sensory response profiles specifically of adult-born neurons, making them significantly more selective (Figure 6C). Additionally, following enrichment, spike rate responses of 8–9 wpi adult-born neurons were exclusively excitatory, while those of enriched resident neurons were both excitatory and inhibitory (but mostly excitatory), similar to resident neurons from naive mice (Figure 6D). Odor enrichment did not affect respiration phase-tuning odor responses of both groups (Figure 6E). Despite the significant effects of odor enrichment on the sensory response profiles of adult-born neurons, their spontaneous firing properties were similar to those of 8–9 wpi neurons from naive mice (Figures S5A–S5D). Additionally, the dendritic morphology of enriched 8–9 wpi neurons was similar to that of 8–9 wpi neurons from naive mice, suggesting that their different response profiles are not a result of altered dendritic development due to enrichment or a bias to a specific morphological subtype (Figure S5E). Here too, functional sampling bias did not affect the results as neurons in both groups were sampled similarly and predominantly in odor-enriched regions (Figure S5F). Furthermore, the response profiles of the glomerular region surrounding the neurons did not predict their response profiles (Figure S5G).

Finally, we tested whether the effects of enrichment were specific to the enriched OB region. To this end, we used a different odor panel of seven odors that do not activate the OB region that we recorded from. We refer to this odor panel as "external," given that it activated only the lateral OB outside our craniotomy recording region, as verified by ISI (Figures 7A and 7B). More specifically, five of the "external" odors activated glomeruli in the dorsolateral OB at this concentration (butyl-formate, ethyl-lactate, acetophenone, isoamyl-acetate, and hexyl-acetate), while two of them did not activate the dorsal OB (limonene and diethyl-maleate). In contrast to the previous enrichment experiment, we now enriched mice with the "external" odor panel (Figure 7A). Similar to the previous experiment, at 8–9 wpi we recorded odor responses to the odors activating the dorsomedial OB ("internal" odors; Figure 2A). We performed ISI mapping on every mouse that we recorded from to verify that all the recorded neurons were at least 0.5 mm away (usually ~1 mm away) from glomeruli activated by the "external" odors. We recorded from 13 adult-born neurons (n = 6 mice). The odor responsiveness and selectivity of 8–9 wpi adult-born neurons enriched with the "external" odors were similar to those of 8–9 wpi from naive mice (Figure 7C). These results show that adult-born neurons developing in nonenriched regions are not affected by odor enrichment in distal regions of the OB. Further, these results imply that the effects of enrichment presented in Figure 6 are odor specific. To our knowledge, these experiments are a first demonstration that adult-born neurons can become functionally distinct from their resident counterparts.

DISCUSSION

To date, the involvement of adult-born neurons in sensory processing has been implicated only indirectly. Experiments

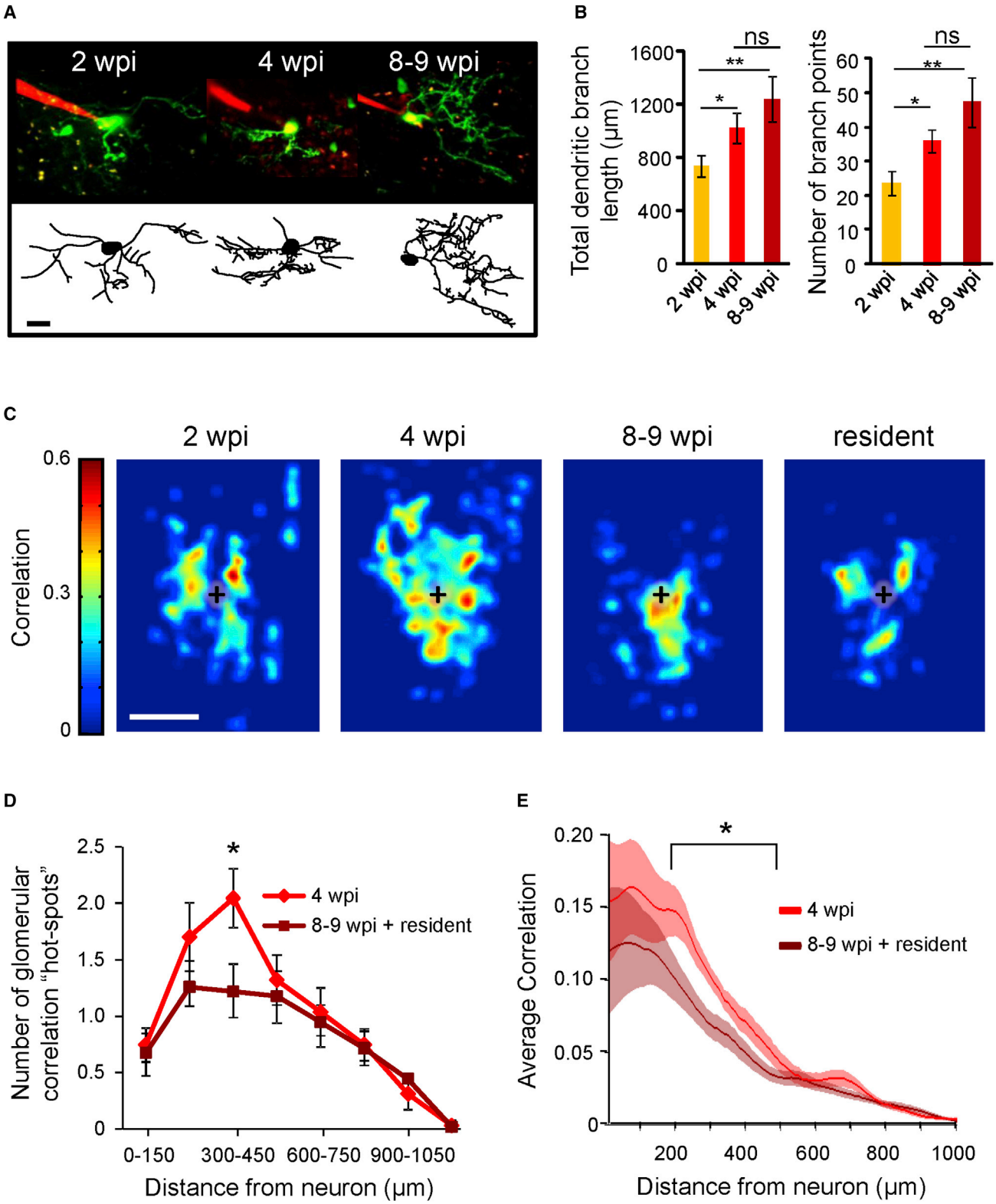

Figure 5. Correlations with Additional Glomeruli, Rather Than Morphology, Are Associated with High Sensitivity in Young Neurons

(A) Examples of GFP-expressing adult-born neurons at different ages. Top: projections of the in vivo two-photon images; bottom: 2D projections of the 3D reconstructions. Scale bar, 20 μm.

(legend continued on next page)

suggesting involvement in sensory processing include olfactory behavioral deficits following reduced or halted adult neurogenesis (Breton-Provencher et al., 2009; Imayoshi et al., 2008; Lazarini et al., 2009; Moreno et al., 2009; Sultan et al., 2010; Valley et al., 2009). Immediate-early gene expression studies provided further indications for sensory-induced activation (Alonso et al., 2006; Magavi et al., 2005) but direct in vivo electrophysiological recordings from adult-born neurons in response to sensory stimulation have not been reported. Our in vivo recordings from adult-born neurons showed that young developing neurons are more sensitive to sensory stimuli, as compared to mature adult-born neurons. Furthermore, sensory enrichment during development caused long-term modification of their sensory response profiles, showing that adult-born neurons can become functionally distinct from their neighbors via an experience-dependent mechanism. This argues against the view that adult neurogenesis serves mainly as a neuronal replacement mechanism and favors its role in dynamically shaping network plasticity.

Transient Period of Enhanced Sensory Promiscuity during Development

Early in their development, adult-born neurons respond to sensory stimuli in a more promiscuous manner, as compared to maturity (Figure 3). It is unclear whether this transient period of sensitivity reflects a mechanism for regulating the neurons' own development per se, or whether it reflects a differential active involvement in sensory processing. In the hippocampus, young and mature adult-born neurons seem to have a different contribution to hippocampal function (Gu et al., 2012; Marín-Burgin et al., 2012; Nakashiba et al., 2012), but no such division has been demonstrated in olfaction. While the active participation in OB odor processing of the young neurons is an attractive and prevalent idea, our results provide a number of arguments against it.

In contrast to the hippocampus, our data appear to favor a different scenario in the OB, in which the circuit initially affects young adult-born neurons without them having any substantial contribution to its function. In this scenario, only after maturation do adult-born neurons contribute to network function in return. The first observation supporting this scenario is the lower number of presynapses of young neurons, as compared to mature ones, suggesting lower potential impact on the circuit (Figures S2A and S2B; Bardy et al., 2010; Kelsch et al., 2008). Second, under control conditions, when no particular sensory events occur, morphology and physiology of young adult-born neurons are distinct and highly dynamic but then naturally recede to become similar to resident neurons (Figure 3; Carleton et al., 2003; Grubb et al., 2008; Kelsch et al., 2008, 2009; Livneh et al., 2009; Livneh and Mizrahi, 2011, 2012; Mizrahi, 2007; Nissant et al., 2009; Whitman and Greer, 2007b). Thus, it seems unbeneficial for a circuit to allow such dynamic neurons to have a prominent role in its immediate function only to be withdrawn later on. Third, our ISI-electrophysiology correlation analysis suggests that the enhanced promiscuity is a result of inputs from additional glomeruli (Figure 5). Thus, young adult-born neurons may receive a different pattern of inputs as compared to their immediate resident neighbors (see also Whitman and Greer, 2007b). Although we cannot rule out that the early developmental period may still serve some different type of glomerular computation, another explanation seems more plausible. Specifically, when more glomeruli provide input to a neuron concomitantly with a period of enhanced plasticity (Kelsch et al., 2009; Livneh et al., 2009; Nissant et al., 2009), it would facilitate a broader selection of possible glomerular inputs through Hebbian and spike-timing-dependent plasticity. Lastly, since "functional fate" is sensitive to the specific sensory experience (Figure 7), it is reasonable to hypothesize that the enhanced promiscuity provides an important developmental phase to promote the unique response selectivity a neuron will acquire. We therefore propose a scenario in which the unique properties of young adult-born neurons are not directly essential for OB function during maturation. Rather, their unique properties during maturation serve their own functional fate by facilitating the selection of the specific synaptic inputs that will determine their function at maturity.

Multiple Functions for Adult-Born Neurons

The functional significance of adult neurogenesis in the OB remains persistently unclear (Lazarini and Lledo, 2011). Chemical ablation, genetic ablation, irradiation-based ablation, and optogenetic activation experiments in the OB have yielded contradictory results in search for a single and specific function of OB adult neurogenesis (Alonso et al., 2012; Breton-Provencher et al., 2009; Imayoshi et al., 2008; Lazarini et al., 2009; Moreno et al., 2009; Sultan et al., 2010; Valley et al., 2009). For example, Imayoshi et al. (2008) found no effects on olfactory discrimination and long-term associative memory following genetic ablation of adult-born neurons. Breton-Provencher et al. (2009) and Sultan et al. (2010) also ablated adult-born neurons but found conflicting results. The latter found impaired olfactory long-term associative memory, while the former found normal long-term memory but impaired short-term memory, both using chemical ablation of neurogenesis. Perhaps, then, adult neurogenesis does not serve only a single predetermined function but can support the circuit in more than one way?

(B) Quantitative morphometric analysis of dendritic structure: total dendritic branch length (left), number of branch points per neuron (right). All values are represented as mean ± SEM. *$p < 0.047$, **$p < 0.009$; ns, not significant, Mann-Whitney test; 2 wpi: n = 7 neurons from 4 mice; 4 wpi: n = 8 neurons from 7 mice; 8–9 wpi: n = 7 neurons from 4 mice.

(C) Average neuron-centered correlation maps (see text for details). Scale bar, 0.5 mm. Black crosses indicate the location of the neurons; 2 wpi: n = 16 neurons from 6 mice; 4 wpi: n = 21 neurons from 11 mice; 8–9 wpi: n = 13 neurons from 6 mice; resident: n = 13 neurons from 12 mice.

(D) Number of glomerular correlation "hot spots" as a function of the distance from the neuron. All values are represented as mean ± SEM. *$p < 0.03$, Mann-Whitney test; 4 wpi: n = 21 neurons from 11 mice; 8–9 wpi: n = 13 neurons from 6 mice; resident: n = 13 neurons from 12 mice.

(E) Average pixel correlation values as a function of the distance from the neuron. All values are represented as mean ± SEM. *$p < 0.04$; ns, not significant, Mann-Whitney test; 4 wpi: n = 21 neurons from 11 mice; 8–9 wpi: n = 13 neurons from 6 mice; resident: n = 13 neurons from 12 mice. See also Figures S3 and S4.

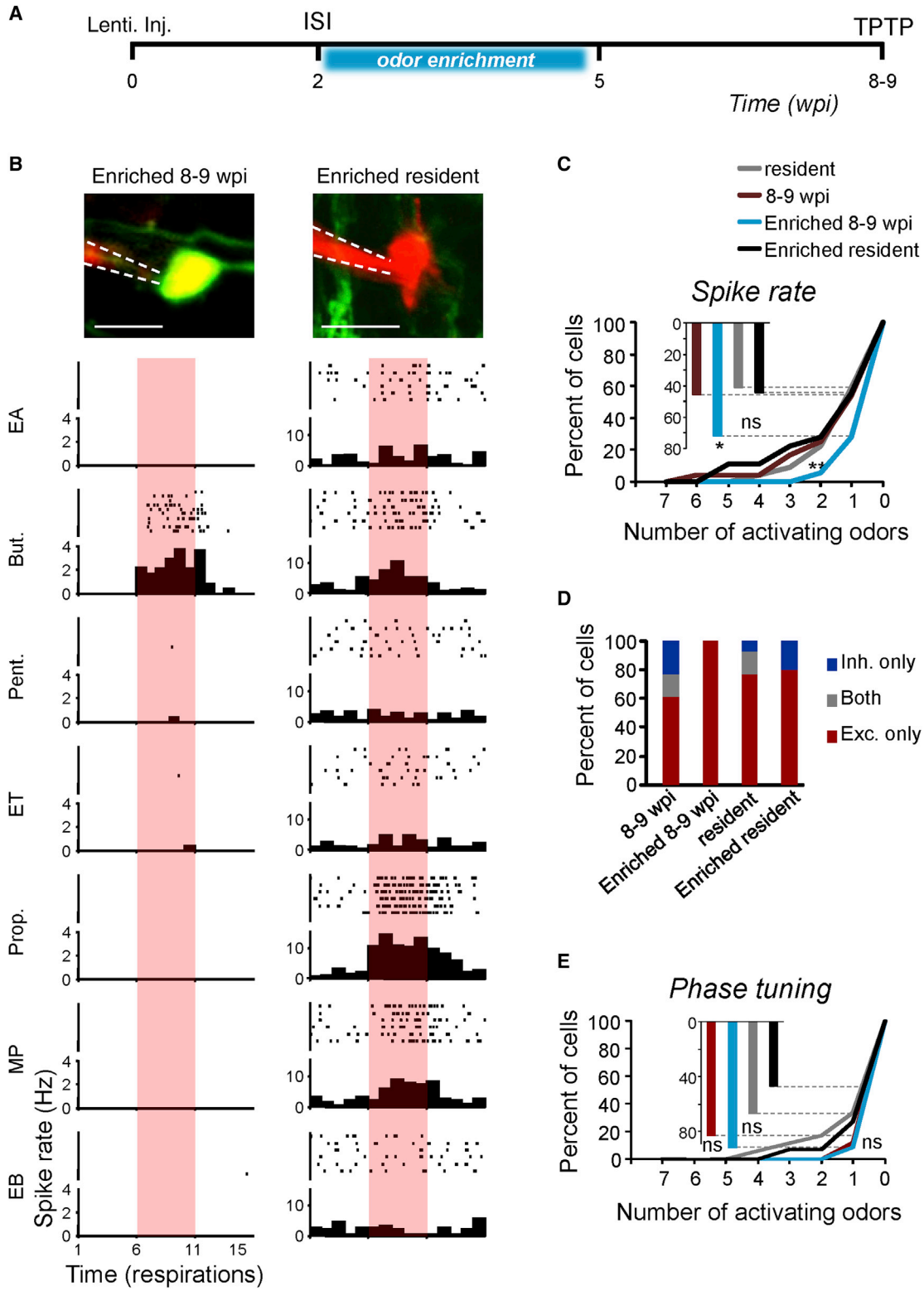

Figure 6. Sensory Experience during Development Induces Distinct Response Profiles

(A) Schematic depiction of the experimental timeline (see text).

(B) Examples of neurons from the two enriched experimental groups. Top: two-photon micrographs of the recorded neurons. Bottom: raster plots (time in seconds) and PSTHs (time in respirations) of each of the seven odors. Scale bar, 10 μm.

(legend continued on next page)

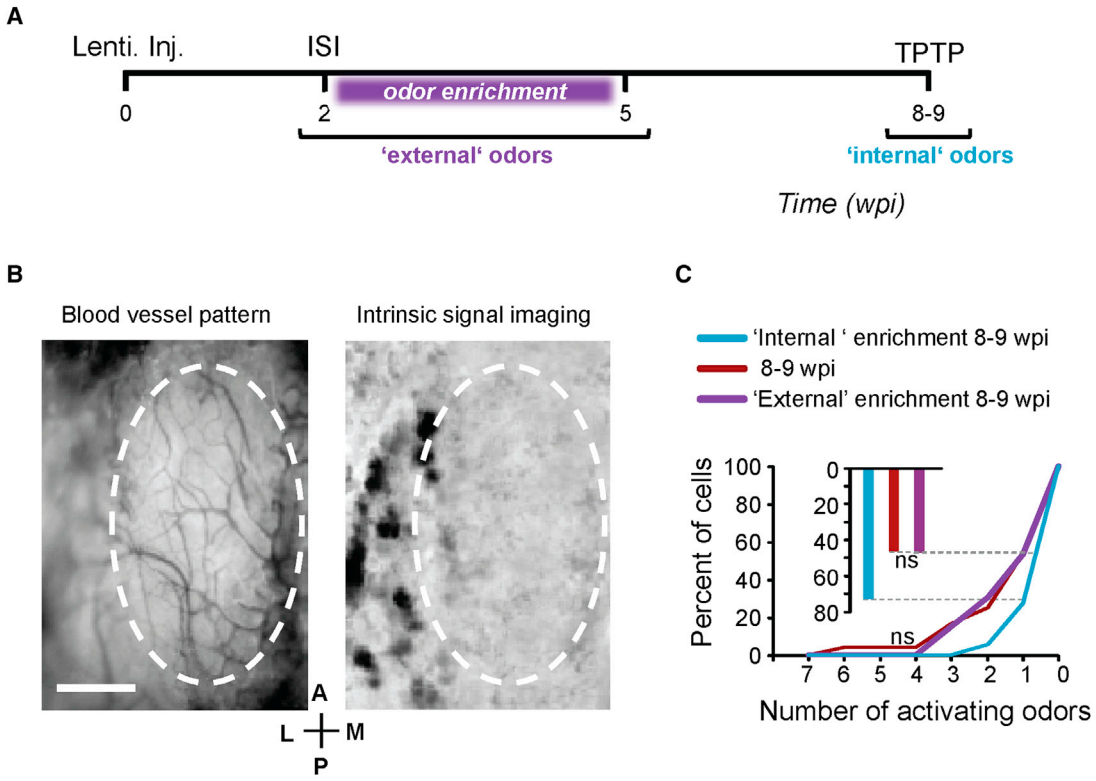

Figure 7. Enrichment-Induced Changes in Response Profiles Are Specific to the Enriched OB Region

(A) Schematic depiction of the experimental timeline. Note that mice were enriched with "external" odors at 2–5 wpi but were recorded from during stimulation with "internal" odors at 8–9 wpi (see text and Figure 2A for the ISI activation map of the "internal" odors).

(B) Left: blood vessel pattern of the dorsal OB. Right: ISI glomerular activation pattern for all seven "external" odors. The image is a composite sum of the seven individual ISI maps. The dashed line indicates the approximate region of craniotomy used for TPTP. M, medial; L, lateral; A, anterior; P, posterior. Scale bar, 0.5 mm.

(C) Cumulative distribution of the spike rate odor responses. Inset: percentage of cells that did not respond to any odor. "External" enrichment 8–9 wpi versus 8–9 wpi: binomial proportions test ($p = 0.49$) and Mann-Whitney test ($p = 0.48$); ns, not significant. "External" enrichment 8–9 wpi: n = 13 neurons from 6 mice. Note that the "Internal" enrichment 8–9 wpi data are presented in Figure 6 as enriched 8–9 wpi. These data and the 8–9 wpi data are the same as in Figure 6 and are shown for comparison.

We hypothesize that adult-born neurons do not have a predetermined role in OB function. Rather, adult neurogenesis acts as a continuous source of neurons for serving ad hoc, experience-based, circuit demands. As such, adult-born neurons are a readily available pool of neurons with an adaptive functional fate that can be shaped based on specific sensory experience. Our sensory enrichment experiments (Figures 6 and 7) support this idea, as novel stimuli can induce adult-born neurons to acquire more narrowly tuned sensory response profiles at maturity (Figure 6), and these effects are specific to the enrichment odors (Figure 7).

It is well established that newborn neurons become one of many different types of cells, as determined by anatomy and molecular markers. This includes primarily granule cells (GCs) and all known subtypes of PGNs (Ninkovic et al., 2007; Whitman and Greer, 2007a). Thus, different neuronal subtypes are expected to serve distinct OB computations. Consequently, based on our data here, we suggest that sensory experience will shape each adult-born neuron's sensory response profile within the boundaries of the properties of its distinct subtype, which is predetermined by early developmental events (e.g., Hack et al., 2005). For example, for adult-born PGNs, synaptic connectivity

(C) Cumulative distribution of the spike rate odor responses. Inset: percentage of cells that did not respond to any odor. *$p < 0.045$ (enriched 8–9 wpi versus 8–9 wpi), **$p < 0.028$ (enriched 8–9 wpi versus 8–9 wpi); ns, not significant, binomial proportions test and Mann-Whitney test. Enriched 8–9 wpi: n = 18 neurons from 8 mice; enriched resident: n = 18 neurons from 10 mice.

(D) Percentage of cells in each group with either excitatory or inhibitory responses, or both. Enriched 8–9 wpi: n = 5 neurons from 5 mice; enriched resident: n = 10 neurons from 7 mice.

(E) Cumulative distribution of the respiration phase-tuning odor responses. Inset: percentage of cells that did not respond to any odor. There was no significant difference between the groups ($p > 0.16$ for all comparisons; ns, not significant, binomial proportions test and Mann-Whitney test). Enriched 8–9 wpi: n = 18 neurons from 8 mice; enriched resident: n = 18 neurons from 10 mice. Note that 8–9 wpi and resident data are the same as in Figure 3 and are shown for comparison. See also Figure S5.

shaped by sensory experience might determine their intra- or interglomerular function. For GCs, sensory experience might affect the specific identities of their mitral cells synaptic partners and also perhaps the level of centrifugal inputs that they receive (Whitman and Greer, 2007b). Thus, sensory experience might have different effects on different neuronal populations. In our data, we detect relatively consistent effects on a potentially heterogeneous population (Figures S2E and S2F). This suggests that adult-born PGNs, at least to some extent, might have a generally similar pattern of maturation and sensitivity to sensory enrichment. Nevertheless, how each molecular subtype (adult-born or not) contributes to the OB circuit odor processing remains to be discovered.

It has been suggested that adult neurogenesis offers the OB a dramatic form of structural and functional plasticity by the addition of new neurons to the network (Lledo et al., 2006). We build upon this idea and argue that these new neurons can also be induced to acquire different functional fates. Furthermore, the significant but less pronounced plasticity of adult-born GCs and PGNs after they mature (Alonso et al., 2012; Livneh and Mizrahi, 2012) might allow them to undergo additional short-term "fine-tuning" of their response profiles to further adjust to changes in the environment. As such, adult neurogenesis may endow the adult OB with two complementary forms of circuit plasticity—an early long-term plasticity of neuronal functional fate and a later finer plasticity of synaptic strength tuning.

Potential Contribution of Adult-Born PGNs to Odor Coding Plasticity

Here we limited our study to PGNs, which are more readily accessible for two-photon imaging, allowing us to perform targeted recordings of their electrophysiological activity. Another advantage of PGNs is that they form synapses in discrete functional-anatomical units (i.e., glomeruli). This anatomical simplicity enables relatively precise targeting to sensory-active regions and subsequent analysis of their relation with their immediate glomerular input (Figures 5 and 7). Sensory enrichment during adult-born PGNs' development increased their selectivity (Figure 6), but what might be the consequences of this increased selectivity for odor coding?

Sensory enrichment has been shown to enhance olfactory perception (Mandairon and Linster, 2009; Moreno et al., 2009) and concomitantly also to cause long-term changes in the response profiles of OB neurons. Electrophysiological recordings from mitral/tufted cells (M/Ts) revealed decreased responsiveness following enrichment (Buonviso and Chaput, 2000). Furthermore, a recent study using time-lapse calcium imaging of mitral cells demonstrated that this decreased responsiveness persists for several weeks, without a complementary elevation in GC responses, suggesting that other inhibitory neurons might be involved (Kato et al., 2012). Indeed, the involvement of glomerular inhibitory interneurons in similar enrichment-induced effects has been directly demonstrated in *Drosophila* (Sachse et al., 2007). Finally, the enrichment-induced enhancement of olfactory perception was found to require adult neurogenesis (Moreno et al., 2009). Together with our results (Figure 6), these studies suggest that at least some long-term effects of odor enrichment on M/T response profiles might be due to changes in adult-born PGNs' response profiles. Interestingly, GABAergic short-axon cells have been recently shown to provide interglomerular lateral inhibition (Whitesell et al., 2013). As such, one possibility is that the enriched adult-born PGNs provide reduced inhibition onto GABAergic short-axon cells, resulting in increased inhibition onto M/Ts via tufted cells, thus reducing their responsiveness. Notably, because we do not know the postsynaptic partners of each neuron that we recorded from, and resident PGNs have been shown to provide inhibition to all glomerular layer neurons and to M/Ts (e.g., Aroniadou-Anderjaska et al., 2000; Gire and Schoppa, 2009; Murphy et al., 2005), this hypothesis remains to be tested directly. Future work, using cell-type-specific molecular tools (Luo et al., 2008), should allow direct tracking of the distinct functional and synaptic connectivity changes that each neuron will undergo to facilitate adjustment to the ever-changing environment of the mammalian OB.

EXPERIMENTAL PROCEDURES

Animals and Lentivirus Injections

We used C57BL/6 mice (8–13 weeks old at the beginning of the experiment). Animal care and experiments were approved by the Hebrew University Animal Care and Use Committee. To label adult-born neurons, we injected a lentivirus encoding either GFP (for targeted recordings) or Syp-GFP (for labeling putative presynapses; Kelsch et al., 2008; Kopel et al., 2012) into the RMS, as described previously (Bardy et al., 2010; Livneh and Mizrahi, 2012; Nissant et al., 2009). See Supplemental Experimental Procedures for further details.

Two-Photon-Targeted Recordings from Adult-Born Neurons

We anesthetized mice with an intraperitoneal injection of ketamine and medetomidine (100 mg/kg and 0.83 mg/kg, respectively). We performed a craniotomy (~1 × 2 mm) over the OB while ensuring that the dura remained intact and surrounded the OB with a recording well made of dental cement. We placed the animals under the microscope in a custom-made stereotaxic device for two-photon imaging. We performed imaging using an Ultima two-photon microscope (Prairie Technologies), with a 16× water-immersion objective lens (0.8 NA; CF175, Nikon), and delivered two-photon excitation (920 nm) with a DeepSee femtosecond laser (Spectraphysics).

We obtained targeted cell-attached recordings using two-photon visual guidance (Komai et al., 2006). We performed all recordings in the glomerular layer (depth 20–80 μm) and verified for each recorded cell that its cell body was indeed in the glomerular layer by filling it by electroporation of the electrode dye (e.g., Figure 1B). For the resident neurons group, we collected data by "shadow patching" (Kitamura et al., 2008) or "blind" patching in the vicinity of GFP-labeled cells and filled the recorded neuron's soma after the recording to verify that it was in the glomerular layer (e.g., Figures 3A and 6B). For further details of electrophysiological recordings and their data analysis, see Supplemental Experimental Procedures.

Odor Delivery and Enrichment

We used a custom-made seven-channel olfactometer. To avoid any cross-contamination between odorants, we used separate tubing for each channel, from the odor vial to the animal's nose. We presented odors at a final concentration of 50 ppm for 2 s, with an interstimulus interval of 18 s, usually seven to ten trials (at least four trials) in pseudorandom order. We monitored the animal's respiration throughout the experiment by a low pressure sensor (1-INCH-D1-4V-MINI, "All sensors") connected to a thin stainless steel tubing (OD 0.7 mm) and placed at the entrance of the animals' contralateral nostril. We identified inhalation onset using an analog converter (window discriminator) and used a custom-written MATLAB program to trigger odor delivery at inhalation onset.

Odor-enriched mice were housed in their home cages with a tea ball, containing all seven odors (either the "internal" or "external" enrichment odors), hanging from the top of the cage following standard odor enrichment

procedures (Moreno et al., 2009; Rochefort et al., 2002). This tea ball contained seven swabs, each soaked with 150 μl of 50 ppm of one of the seven odors in our enrichment panel. We replaced odorants daily, approximately every 24 hr. The tea ball was present in the cage 2–5 wpi. See Supplemental Experimental Procedures for further details.

Intrinsic Signal Imaging and Data Analysis

ISI of the dorsal surface of the OB was performed using an Imager 3001 system (Optical Imaging) via thinned bone, as described previously (Livneh et al., 2009). We used the surface blood vessel pattern to align ISI maps with two-photon images, as described previously (Livneh et al., 2009; Livneh and Mizrahi, 2012).

We analyzed ISI maps offline, using custom-written scripts in MATLAB. We obtained the normalized ISI signal by $\Delta R/R = (R_{odor} - R_{air})/R_{air}$; where R_{odor} is the ISI signal during the last 2 s of the 4 s odor presentation, and R_{air} is the ISI signal during the 2 s before odor presentation, averaging four trials. We filtered the $\Delta R/R$ image to remove contamination from a large-scale hemodynamic signal by subtracting a copy convolved with a Gaussian spatial kernel (STD = 315 μm). For quantitative analysis, we set the threshold for activation at 1.65 SDs above the mean signal. This image processing yields ISI maps that reflect mostly olfactory receptor neuron input to the OB (Fantana et al., 2008; Meister and Bonhoeffer, 2001; Soucy et al., 2009; Uchida and Mainen, 2003; Wachowiak and Cohen, 2003). See Supplemental Experimental Procedures for further details.

Further details regarding immunohistochemistry, confocal microcopy, and morphological analysis can be found in the Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and five figures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2014.01.007.

AUTHOR CONTRIBUTIONS

Y.L. and A.M. designed the experiments. Y.L. performed the experiments and analyzed the data with contributions from Y.A. Y.L. and A.M. wrote the manuscript.

ACKNOWLEDGMENTS

We thank the members of the Mizrahi Lab and D. Nachmani for discussions and critical reading of the manuscript. We thank N. Book, A. Vinograd, and G. Tasaka for technical assistance. Y.L. is supported by the Adams Fellowship Program of the Israel Academy of Sciences and Humanities. This work was supported by the European Research Council Grant (number 203994) and Israeli Science Foundation grant (number 1284/10) to A.M.

Accepted: December 17, 2013
Published: February 6, 2014

REFERENCES

Adam, Y., and Mizrahi, A. (2010). Circuit formation and maintenance—perspectives from the mammalian olfactory bulb. Curr. Opin. Neurobiol. *20*, 134–140.

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

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

Altman, J. (1969). Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J. Comp. Neurol. *137*, 433–457.

Aroniadou-Anderjaska, V., Zhou, F.M., Priest, C.A., Ennis, M., and Shipley, M.T. (2000). Tonic and synaptically evoked presynaptic inhibition of sensory input to the rat olfactory bulb via GABA(B) heteroreceptors. J. Neurophysiol. *84*, 1194–1203.

Aungst, J.L., Heyward, P.M., Puche, A.C., Karnup, S.V., Hayar, A., Szabo, G., and Shipley, M.T. (2003). Centre-surround inhibition among olfactory bulb glomeruli. Nature *426*, 623–629.

Bagley, J., LaRocca, G., Jimenez, D.A., and Urban, N.N. (2007). Adult neurogenesis and specific replacement of interneuron subtypes in the mouse main olfactory bulb. BMC Neurosci. *8*, 92.

Bardy, C., Alonso, M., Bouthour, W., and Lledo, P.M. (2010). How, when, and where new inhibitory neurons release neurotransmitters in the adult olfactory bulb. J. Neurosci. *30*, 17023–17034.

Bathellier, B., Buhl, D.L., Accolla, R., and Carleton, A. (2008). Dynamic ensemble odor coding in the mammalian olfactory bulb: sensory information at different timescales. Neuron *57*, 586–598.

Breton-Provencher, V., Lemasson, M., Peralta, M.R., 3rd, and Saghatelyan, A. (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*, 15245–15257.

Buonviso, N., and Chaput, M. (2000). Olfactory experience decreases responsiveness of the olfactory bulb in the adult rat. Neuroscience *95*, 325–332.

Cang, J., and Isaacson, J.S. (2003). In vivo whole-cell recording of odor-evoked synaptic transmission in the rat olfactory bulb. J. Neurosci. *23*, 4108–4116.

Carleton, A., Petreanu, L.T., Lansford, R., Alvarez-Buylla, A., and Lledo, P.M. (2003). Becoming a new neuron in the adult olfactory bulb. Nat. Neurosci. *6*, 507–518.

Dhawale, A.K., Hagiwara, A., Bhalla, U.S., Murthy, V.N., and Albeanu, D.F. (2010). Non-redundant odor coding by sister mitral cells revealed by light addressable glomeruli in the mouse. Nat. Neurosci. *13*, 1404–1412.

Fantana, A.L., Soucy, E.R., and Meister, M. (2008). Rat olfactory bulb mitral cells receive sparse glomerular inputs. Neuron *59*, 802–814.

Fukunaga, I., Berning, M., Kollo, M., Schmaltz, A., and Schaefer, A.T. (2012). Two distinct channels of olfactory bulb output. Neuron *75*, 320–329.

Ge, S., Yang, C.H., Hsu, K.S., Ming, G.L., and Song, H. (2007). A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. Neuron *54*, 559–566.

Gire, D.H., and Schoppa, N.E. (2009). Control of on/off glomerular signaling by a local GABAergic microcircuit in the olfactory bulb. J. Neurosci. *29*, 13454–13464.

Grubb, M.S., Nissant, A., Murray, K., and Lledo, P.M. (2008). Functional maturation of the first synapse in olfaction: development and adult neurogenesis. J. Neurosci. *28*, 2919–2932.

Gu, Y., Arruda-Carvalho, M., Wang, J., Janoschka, S.R., Josselyn, S.A., Frankland, P.W., and Ge, S. (2012). Optical controlling reveals time-dependent roles for adult-born dentate granule cells. Nat. Neurosci. *15*, 1700–1706.

Hack, M.A., Saghatelyan, A., de Chevigny, A., Pfeifer, A., Ashery-Padan, R., Lledo, P.M., and Götz, M. (2005). Neuronal fate determinants of adult olfactory bulb neurogenesis. Nat. Neurosci. *8*, 865–872.

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

Kato, H.K., Chu, M.W., Isaacson, J.S., and Komiyama, T. (2012). Dynamic sensory representations in the olfactory bulb: modulation by wakefulness and experience. Neuron *76*, 962–975.

Kelsch, W., Lin, C.W., and Lois, C. (2008). Sequential development of synapses in dendritic domains during adult neurogenesis. Proc. Natl. Acad. Sci. USA *105*, 16803–16808.

Kelsch, W., Lin, C.W., Mosley, C.P., and Lois, C. (2009). A critical period for activity-dependent synaptic development during olfactory bulb adult neurogenesis. J. Neurosci. *29*, 11852–11858.

Kelsch, W., Sim, S., and Lois, C. (2010). Watching synaptogenesis in the adult brain. Annu. Rev. Neurosci. *33*, 131–149.

Kernell, D. (1966). Input resistance, electrical excitability, and size of ventral horn cells in cat spinal cord. Science *152*, 1637–1640.

Kitamura, K., Judkewitz, B., Kano, M., Denk, W., and Häusser, M. (2008). Targeted patch-clamp recordings and single-cell electroporation of unlabeled neurons in vivo. Nat. Methods *5*, 61–67.

Komai, S., Denk, W., Osten, P., Brecht, M., and Margrie, T.W. (2006). Two-photon targeted patching (TPTP) in vivo. Nat. Protoc. *1*, 647–652.

Kopel, H., Schechtman, E., Groysman, M., and Mizrahi, A. (2012). Enhanced synaptic integration of adult-born neurons in the olfactory bulb of lactating mothers. J. Neurosci. *32*, 7519–7527.

Kosaka, K., and Kosaka, T. (2007). Chemical properties of type 1 and type 2 periglomerular cells in the mouse olfactory bulb are different from those in the rat olfactory bulb. Brain Res. *1167*, 42–55.

Lazarini, F., and Lledo, P.M. (2011). Is adult neurogenesis essential for olfaction? Trends Neurosci. *34*, 20–30.

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

Lepousez, G., Valley, M.T., and Lledo, P.M. (2013). The impact of adult neurogenesis on olfactory bulb circuits and computations. Annu. Rev. Physiol. *75*, 339–363.

Livneh, Y., and Mizrahi, A. (2011). Long-term changes in the morphology and synaptic distributions of adult-born neurons. J. Comp. Neurol. *519*, 2212–2224.

Livneh, Y., and Mizrahi, A. (2012). Experience-dependent plasticity of mature adult-born neurons. Nat. Neurosci. *15*, 26–28.

Livneh, Y., Feinstein, N., Klein, M., and Mizrahi, A. (2009). Sensory input enhances synaptogenesis of adult-born neurons. J. Neurosci. *29*, 86–97.

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

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

Luo, M., and Katz, L.C. (2001). Response correlation maps of neurons in the mammalian olfactory bulb. Neuron *32*, 1165–1179.

Luo, L., Callaway, E.M., and Svoboda, K. (2008). Genetic dissection of neural circuits. Neuron *57*, 634–660.

Macrides, F., and Chorover, S.L. (1972). Olfactory bulb units: activity correlated with inhalation cycles and odor quality. Science *175*, 84–87.

Magavi, S.S., Mitchell, B.D., Szentirmai, O., Carter, B.S., and Macklis, J.D. (2005). Adult-born and preexisting olfactory granule neurons undergo distinct experience-dependent modifications of their olfactory responses in vivo. J. Neurosci. *25*, 10729–10739.

Mandairon, N., and Linster, C. (2009). Odor perception and olfactory bulb plasticity in adult mammals. J. Neurophysiol. *101*, 2204–2209.

Marín-Burgin, A., Mongiat, L.A., Pardi, M.B., and Schinder, A.F. (2012). Unique processing during a period of high excitation/inhibition balance in adult-born neurons. Science *335*, 1238–1242.

Meister, M., and Bonhoeffer, T. (2001). Tuning and topography in an odor map on the rat olfactory bulb. J. Neurosci. *21*, 1351–1360.

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

Mizrahi, A., Lu, J., Irving, R., Feng, G., and Katz, L.C. (2006). In vivo imaging of juxtaglomerular neuron turnover in the mouse olfactory bulb. Proc. Natl. Acad. Sci. USA *103*, 1912–1917.

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

Murphy, G.J., Darcy, D.P., and Isaacson, J.S. (2005). Intraglomerular inhibition: signaling mechanisms of an olfactory microcircuit. Nat. Neurosci. *8*, 354–364.

Nakashiba, T., Cushman, J.D., Pelkey, K.A., Renaudineau, S., Buhl, D.L., McHugh, T.J., Rodriguez Barrera, V., Chittajallu, R., Iwamoto, K.S., McBain, C.J., et al. (2012). Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion. Cell *149*, 188–201.

Ninkovic, J., Mori, T., and Götz, M. (2007). Distinct modes of neuron addition in adult mouse neurogenesis. J. Neurosci. *27*, 10906–10911.

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

Parrish-Aungst, S., Shipley, M.T., Erdelyi, F., Szabo, G., and Puche, A.C. (2007). Quantitative analysis of neuronal diversity in the mouse olfactory bulb. J. Comp. Neurol. *501*, 825–836.

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

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

Sachse, S., Rueckert, E., Keller, A., Okada, R., Tanaka, N.K., Ito, K., and Vosshall, L.B. (2007). Activity-dependent plasticity in an olfactory circuit. Neuron *56*, 838–850.

Saghatelyan, A., Roux, P., Migliore, M., Rochefort, C., Desmaisons, D., Charneau, P., Shepherd, G.M., and Lledo, P.M. (2005). Activity-dependent adjustments of the inhibitory network in the olfactory bulb following early postnatal deprivation. Neuron *46*, 103–116.

Smear, M., Shusterman, R., O'Connor, R., Bozza, T., and Rinberg, D. (2011). Perception of sniff phase in mouse olfaction. Nature *479*, 397–400.

Soucy, E.R., Albeanu, D.F., Fantana, A.L., Murthy, V.N., and Meister, M. (2009). Precision and diversity in an odor map on the olfactory bulb. Nat. Neurosci. *12*, 210–220.

Sultan, S., Mandairon, N., Kermen, F., Garcia, S., Sacquet, J., and Didier, A. (2010). Learning-dependent neurogenesis in the olfactory bulb determines long-term olfactory memory. FASEB J. *24*, 2355–2363.

Tan, J., Savigner, A., Ma, M., and Luo, M. (2010). Odor information processing by the olfactory bulb analyzed in gene-targeted mice. Neuron *65*, 912–926.

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

Valley, M.T., Mullen, T.R., Schultz, L.C., Sagdullaev, B.T., and Firestein, S. (2009). Ablation of mouse adult neurogenesis alters olfactory bulb structure and olfactory fear conditioning. Front. Neurosci. *3*, 51.

Wachowiak, M., and Cohen, L.B. (2003). Correspondence between odorant-evoked patterns of receptor neuron input and intrinsic optical signals in the mouse olfactory bulb. J. Neurophysiol. *89*, 1623–1639.

Whitesell, J.D., Sorensen, K.A., Jarvie, B.C., Hentges, S.T., and Schoppa, N.E. (2013). Interglomerular lateral inhibition targeted on external tufted cells in the olfactory bulb. J. Neurosci. *33*, 1552–1563.

Whitman, M.C., and Greer, C.A. (2007a). Adult-generated neurons exhibit diverse developmental fates. Dev. Neurobiol. *67*, 1079–1093.

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

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