A Critical Period for Activity-Dependent Synaptic Development during Olfactory Bulb Adult Neurogenesis

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New neurons integrate in large numbers into the mature olfactory bulb circuit throughout life. The factors controlling the synaptic development of adult-born neurons and their connectivity remain essentially unknown. We examined the role of activity-dependent mechanisms in the synaptic development of adult-born neurons by genetic labeling of synapses while manipulating sensory input or cell-intrinsic excitability. Sensory deprivation induced marked changes in the density of input and output synapses during the period when new neurons develop most of their synapses. In contrast, when sensory deprivation started after synaptic formation was complete, input synapses increased in one domain without detectable changes in the other dendritic domains. We then investigated the effects of genetically raising the intrinsic excitability of new neurons on their synaptic development by delivering a voltage-gated sodium channel that triggers long depolarizations. Surprisingly, genetically increasing excitability did not affect synaptic development but rescued the changes in glutamatergic input synapses caused by sensory deprivation. These experiments show that, during adult neurogenesis in the olfactory bulb, synaptic plasticity is primarily restricted to an early period during the maturation of new neurons when they are still forming synapses. The addition of cells endowed with such an initial short-lived flexibility and long-term stability may enable the processing of information by the olfactory bulb to be both versatile and reliable in the face of changing behavioral demands.

Introduction

The main olfactory bulb (OB) in the mammalian brain receives new neurons throughout life (Altman, 1962; Lois and Alvarez-Buylla, 1993; Luskin, 1993). All new neurons added to the adult olfactory bulb are interneurons (Lledo et al., 2008); ~95% of these are granule cell neurons (GCs), and the rest are periglomerular neurons. Considerable progress has been made recently in identifying the factors that regulate the specification of the different subtypes of adult-born interneurons in the olfactory bulb (Hack et al., 2005; Kelsch et al., 2007; Merkle et al., 2007; Batista-Brito et al., 2008). In contrast, relatively little is known about the mechanisms that regulate the synaptic development of adult-born neurons and their connectivity within the mature circuit.

Neuronal activity is thought to be an important factor shaping the wiring of new neurons. In neonatal animals, sensory deprivation reduces the survival of new GCs (Brunjes, 1994; Saghatelian et al., 2005) and triggers a decrease in spine density and excitatory inputs (Saghatelian et al., 2005), whereas preexisting, mature GCs maintain their spine density (Frazier-Cierpial and Brunjes, 1989; Saghatelian et al., 2005). Adult-born GCs also have a critical period during their differentiation such that sensory deprivation reduces the survival of new GCs mainly in the third and fourth week after new neurons are

born in the subventricular zone (SVZ) (Petreanu and Alvarez-Buylla, 2002; Yamaguchi and Mori, 2005). Before this critical period, the survival and dendritic growth of new GCs is independent of sensory input (Petreanu and Alvarez-Buylla, 2002). Interestingly, the beginning of the critical period for survival [~14 d after the birth of a new neuron (Yamaguchi and Mori, 2005)] coincides with the time when glutamatergic input synapses start to develop in adult-born GCs (Kelsch et al., 2008).

Here we investigate whether, in addition to the critical period for survival, there exists a critical period during which the synapses of new neurons are plastic. Toward this goal, we used a genetic labeling technique that allowed us to examine how synaptic development is affected by manipulating sensory input and intrinsic excitability of adult-born neurons. To reduce sensory input, we performed unilateral naris occlusion and observed that adult-born neurons that developed in a sensory-deprived bulb experienced synaptic losses in their distal and basal dendritic domains and gain of input sites in the proximal domain. In contrast, when sensory deprivation started after their synaptic development was complete, adult-born GCs only gained synapses in the proximal domain, with no changes in the other domains. Next we examined whether synapse development was affected by manipulating the intrinsic excitability of new GCs. To increase cell-intrinsic neuronal excitability, we genetically delivered NaChBac, a bacterial voltage-gated sodium channel (Ren et al., 2001; Luan et al., 2006; Nitabach et al., 2006), into the progenitor cells in the SVZ that give rise to new GCs. Interestingly, this manipulation did not induce detectable synaptic changes in new neurons generated in adult animals under normal conditions but rescued the changes in glutamatergic input synapses induced by sensory deprivation.

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Figure 1. Sensory deprivation changes glutamatergic input synapse development in adult-born GCs. **A**, Progenitor cells were infected with retroviruses in the SVZ in combination with unilateral naris occlusion either at the same day or after synaptic development was complete (starting at 42 dpi). Genetically labeled GCs were examined at different days after infection. **B**, GCs have three main dendritic domains: a basal and a proximal domain that receive glutamatergic input synapses and a distal dendritic domain that contains recurrent input and output synapses. **C**, At 28 dpi, PSDG⁺ clusters were examined in adult-born GCs in sensory-deprived (starting on the day of retroviral labeling) or contralateral control olfactory bulbs. To attribute PSDG⁺ clusters (green) to a particular GC, dendritic morphology was visualized by red dye-labeled GFP immunofluorescence against the diffuse PSD-95:GFP present in the cytosol that was otherwise undetectable. The three main dendritic domains were analyzed separately (from top): distal, proximal, or basal domain. Scale bar, 10 μ m.

These observations indicate that activity-dependent increases or decreases in synaptic densities are primarily restricted to an early period during the maturation of new neurons when they are still forming synapses.

Materials and Methods

Generation of retroviral vectors. Recombinant retroviral vectors under the control of the Rous sarcoma virus (RSV) promoter for PSDG (PSD-95:GFP) and SypG (synaptophysin:GFP) (*Mpsdg* and *Msypg*) were prepared and stored as described previously (Kelsch et al., 2008). All constructs described here were generated by inserting the following cDNAs or cassettes downstream from the RSV promoter in the Molar retroviral vector: *Mmcherry*, mCherry cDNA; *Mnachbacg*, a fusion protein between the N terminus of NaChBac and the C terminus of green fluorescent protein (GFP); *Mpsdg:2A:nachbac*, a bicistronic cassette encoding a fusion between postsynaptic density 95 (PSD-95) and GFP linked by a picornavirus 2A sequence to the NaChBac cDNA; *Msypg:2A:nachbac*, a bicistronic cassette encoding a fusion between synaptophysin and GFP linked by a picornavirus 2A sequence to the NaChBac cDNA; *Mpsdg:2A:e191k*, a bicistronic cassette encoding a fusion between PSD-95 and GFP linked by a picornavirus 2A sequence to the nonconducting e191k mutant of NaChBac.

Retroviral labeling in vivo. All experiments were performed with 2-month-old Sprague Dawley rats. All animal procedures were approved by the local Animal Welfare Committee and National Institutes of Health guidelines. Retroviral injections were performed as described recently (Kelsch et al., 2008). The stereotaxic coordinates are in millimeters in reference to bregma: anterior, +1.2; lateral, $\pm$ 1.6; ventral, -3.1 . Animals were kept in a 12 h daylight cycle and under the same housing conditions.

Olfactory deprivation. For sensory deprivation experiments, unilateral surgical naris occlusion was performed on the day of intracerebral injection of viruses *Mpsdg*, *Msypg*, and *Mpsdg:2A:nachbac* into the subventricular zone. On the day of surgery, adult rats obtained a single intraperitoneal injection of bromodeoxyuridine (BrdU) (100 mg/kg bodyweight) to monitor the success of sensory deprivation by an external criterion (Yamaguchi and Mori, 2005). Only animals were included in the analysis of early deprivation that displayed a significant decrease in BrdU-positive (BrdU⁺) cells in the deprived bulb (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). As expected (Yamaguchi and Mori, 2005), after late deprivation, no decrease in BrdU⁺ cells in the deprived bulb (BrdU injection at the day of retroviral infection) was observed (0.98 ± 0.07), but the deprived site displayed a

strong reduction in c-Fos expression in the granule cell layer and reduction of tyrosine hydroxylase expression in the glomerular layer (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). For this selection, process immunofluorescence was performed with antibodies against BrdU (Accurate), c-Fos (Oncogene), and tyrosine hydroxylase (Millipore Bioscience Research Reagents).

Analysis of synaptic markers. Tissue processing and analysis of SypG⁺ and PSDG⁺ clusters was performed as described previously (Kelsch et al., 2008). In brief, 50- μ m-thick coronal slices were incubated in primary rabbit anti-GFP (1:4000; Millipore Bioscience Research Reagents) and Alexa-555 secondary antibodies (1:750; Invitrogen). Confocal image stacks were acquired using an Olympus Fluoview confocal microscope (60 $\times$ oil-immersion lens, 1.4 numerical aperture) (pixel size, $0.23 \times 0.23 \mu$ m, 1024×1024 pixel) and with z-step of 0.25 μ m (80–150 sections). Maximal intensity projections were used to measure the density of PSDG⁺ or SypG⁺ clusters of a dendritic segment with the integrated morphometry analysis of MetaMorph software (Molecular Devices).

Statistical analysis. Each analyzed data point [e.g., sensory deprivation and basal domain, 17 d postinfection (dpi)] contained normally distributed PSDG⁺ cluster densities from 14 cells. GCs with deep and superficial dendritic targeting in the external plexiform layer (Kelsch et al., 2007) showed the same activity-dependent plasticity in their dendritic domains (data not shown); therefore, presented data were pooled. Statistical significance was determined using a Student's *t* test for pairwise comparisons at the same days postinfection.

Electrophysiological recordings. Whole-cell recordings were performed as described previously (Kelsch et al., 2007, 2008). In brief, 350 μ m horizontal acute slices were prepared from adult olfactory bulbs and recovered in recording solution (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 1 MgCl₂, 2 CaCl₂, and 20 glucose, pH 7.3 (312 mOsm). Fluorescence-guided whole-cell patch-clamp recordings were performed and analyzed with a MultiClamp 700B amplifier and pClamp9 software (Molecular Devices). The pipette solution contained the following (in mM): 2 NaCl, 4 KCl, 130 Kgluconate, 10 HEPES, 0.2 EGTA, 4 ATP-Mg, 0.3 GTP-Tris, and 14 phosphocreatine, pH 7.3 with KOH. Access resistance was $<20 \text{ M}\Omega$, and junction potential was not corrected. To determine the current–voltage relationship of NaChBac-expressing GCs, 1 μ M tetrodotoxin was used. Because fluorescence of the fusion protein was too weak to detect constructs containing both NaChBac and a synaptic marker in acute slices, *in vitro* retroviral expression in human embryonic kidney cell lines was used to confirm that the current was pre-

served. A slow inactivating inward current was activated by depolarization as described previously (Ren et al., 2001) for *Mpsdg:2A:nachbac* and *Msyng:2A:nachbac* but not for mutant *Mpsdg:2A:e191k* (data not shown).

Results

Adult-generated neurons display different synaptic changes in specific dendritic domains in response to sensory deprivation

To determine how neuronal activity affects the synaptic development of adult-born neurons in the rat olfactory bulb, we blocked sensory input to the bulb by performing unilateral naris occlusion (Fig. 1A) and compared the synaptic structure and organization of GCs in the deprived and contralateral control olfactory bulb. We measured the development of glutamatergic input synapses of new adult-born GCs using PSDG, a genetic marker consisting of a fusion protein between PSD-95 and GFP. PSD-95 is a protein localized to the postsynaptic density of glutamatergic input synapses (Sheng, 2001), and PSDG delivered into new neurons with retroviral vectors (*Mpsdg*) can be used to genetically label these synapses (Niell et al., 2004; Gray et al., 2006; Kelsch et al., 2008; Livneh et al., 2009). We have shown previously that expression of PSDG at the modest levels yielded by retroviral expression does not alter synaptic properties as measured by electrophysiological methods (Kelsch et al., 2008). To analyze the development of output synapses, we used SypG, a fusion protein between synaptophysin and GFP. Synaptophysin is a protein localized to presynaptic neurotransmitter vesicles (Südhof and Jahn, 1991), and SypG expressed with retroviral vectors (*Msyng*) can be used to genetically label output synapses (Li and Murthy, 2001; Meyer and Smith, 2006; Kelsch et al., 2008). We examined SypG⁺ cluster densities at 28 dpi, once GCs had completed their maturation and SypG⁺ clusters were confined to spines of the distal dendritic domain (supplemental Fig. 1, available at www.jneurosci.org as supplemental material).

GCs are axonless inhibitory interneurons, and their synapses are distributed in three dendritic domains, known as basal, proximal, and distal dendritic domains (Fig. 1B). GCs receive glutamatergic input synapses through the basal, proximal, and distal domains. The distal dendritic domain of GCs contains both input and output synapses, whereas the basal and proximal dendritic domains do not release neurotransmitter (Mori, 1987).

Blocking sensory input during a period in which new neurons are undergoing synaptic development, between 14 and 28 dpi, led to reductions in the density of PSDG⁺ input synapses (Fig. 1C) in the distal and basal dendritic domains (Fig. 2A). Similarly, the density of SypG⁺ output synapses in the distal dendritic domain decreased significantly under sensory deprivation (Fig. 3). Interestingly, sen-

Figure 2. Sensory deprivation changes glutamatergic synapses in specific dendritic domains during a critical period. **A**, Scatter plot and mean density of PSDG⁺ clusters (clusters per micrometer) of adult-born GCs in sensory-deprived (starting on the day of retroviral labeling) and contralateral control (red and black circles, respectively) olfactory bulbs at different days after infection. Statistical significance is only indicated if $p < 0.05$ (t test). The bottom graph shows the ratio of the mean cluster density of sensory-deprived over control GCs in a specific domain at a given day after infection. **B**, When sensory deprivation started after synaptic development was complete, the mean density of PSDG⁺ clusters (clusters per micrometer) only changed in the proximal domain (unilateral naris occlusion at 42 dpi and examined at 63 dpi).

sory deprivation during synaptic development increased the density of PSDG⁺ input synapses in the proximal dendritic domain of new GCs (Fig. 2A). The changes in synaptic densities became statistically significant at the time when new neurons reached maturity (28 dpi) and remained so thereafter until the longest time we monitored the new neurons (56 dpi) (Fig. 2A). For this and the following experiments, data from GCs with different dendritic targeting were pooled as an initial analysis and indicated no significant differences between these subpopulations. These observations indicate that sensory deprivation to the bulb triggered upregulation and downregulation of the synaptic input and outputs of new GCs. In addition, these activity-dependent synaptic changes were not uniform throughout the cells but confined to specific dendritic domains.

We next examined whether changes in synaptic organization also occurred when sensory deprivation was started after synaptic development of new GCs was complete. Toward this end, unilateral naris occlusion was started 42 d after a cohort of genetically labeled new neurons had been born in the SVZ (Fig. 1A). Adult-born GCs de-

Figure 3. Sensory deprivation during synaptic development reduces output synapse density. **A**, At 28 dpi, synaptophysin:GFP⁺ clusters were examined in the distal domain of adult-born GCs from sensory-deprived (starting on the day of retroviral labeling) and contralateral control olfactory bulbs. Scale bars, 10 μ m. **B**, Scatter plot and mean density of SypG⁺ clusters (clusters per micrometer) of adult-born GCs from sensory-deprived (starting on the day of retroviral labeling) and contralateral control (red and black circles, respectively) olfactory bulbs at 28 dpi (t test). **C**, When sensory deprivation started after synaptic development was complete, there were no changes in the mean density of SypG⁺ clusters (clusters per micrometer) (unilateral naris occlusion at 42 dpi and examined at 63 dpi).

prived of sensory input for 3 weeks (between 42 and 63 dpi), starting after completion of synaptic development of a cohort of labeled neurons, only resulted in an increase in synaptic input in the proximal domain (Fig. 2*B*), with no detectable changes in synaptic input or output densities in the distal and basal domains (Figs. 2*B*, 3). These experiments suggest the existence of a critical period during the synaptic development of new neurons when the organization of synapses throughout the cell can be strongly shaped by sensory experience. In addition, these results indicate that the proximal dendritic domain of new neurons has special properties that enable it to alter its synaptic organization after all other dendritic domains become unresponsive to manipulations of sensory input.

A genetic method to increase cell-intrinsic excitability

The activity of neurons is primarily regulated by two factors, the synaptic input that they receive and their intrinsic excitability determined by the set of ion channels present in their membranes. To investigate the role of intrinsic excitability in synaptic development, we tested whether increasing cell-intrinsic excitability would change the formation and maintenance of synapses as they integrate into the circuits of the bulb. Toward this goal, we devised a genetic strategy that would selectively render these new neurons more excitable without significantly altering the properties of the rest of the circuit into which they integrate. To increase the intrinsic excitability of single neurons, we used NaChBac, a voltage-gated sodium channel from the halophilic bacteria *Bacillus halodurans*. Expression of NaChBac increases the intrinsic excitability of neurons because, first, its activation threshold is more negative than that of native sodium channels, and second, NaChBac produces a slowly inactivating depolarizing current that lasts on the order of hundreds of milliseconds (Ren et al., 2001) compared with the ~ 1 ms inactivation time of the endogenous mammalian sodium channels responsible for the fast action potentials (Llinas, 1988). To visualize neurons expressing NaChBac, we generated a retroviral vector encoding a fusion protein

Figure 4. Genetically increased excitability does not change glutamatergic input synapse development. **A**, Whole-cell recordings were obtained from acute slices containing GCs that either expressed mCherry as control or NaChBac:GFP. Bottom left, At 28 dpi, a short current injection (4 nA, 5 ms) evoked a sustained depolarization in NaChBac-expressing GCs but not in controls of adult-born GCs. Right, The current–voltage relationship revealed a voltage-dependent inward current that was only observed in NaChBac-expressing GCs (10 mV steps, $V_h = -70$ mV, 16 dpi). **B**, At 28 dpi, PSDG⁺ clusters were examined in adult-born GCs expressing either the synaptic marker alone or with NaChBac. Scale bar, 10 μ m.

between GFP and NaChBac (*Mnachbac*). To study the electrical properties of new neurons expressing NaChBac, we delivered *Mnachbac* into GC progenitors in the SVZ. As controls, we coinjected a retroviral vector encoding the red fluorescent protein mCherry (*Mmcherry*) into the same animals and performed fluorescence-guided whole-cell recordings of either mCherry⁺ or NaChBac⁺ cells in acute slices. Unlike control neurons ($n = 6$ GCs), NaChBac⁺ neurons expressed a TTX-resistant slow-inactivating inward current that activated at -40 mV ($n = 5$ GCs) (Fig. 4*A*). NaChBac expression did not alter other passive membrane properties measured at -70 mV such as membrane time constant, input resistance, and resting membrane potential (data not shown). Whereas a 5 ms pulse of suprathreshold current injection (4 nA) evoked a single spike in mCherry⁺ control GCs ($n = 9$ GCs), the same current injection reliably caused an additional depolarization lasting on average 566 ± 49 ms in NaChBac⁺ GCs ($n = 8$ GCs) (Fig. 4*A*). This suprathreshold current injection (4 nA, 5 ms) elicited long depolarizations in NaChBac⁺ GCs as early as 14–18 dpi ($n = 8$ GCs), which were never observed in control neurons at any of the time points studied. At 28 dpi, the activation threshold for spiking in NaChBac⁺ GCs was -41 ± 2.0 mV ($n = 9$ GCs), whereas for mCherry⁺, control GCs was -30 ± 1.9 mV ($n = 8$ GCs) measured

at 26–28 dpi in adult rats. NaChBac expression did not affect the migration of new neurons into the olfactory bulb and increased their survival (our unpublished observations).

Genetically increasing the intrinsic excitability of adult-born neurons does not affect synapse formation

Having established a method to genetically increase the intrinsic excitability of newly generated neurons, we examined how this manipulation affected the formation of synapses using a bicistronic construct that expressed both PSDG, the fusion between GFP and PSD-95, and NaChBac (*Mpsdg:2A:nachbac*) (Fig. 4B). We measured the density of PSDG⁺ synapses in new GCs infected with *Mpsdg:2A:nachbac* and compared them with neurons expressing PSDG either alone (Fig. 4B) or with a nonconducting E191K NaChBac mutant (*Mpsdg:2A:e191k*) (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). GCs expressing both PSDG and the nonconducting E191K NaChBac mutant (*Mpsdg:2A:e191k*) had the same PSDG⁺ synapse density as control neurons expressing only PSDG in adult-born GCs (supplemental Fig. 1, available at www.jneurosci.org as supplemental material), indicating that expression of the nonconducting channel did not change PSDG⁺ synapse density by itself. To examine whether increased membrane excitability affected output synapses, we generated a bicistronic construct that expressed both synaptophysin:GFP (*Sypg*), the fusion protein between synaptophysin and GFP, and NaChBac (*Msypg:2A:nachbac*) (see Fig. 6).

Unexpectedly, increasing the intrinsic excitability by NaChBac expression in adult-born GCs did not change the density of PSDG⁺ synapses in any of their dendritic domains (Fig. 5A), although NaChBac remained active during synaptic development of adult-born GCs (Fig. 4A). Similarly, the density of SypG⁺ output synapses in adult-generated GCs was not changed by NaChBac expression (*Msypg:2A:nachbac* vs *Msypg*) (Fig. 6). These observations indicate that the formation of synapses is not affected by perturbations in the cell-intrinsic excitability of new neurons.

Increased intrinsic excitability blocks the synaptic changes evoked by sensory deprivation

The observation that strong synaptic changes were triggered by sensory deprivation, but not by raising the intrinsic excitability of new neurons could be at-

Figure 5. Genetically increased excitability does not change glutamatergic input synapse development and blocks the synaptic changes induced by sensory deprivation. **A**, Scatter plot and mean density of PSDG⁺ clusters (clusters per micrometer) in a dendritic domain of new control or NaChBac-expressing GCs (black and red circles, respectively) born in the adult and examined at different dpi. Statistical significance is only indicated if $p < 0.05$ (t test). The bottom graph shows the ratio of the mean cluster density of NaChBac-expressing over control GCs in a specific dendritic domain at a given day after infection. **B**, Same as **A**, but instead, adult-born GCs coexpressed PSD-95:GFP and NaChBac in both the sensory-deprived (starting on the day of retroviral labeling) and contralateral control olfactory bulb. Statistical significance is only indicated if $p < 0.05$ (t test).

Figure 6. Genetically increased excitability does not change output synapse development. **A**, At 28 dpi, SypG⁺ clusters were examined in the distal domain of adult-born GCs expressing either the synaptic marker alone or with NaChBac. Scale bar, 10 μ m. **B**, Scatter plot and mean density of SypG⁺ clusters (clusters per micrometer) of new control or NaChBac-expressing GCs (black and red circles, respectively) born in the adult at 28 dpi. No significant differences were detected (t test).

Figure 7. Sensory deprivation and intrinsic excitability differently control synaptic development in the dendritic domains of adult-born neurons. Reduced sensory input during synaptic development changed synaptic densities in all dendritic domains. When sensory deprivation started after the completion of synaptic development, the only detectable changes were increases in the density of glutamatergic input synapses in the proximal domain. Genetically increasing the excitability of new neurons did not affect the synaptic development of cells that matured in a normal sensory stimulation but rescued the synaptic changes triggered by sensory deprivation.

tributable to the ability of these new neurons to implement mechanisms of plasticity when their synaptic inputs are modified (by sensory deprivation) but not when their intrinsic membrane excitability is perturbed (by NaChBac expression). Alternatively, synaptic changes attributable to increased excitability of adult-generated neurons may only come into play if their synaptic inputs are perturbed. To test these possibilities, we analyzed the distribution of synapses in new NaChBac⁺ adult-born GCs that had also been deprived of sensory input. As indicated above, sensory deprivation caused a decrease in the number of glutamatergic input synapses in the distal and basal dendritic domains of adult-born neurons and an increase in the proximal domain (Fig. 2). Surprisingly, NaChBac blocked the changes in PSDG⁺ synapse densities of adult-born neurons triggered by sensory deprivation. In the proximal domain, NaChBac expression completely eliminated the synaptic increases induced by sensory deprivation at all times studied (Fig. 5B). In the distal domain, NaChBac expression blocked the decrease in synaptic density induced by naris occlusion partially by 28 dpi and completely by 56 dpi (Fig. 5B). These observations indicate that increasing the intrinsic excitability of new neurons in the adult brain can trigger synaptic changes in glutamatergic input synapses but only after their activity is modified by sensory deprivation.

Discussion

The connectivity of neurons within brain circuits is primarily regulated at two levels: first, by determining the identity of the neurons that will become synaptic partners, and second, by controlling the number of synapses formed between these partners. During adult neurogenesis in the OB, the identity of the synaptic partners for new GCs (mitral or tufted cells) appears to be primarily determined by genetic factors, such that separate precursors exist in the SVZ that give rise to new GCs apparently committed to make synapses with either mitral or tufted cells (Kelsch et al., 2007). In contrast, the density of synaptic connections can be strongly regulated by neuronal activity (Brunjes, 1994; Saghatelian et al., 2005). Such a dual control of neuronal connectivity during adult neurogenesis may simultaneously provide stereotypy of the circuit and flexibility to form novel connections for processing information in response to sensory-driven behavioral demands.

In this study, we analyzed how neuronal activity sculpts the synaptic development and connectivity of new GCs added to an adult brain circuit. We observed that new neurons added to the adult olfactory bulb under sensory deprivation experience dramatic changes in their synaptic development. In particular, we focused in this study on how and when neuronal activity can change the density and distribution of synaptic input and output sites of adult-born neurons. These changes are non-uniform in that either increases or decreases in synapse density occurred in different dendritic domains (Fig. 7). Sensory deprivation caused decreases in synapses in the distal and basal domain and increases in the proximal domain. Thus, each dendritic domain appears to act as an independent unit of synaptic plasticity.

Interestingly, sensory input-dependent changes in synaptic wiring are primarily restricted to the period when the new adult-born neurons develop their synapses. The synapses in the distal and basal domain are modifiable by sensory activity only for a limited time during the maturation of new GCs. After GCs have completed their maturation, the only synapses that appear to retain their modifiability in response to sensory deprivation are located in the proximal dendritic domain. In line with the limited period of activity-dependent synaptic changes that we observed, it has been shown recently that the ability to induce long-term potentiation was also lost after the first month in the life of new adult-born GCs (Nissant et al., 2009).

Given the strong synaptic changes induced by sensory deprivation on new GCs, it is surprising that genetically triggering long depolarizations (~600 ms) in these new neurons throughout their maturation did not interfere with the formation and maintenance of synapses. Despite the strong electrical perturbation induced by NaChBac expression, we could not detect any morphological changes in the synapse in any of the dendritic domains of new GCs. Similarly, the frequency and amplitudes of spontaneous EPSC inputs of adult-born GCs expressing NaChBac were not altered compared with matched controls (our unpublished observations). These results were surprising because many neurons have homeostatic mechanisms that regulate their synaptic input so that, when their excitability is raised, they reduce the number or strength of their excitatory synapses to prevent runaway activity. Interestingly, although GCs in the OB do not exhibit this compensatory behavior, we have recently observed that

new neurons in the dentate gyrus with NaChBac-enhanced excitability experience a strong reduction in their excitatory synapses (our unpublished results). The observation that increased excitability does not affect the density or distribution of excitatory synapses in GCs in the OB could be explained by other compensatory mechanisms, such as increased synaptic inhibition or changes in ion channels that could balance the increased intrinsic excitability. However, we did not observe any changes in intrinsic membrane or synaptic properties, suggesting either the existence of subtle compensatory mechanisms that escaped our detection methods or, alternatively, that these neurons do not compensate for this form of increased cell-intrinsic excitability.

Adult-born neurons, however, were not completely unresponsive to manipulations of their intrinsic excitability, because NaChBac-enhanced excitability counteracted the changes in glutamatergic input synapses caused by sensory deprivation. These observations also suggest the possibility that there may exist a program regulating the formation and maintenance of synapses in new GCs that requires a minimum threshold of neuronal activity contributed by the combined action of synaptic drive and cell-intrinsic excitability. If neuronal activity falls below that putative threshold attributable to a reduction in synaptic inputs (as a result of sensory deprivation), new neurons implement changes in the organization of their synapses. If new GCs with reduced synaptic input are simultaneously rendered hyperexcitable (as a result of NaChBac expression), the cells may still be over the threshold so that no synaptic changes occur.

The ability of new neurons to adapt their connectivity to the state of the adult circuit (e.g., sensory deprivation vs normal environment) may shed light on the contribution of continuous neurogenesis to learning and memory (Nottebohm, 2002; Lledo and Saghatelian, 2005; Bischofberger, 2007; Aimone et al., 2009). Several studies have demonstrated that the plasticity of adult-born neurons is maximal during their initial development and that it is progressively lost as the cells mature (Schmidt-Hieber et al., 2004; Ge et al., 2007; Nissant et al., 2009). Our results extend these observations by demonstrating that the synapses of new neurons have a high degree of structural plasticity during a time window when they are initially added into the circuit. Once they mature, activity-dependent plasticity in new neurons becomes more limited. This window of plasticity may allow the bulb to capture new neurons into stable patterns of connectivity while the circuit is in a certain state and thus generate stable information processing modules, whereas the next wave of new neurons may capture subsequent “circuit states.” The addition of cells endowed with such an initial short-lived flexibility and long-term stability may enable the processing of information by the olfactory bulb to be both versatile and reliable in the face of changing behavioral demands.

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