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Functional Properties of Adult-born Juxtaglomerular Cells in the Mammalian Olfactory Bulb

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It is now generally acknowledged that two regions of the brain maintain the capacity to produce new cells in adulthood (Gross, 2000): throughout all postnatal life new neurons are added to the dentate gyrus of the hippocampus and to the olfactory bulb (OB) of all mammals (Hastings *et al.*, 2000), including primates (Kornack and Rakic, 2001). In particular, neuroblasts bound to the OB originate in the more anterior part of the lateral ventricles, in a region called subventricular zone (SVZ), and migrate to the OB, where they end in the glomerular and granule cell layers (Alvarez-Buylla and Garcia-Verdugo, 2002; Winner *et al.*, 2002).

In order to establish that postnatally generated cells have become functional neurons three conditions should be met: first, it should be identified the point in time at or after which the cell was generated; secondly, evidence should be provided that the new cell has the functional properties of a neuron (e.g. capacity to generate action potentials); and thirdly, it should be demonstrated that the new cells are capable of entering into synaptic relationship with the existing neuronal network. This kind of evidence has been provided for cells generated in the SGZ in adult hippocampus, where it has been shown that the new cells differentiate into granule cells that integrate into hippocampal circuitry (van Praag *et al.*, 2002). For the new cells generated in the SVZ, in contrast, direct experimental evidence of functionality is more limited (Carlén *et al.*, 2002; Carleton *et al.*, 2003).

In the OB, postnatally generated interneurons have been well characterized morphologically, but very little is known concerning their physiology and function. Obtaining recordings from neurons identified as newly generated has been primarily impeded by the difficulty in distinguishing between previously generated and newly generated neurons in living brain tissue. Our experimental approach was to use replication-incompetent recombinant retrovirus to infect newly generated cells in the SVZ and genetically mark them with a reporter protein [an enhanced version of the green fluorescent protein, engineered with the membrane targeted domain of gap43 (Moriyoshi *et al.*, 1996; Okada *et al.*, 1999)]. Two to five days after virus injection GFP+ cells were found in the SVZ and in the rostral migratory stream (RMS). Time lapse imaging of labelled cells in RMS indicated that they were migratory neurons moving at the average speed of ~30 μm/h. After 10–14 days virally infected cells were found in the granular and glomerular layers of the OB. Our work mainly focused on the fraction of cells (20–25%) that reached the glomerular layer. Soon after the cells have reached their final destination in the OB, they display voltage-dependent currents typical of more mature neurons. Recordings from GFP+ cells in the glomerular layer indicated that most new periglomerular (PG) neurons have electrophysiological properties typical of a specific PG cell subtype (Puopolo and Belluzzi, 1998), characterized by a prominent A-type K^+ current, a large I_{Na} and the absence of delayed rectifier K^+ current. This pattern of conductances confers to these cells a unique excitability profile, denoted by the absence of outward rectification. An interesting aspect of the electrophysiological properties of newly generated cells, both PG and granule cells, is that they tend to have a sodium current which is significantly larger than in controls, with a steeper conductance–voltage relationship and more negative activation voltages. This difference as well as the higher g_{Na}/g_K in new cells may result in greater excitability to better respond to immature excitatory synaptic inputs. If new cells do in fact represent a distinct electrophysiological class then this suggests that newly added neurons to the bulb may have a specific role within the bulb. The larger implication for bulb function is not clear at this point; however, the specificity of the physiological type suggests that new neurons may be more critical to some aspects of bulb functioning than to others (Gheusi *et al.*, 2000).

In the glomerular layer we have also found a second GFP+ cell type, less frequent, that we have subsequently identified as dopaminergic (A. Pignatelli *et al.*, submitted for publication). These cells showed a prominent delayed rectifier potassium current, a large sodium current, and responded with multiple spikes to the injection of depolarizing current. The main feature of this small group of cells was autorhythmicity: they showed spontaneous firing at a 5–18 Hz, in most cases at regular intervals.

The progressive maturation of voltage-gated currents was determined by analysing the currents present in labelled cells from the migratory route to the glomerular layer. Cells in the RMS express only a small (1 nS) TEA-sensitive, delayed rectifier-type K^+ current. A large, transient A-type K^+-current (10.5 nS) is then added when the cells, once reached the OB, initiate their radial migration towards the glomerular layer. The last transformation is the loss of the delayed rectifier K^+-current and the development of a large, fast, TTX-sensitive Na^+-current (15.9 nS).

We found clear evidence of functional synaptic contacts in most of the new PG cells tested. Shortly afterwards they have reached their final destination, PG cells frequently show spontaneous synaptic currents, which can be blocked by the classical GABA(A) antagonists bicuculline and picrotoxin. Focal application of different neurotransmitters showed that these cells always respond to GABA, and only subsequently to glutamate. The presence of synaptic contacts onto GFP+ cells was further confirmed by immunohistochemical studies. Synapsin- and synaptophysin-positive presynaptic terminals were found adjacent to GFP+ dendrites in PG cells. We found clear evidence of functional synaptic contacts in approximately half of all new PG cells tested. For the first time we have provided the demonstration that neurons generated in adulthood show synaptic responses following stimulation of the olfactory nerve, indicating functional integration with existing bulb circuitry: under current-clamp conditions action potentials can be evoked with delays compatible with monosynaptic contacts, and under current clamp we recorded synaptic currents that could be blocked by kynurenate. While this shows that many new cells may functionally integrate with existing bulb circuitry, it also suggests that some new cells are

blocked or delayed in connecting to existing synaptic circuitry. As many new cells appear to die shortly after their arrival in the OB (Biebl *et al.*, 2000; Winner *et al.*, 2002; Petreanu and Alvarez-Buylla, 2002), one possibility is that new cells compete for synaptic contacts and those that do not receive such contact may eventually die.

A prominent feature of OB circuits are reciprocal dendro-dendritic synapses among interneurons and projection neurons: using the vesicle glycoprotein SV2 as synaptic marker we have found strong co-localization of SV2 and GFP in dendrite terminals, and SV2 labelling in structures just outside them, with a faint gap dividing the two regions.

Functional GABA receptors develop before glutamate receptors in newly generated OB neurons: focally applied GABA (100 μM) caused currents in all GFP+ cells at all ages and post infection survivals studied; in contrast, responses to glutamate (100 μM) appeared in GFP+ cells only 4 weeks after infection.

Several important maturational changes are known to take place in the GABAergic systems during embryonic development (reviewed in Owens and Kriegstein, 2002); among these, there is a developmental shift in $E_{GABA(A)}$, due to a developmental decrease in the intracellular chloride concentration, a decrease in the apparent affinity to GABA, which is reflected in greater sensitivity to GABA, and synaptic currents with slow decay kinetics. We compared functional properties of GABA receptor in GFP+ and in unlabelled PG cells. To check the concentration of chloride ions in newly generated cells we performed perforated patch recordings using gramicidin, an antibiotic creating channels which are not permeable to anions. At 12 days post-injection the reversal for GABA induced Cl^- currents is –61.0 ± 4.7 mV, corresponding to an intracellular chloride concentration of 11.83 mM ($n = 5$).

The sensitivity of GABAergic receptors in controls and mature GFP+ neurons was essentially identical: the effective concentrations of GABA giving 50% maximal response was 28.0 and 27.7 μM, and Hill coefficients were 1.55 and 1.23, respectively. Finally, the kinetics of GABA currents in unlabelled and GFP+ PG cells were similar. GABA responses showed a rapid desensitisation which was similar in amplitude (73.3 ± 1.36 and 79.9 ± 2.0%, measured at the end of 10 s application) and time constant (2.22 ± 0.22 and 2.46 ± 0.32 s) in control and GFP+ PG cell cells, respectively.

These data provide a basic description of the physiology of newly generated cells in the OB, and show that they become functional neurons which synaptically integrate into OB circuitry soon after their arrival. The focus now is on the molecular mechanisms that regulate efficient electrophysiological differentiation and synaptic integration of new neurons: understanding these mechanisms may be critical to future strategies of neuronal repair.

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