Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Temporal Patterns of Olfactory Bulb Interneuron Neurogenesis

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Review of Batista-Brito et al. (<http://www.jneurosci.org/cgi/content/full/28/15/3966>)

Interneurons are among the most diverse cell populations in the CNS, exhibiting a wide range of morphological, physiological, and molecular properties. Efforts to describe the origins of interneuron diversity have produced varying degrees of success in the spinal cord and cerebral cortex, but have proven more elusive in the olfactory bulb, where new interneurons are integrated during both embryogenesis and adulthood. To date, the most successful attempts to discover the origins of interneuron subtypes have involved birthdating techniques and genetic manipulations using interneuron-specific transcription factors. Recent efforts to determine specific spatial and temporal origins of distinct interneuron subtypes have helped develop a model in which interneuron diversity arises not from one homogeneous progenitor pool, but rather from a mosaic of spatially and temporally restricted progenitors, each contributing to a different interneuron subtype population.

Until recently, analyses of the origins of olfactory bulb interneuron subtypes were limited to profiling transcription factors that define specific interneuron populations. Molecular specification of

different subtypes occurs early in development in the dorsal lateral ganglionic eminence, where embryonic olfactory bulb interneurons originate, and continues later in the subventricular zone, the birthplace of adult olfactory bulb interneurons. There are at least 10 known transcription factors associated with olfactory bulb interneuron specification (for a complete review, see Bovetti et al., 2007). For example, GABAergic and dopaminergic phenotypes are specified by the transcription factors *Dlx1*, *Dlx2*, *Dlx5*, and *Dlx6*. Additionally, the transcription factor *Pax6* regulates the differentiation of periglomerular cells and superficial granule cells that are dopaminergic or tyrosine hydroxylase (TH)-positive.

Merkle et al. (2007) added to this picture of olfactory bulb interneuron diversity by showing that different interneuron subtypes are specified in spatially distinct subregions within the subventricular zone. By labeling targeted, spatially confined radial glial progenitor cells at postnatal day 0 (P0), the authors showed that interneuron subtype production is determined by unique, regionally specific progenitor pools. Importantly, because the progenitors (not migrating neuroblasts) were targeted directly, this study demonstrated that there is a tremendous degree of heterogeneity in the subventricular zone itself, before neurons become postmitotic.

The recent study by Batista-Brito et al. (2008) as well as a previous study by De Marchis et al. (2007) now contribute a

temporal domain to olfactory bulb interneuron subtype specification. To temporally fate map olfactory bulb interneurons, Batista-Brito et al. (2008) created *Dlx1/2-CreER* transgenic animals. *Dlx1/2* is a transcription factor that is transiently expressed in all interneurons when they differentiate. *CreER* mice contain a fusion protein linking Cre and a mutated estrogen receptor that enables Cre activity to be induced with tamoxifen application. *Dlx1/2-CreER* mice were crossed with *RosaYFP* animals to identify cells that underwent recombination. Cells expressing *Dlx1/2* at the time of tamoxifen ingestion or injection undergo tamoxifen-induced Cre recombination then constitutively express yellow fluorescent protein (YFP). In this study, tamoxifen was given at various time points throughout development and labeled cells were analyzed in the olfactory bulb at P30 when different interneuron subtypes were classified by immunohistochemistry and laminar position.

Using this method, the authors determined that the distribution of cells in the various layers of the olfactory bulb varies with the time of tamoxifen injection. A higher percentage of cells fate mapped at early ages [embryonic day 12.5 (E12.5)–E15.5] were located in the glomerular layer, whereas those injected at late embryonic and postnatal ages were more often destined for the granule cell layer. Although these findings are interesting and mostly agree with published studies (Bayer, 1983), it is important to note that the number of YFP-positive cells observed

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at each time point varied greatly, from 3.54 cells/mm² at E12.5, to 294 cells/mm² at P0. Although statistical significance was achieved in this experiment, comparing such dissonant numbers makes interpreting the results more difficult, particularly for the E12.5 injection time.

The authors go on to show that interneurons born at different ages not only migrate to specific layers, but also preferentially differentiate into distinct interneuron subtypes. In the glomerular layer, calbindin-positive cell production peaked in early development and declined after birth, whereas the percentage of calretinin-positive interneurons increased with age and were the major cell type produced postnatally, findings consistent with published literature (De Marchis et al., 2007; Ninkovic et al., 2007).

The data presented by Batista-Brito et al. (2008) also showed that by P30 only 3.9% of the newly generated interneurons were TH-positive. The authors point out that this finding differs significantly from that of De Marchis et al. (2007) and argue that the divergence is likely attributable to biases that arise using dye labeling and transplant studies. These methods target a specific region of the subventricular zone and not the entire neurogenic population, which is broadly distributed around the lateral ventricles. Such approaches could therefore produce a sampling bias because olfactory bulb interneuron diversity is generated by spatially restricted progenitor domains (Merkle et al., 2007), and targeting restricted areas limits analysis to a specific interneuron progenitor population. This reason is not sufficient to explain the discrepancy, however, because many studies have reported substantial TH-positive interneuron production in adult animals (Bagley et al., 2007; Kohwi et al., 2007; Merkle et al., 2007). Instead, the inconsistency might be explained by the method of quantification used by Batista-Brito et al. (2008), because they report all interneuron subtype data as percentages of total YFP-positive interneurons. Perhaps the low cell count of total YFP-positive cells from E12.5 mice overstates the percentage production of TH-positive cells from that age. However, this is unlikely because it seems total YFP-positive cells at P30 (22 ± 6 cells/mm²) is also much lower than that at P10 (248 ± 26 cells/mm²), although not as low as the number at E12.5 (3.54 ± 0.3 cells/mm²). Regardless, because of the discrepancies with previous research, we feel that there is insufficient evidence to determine

whether TH-positive interneurons are produced at very low rates postnatally.

The authors conclude that olfactory bulb interneuron subtypes have unique temporal production patterns and that most of the diversity is generated around the birth of the animal, when olfactory sensation begins. We agree that this study adds a temporal component to known influences over olfactory bulb interneuron generation and suggests there are potential differences in interneuron subtype production. However, we feel important caveats must be addressed for the conclusions to be generalized to all of olfactory bulb interneuron diversity.

Batista-Brito et al. (2008) argue that *Dlx1/2* is transiently expressed in postmitotic neurons and therefore only rapidly dividing neural precursor cells and migrating neuroblasts are labeled. They report no labeled cells near the ventricle 7 d after tamoxifen injection, indicating that the progenitors (presumably radial glial cells) were not labeled by their method. They also report that after P10, no YFP-positive cells were found in the olfactory bulb after tamoxifen injection. We find this curious because at least two studies have used reliable methods to demonstrate abundant *Dlx1/2* expression in the adult olfactory bulb (Porteus et al., 1994; Saino-Saito et al., 2003), including the glomerular layer and granule cell layer. Although it is not clear why Batista-Brito et al. (2008) did not detect YFP-positive interneurons in the olfactory bulb of later postnatal animals, it stands to reason that the labeled cells in the current study do not necessarily reflect neurons that became postmitotic immediately around the time of tamoxifen injection.

This point is particularly important because the authors' interpretation of the data relies on a small window of *Dlx1/2* expression, because the embryonic tamoxifen injections are only separated by 2–3 d. If a cell expresses *Dlx1/2* for longer than that, using this method would temporally fate map the cell for at least two different time points, first at the earliest injection time and again at subsequent injection times, for as long as *Dlx1/2* expression continues. Hence, this method may lack the fine temporal resolution on which the authors' analysis depends. For this reason, we feel that these experiments would have benefited from concurrent BrdU administration with the tamoxifen injection. Colocalization of BrdU and YFP would indicate that a cell was undergoing mitosis at the time of *Dlx1/2* expression and it would be clear that analysis was

confined to newly born cells at each time point. In short, we would like to see the results confirmed with more traditional birthdating methods.

Another important question that arises in any fate mapping study is the issue of cell death. In the current study, Batista-Brito et al. (2008) performed tamoxifen injections at multiple times, but they always analyzed subtype specification at P30. This means that the closer to P30 the tamoxifen injection fell, the more recently the YFP-positive cells became postmitotic. If cell death is a factor in these cell types, particularly if it affects different interneuron subtypes differently (Ninkovic et al., 2007), the differing times between injection and observation may have produced results that do not accurately reflect the overall pattern of interneuron subtype specification.

To address this problem, the authors performed immunohistochemistry for activated caspase-3 (a marker of apoptosis) after fate mapping cells from different injection times. They concluded that so few YFP-positive cells were undergoing cell death that it was not likely to affect their results. However, we believe that this method suffers the same flaw as the fate mapped cell counts, because the authors only analyzed sections at P30 and any evidence of cell death that may have occurred before this time point would no longer be present. We therefore feel that the authors did not sufficiently address the possibility of cell death affecting their results.

In conclusion, Batista-Brito et al. (2008) provide intriguing information regarding the temporal dynamics of olfactory bulb interneuron subtype specification in the context of the *Dlx1/2* lineage. Our primary concern regards the authors' assertion that their method of tamoxifen injection allows precise determination of the time of interneuron specification. We suspect that *Dlx1/2* expression is insufficiently transient to determine an exact birthdate of these neurons and that cell death may blur the endpoint analysis at P30. With these potential confounding factors, we are afraid that the present analysis represents a series of temporally amorphous snapshots of the *Dlx1/2* lineage rather than a precise description of interneuron subtype specification. Despite this, the findings are an important contribution to the emerging model that olfactory bulb interneuron subtypes are derived from a heterogeneous progenitor pool and that olfactory bulb interneuron neurogenesis does not reach a steady state until well into adulthood.

References

- Bagley J, LaRocca G, Jimenez DA, Urban NN (2007) Adult neurogenesis and specific replacement of interneuron subtypes in the mouse main olfactory bulb. *BMC Neurosci* 8:92.
- Batista-Brito R, Close J, Machold R, Fishell G (2008) The distinct temporal origins of olfactory bulb interneuron subtypes. *J Neurosci* 28:3966–3975.
- Bayer SA (1983) ³H-thymidine-radiographic studies of neurogenesis in the rat olfactory bulb. *Exp Brain Res* 50:329–340.
- Bovetti S, Peretto P, Fasolo A, De Marchis S (2007) Spatio-temporal specification of olfactory bulb interneurons. *J Mol Histol* 38:563–569.
- De Marchis S, Bovetti S, Carletti B, Hsieh YC, Garzotto D, Peretto P, Fasolo A, Puche AC, Rossi F (2007) Generation of distinct types of periglomerular olfactory bulb interneurons during development and in adult mice: implication for intrinsic properties of the subventricular zone progenitor population. *J Neurosci* 27:657–664.
- Kohwi M, Petryniak MA, Long JE, Ekker M, Obata K, Yahagawa Y, Rubenstein JL, Alvarez-Buylla A (2007) A subpopulation of olfactory bulb GABAergic interneurons is derived from Emx1- and Dlx5/6-expressing progenitors. *J Neurosci* 27:6878–6891.
- Ninkovic J, Mori T, Götz M (2007) Distinct modes of neuron addition in adult mouse neurogenesis. *J Neurosci* 27:10906–10911.
- Merkle FT, Mizadeh A, Alvarez-Buylla A (2007) Mosaic organization of neural stem cells in the adult brain. *Science* 317:381–384.
- Porteus MH, Bulfone A, Liu JK, Puelles L, Lo LC, Rubenstein JL (1994) DLX-2, MASH-1, and MAP-2 expression and bromodeoxyuridine incorporation define molecularly distinct cell populations in the embryonic mouse forebrain. *J Neurosci* 14:6370–6383.
- Saino-Saito S, Berlin R, Baker H (2003) Dlx-1 and Dlx-2 expression in the adult mouse brain: relationship to dopaminergic phenotypic regulation. *J Comp Neurol* 16:18–30.