animals, HSPCs are identified in virtually all tissues studied except brain. The data from Ajami *et al.*[1] suggest that the abundance of HSPC-derived microglia in the CNS of radiation chimerae[14] is contingent on the use of irradiation to precondition the animals.

In the other new paper, Mildner *et al.*[2] examined other aspects of the radiation chimerism approach. One incisive experiment used a linear accelerator to deliver irradiation that precisely spared the cranium (shown rather sensationally by the retention of hair color only over the head). This maneuver resulted in the exclusion of blood-derived cells from the parenchymal microglial population in healthy adult mice (Experiment 2), after facial axotomy (Experiment 9) and after cuprizone-induced demyelination of the corpus callosum (Experiment 7). Results for healthy adult mice were confirmed by using immunodeficient recipient mice as the chimeric recipients, avoiding altogether the use of radiation (Experiment 4). Thus, where Ajami *et al.*[1] showed that irradiation with facial axotomy was not sufficient to bring a detectable number of blood myeloid cells into the parenchyma as microglia (Experiment 16), even when the majority of circulating cells are labeled, Mildner *et al.*[2] showed (Experiments 2 and 9) that irradiation is nonetheless essential for this to occur. It is fortunate that both groups studied facial axotomy, as well as healthy adult mice, as this overlap renders their data authentically complementary.

Mildner *et al.*[2] also evaluated which monocytic cells in the circulation might generate parenchymal microglia under precise conditions, including irradiation and transfer of bone-marrow cells. There are two functionally distinct populations of circulating monocytes[15]. One, distinguished by expression of the chemokine receptor CCR2 and strong expression of the Ly6C marker, is specialized to generate macrophages in inflamed tissues. The second population poorly expresses CCR2 and regularly replaces the tissue populations of resident macrophages. Surprisingly, chimeric recipients of *Ccr2*$^{-/-}$ bone marrow, even after total body irradiation and imposition of pathology (Experiments 3, 6 and 10), very weakly incorporated blood-derived cells into the microgliosis that accompanied the tissue reaction. Interpretation of this finding is somewhat complex. CCR2 is required for Ly6C^{hi} 'inflammatory' monocytes to exit the bone marrow. Therefore, the blood of *Ccr2*$^{-/-}$ mice is depleted of these cells, but they are present in bone marrow, which is the tissue being transferred. However, when Mildner *et al.*[2] compared blood populations of the *Ccr2*$^{-/-}$ with those of wild-type chimeras (some weeks after the procedure), they found very few circulating Ly6C^{hi} inflammatory monocytes, despite having transferred a large number of these cells. Therefore, the most straightforward conclusion is that when irradiation chimeras are studied the cells contributing to the microgliosis come from circulating inflammatory, not homeostatic, monocytes.

Together, these studies will motivate many new lines of research, which will further characterize the cells that give rise to microglia in the irradiated mice and evaluate the contributions of cranial (and perhaps systemic) irradiation to microgliosis. It will be important to define whether CCR2 contributes to engraftment of circulating cells into the CNS via moving cells from bone marrow to blood, or from blood to tissue. Finally, it will be necessary to filter the data from prior studies using irradiation chimerism through this new lens, to re-evaluate the pathophysiological roles of microglia in CNS diseases.

1. Ajami, B., Bennett, J., Krieger, C., Tetzlaff, W. & Rossi, F. *Nat. Neurosci.* **10**, 1538–1543 (2007).
2. Mildner, A. *et al. Nat. Neurosci.* **10**, 1544–1553 (2007).
3. Ting, J.P., Nixon, D.F., Weiner, L.P. & Frelinger, J.A. *Immunogenetics* **17**, 295–301 (1983).
4. Matsumoto, Y. & Fujiwara, M. *J. Neuroimmunol.* **17**, 71–82 (1987).
5. Bauer, J. *et al. Glia* **15**, 437–446 (1995).
6. Hickey, W.F. & Kimura, H. *Science* **239**, 290–292 (1988).
7. Greter, M. *et al. Nat. Med.* **11**, 328–334 (2005).
8. Ponomarev, E.D., Shriver, L.P., Maresz, K. & Dittel, B.N. *J. Neurosci. Res.* **81**, 374–389 (2005).
9. Flugel, A., Bradl, M., Kreutzberg, G.W. & Graeber, M.B. *J. Neurosci. Res.* **66**, 74–82 (2001).
10. Ford, A.L., Goodsall, A.L., Hickey, W.F. & Sedgwick, J.D. *J. Immunol.* **154**, 4309–4321 (1995).
11. Massengale, M., Wagers, A.J., Vogel, H. & Weissman, I.L. *J. Exp. Med.* **201**, 1579–1589 (2005).
12. Gartner, S. *Science* **287**, 602–604 (2000).
13. Massberg, S. *et al. Cell* (in the press) (2007).
14. Simard, A.R. & Rivest, S. *FASEB J.* **18**, 998–1000 (2004).
15. Tsou, C.L. *et al. J. Clin. Invest.* **117**, 902–909 (2007).

A sharper view from the top

Xiaoqin Wang

Maintaining a precise representation of sound frequency in auditory cortex is difficult because of converging diffuse thalamocortical inputs. A recent study demonstrates that this is accomplished via recurrent intracortical connections.

In every mammalian species that has been examined, the auditory cortex is arranged in tonotopic maps[1,2] on the basis of the frequency tuning (selectivity) of individual cortical neurons. In turn, the precision of this frequency tuning determines the accuracy of the tonotopic organization. However, many thalamocortical inputs carrying different sound frequencies diffusely project onto individual pyramidal neurons of layer IV, which should make the neurons' frequency tuning less sharp. A new study in this issue[3] demonstrates that intracortical connections are critical for maintaining neuronal frequency tuning in response to this challenge.

The tonotopic organization exists at each stage of the ascending auditory pathway, from the receptors (hair cells) to the primary auditory cortex (A1), through the cochlear nucleus, inferior colliculus and thalamus. Because layer IV of A1 receives its primary inputs from the ventral division of the auditory thalamus (medial geniculate body, MGB), it is generally believed that the thalamocortical projection defines the frequency selectivity and tonotopic organization that is observed in A1. However, this functional hypothesis does not completely correlate with the anatomical projections between thalamus and cortex, which are not organized in a point-to-point fashion. MGB neurons tuned to a particular frequency project to a population of A1 neurons distributed over a few hundred microns (**Fig. 1a**), with these recipient neurons showing frequency tuning profiles that progressively differ from that of the MGB neuron[4]. By the same token, an individual A1 neuron does not simply receive inputs from MGB neurons tuned to the same frequency, but also from other MGB neurons with different frequency tuning profiles. This thalamocortical connectivity structure creates

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a problem for the auditory cortex, making it difficult to maintain frequency-tuning precision, as such an organization inevitably leads to a broadening of frequency tuning in A1 as compared with MGB. Adding to the complexity, each A1 neuron also receives a large number of intracortical connections (**Fig. 1a**). Given this anatomical arrangement, it is not intuitive how auditory cortex can maintain precise frequency tuning. Also unknown is how thalamocortical projections interact with the intracortical connections. The new study by Liu *et al.*[3] has shed some light on these puzzling questions.

Liu *et al.*[3] attempted to untangle the contributions of thalamocortical and intracortical inputs to cortical frequency selectivity by using a cleverly designed pharmacological silencing method. Traditional studies have used muscimol (a $GABA_A$ receptor agonist) to silence the cortex while studying thalamocortical inputs onto a cortical neuron. However, the use of muscimol can also lead to the activation of $GABA_B$ receptors, thus also reducing thalamocortical synaptic transmission due to the presence of $GABA_B$ receptors on thalamocortical axons[5]. To avoid this confound, Liu *et al.*[3] developed a pharmacological cocktail to effectively silence intracortical connections while largely preserving thalamocortical synaptic transmission. The study overcame the nonspecific effects of muscimol on presynaptic transmission by applying SCH50911, a specific antagonist of $GABA_B$ receptors, together with muscimol. Using this approach, along with *in vivo* whole-cell voltage-clamp recording techniques, the authors were able to separate the contributions of thalamocortical and intracortical inputs to layer IV neurons in rat auditory cortex.

Liu *et al.*[3] found that the peak of the thalamocortical tuning profile is broad and flat, whereas the tuning profile of intracortical input is much sharper. When combined, these two curves can give rise to narrow frequency tuning, matching the typical frequency tuning of cortical neurons, as observed in the extracellular spiking activity. The authors concluded that weakly tuned thalamocortical input determines the frequency range of A1 neurons, whereas intracortical input defines the shape of the tuning profile of the spiking activity, as one might add a pyramid on to the top of a flat base (**Fig. 1b**). In other words, intracortical input reconstitutes the sharpness of frequency tuning in the cortex. This study provides a beautiful demonstration of how thalamocortical and intracortical inputs interact synergistically.

The sharpening of frequency tuning in auditory cortex could also be achieved through inhibitory inputs and activity. Side-band inhibition that flanks a neuron's

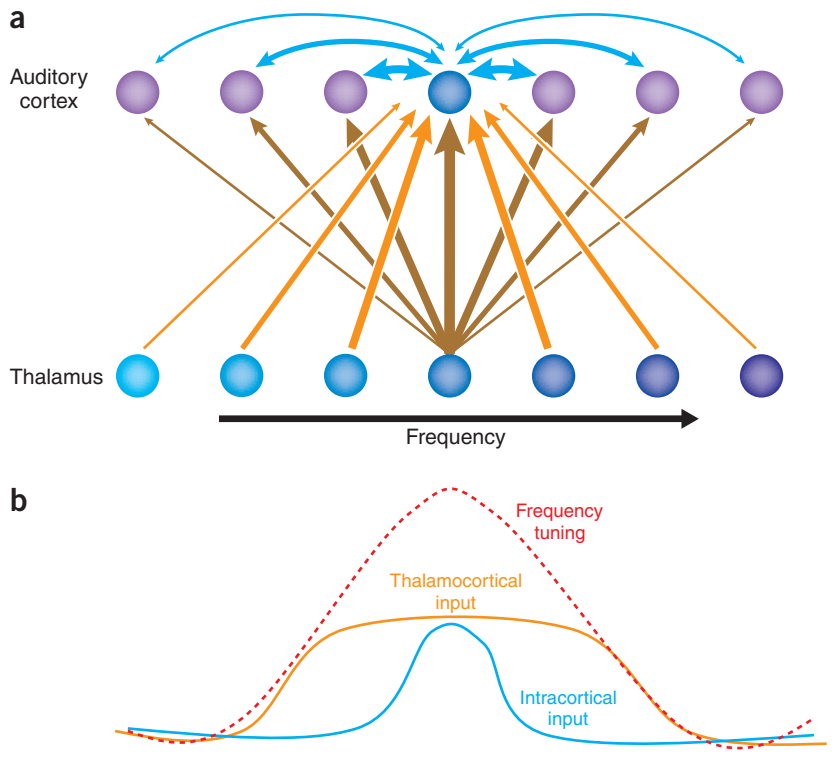

Figure 1 The frequency tuning of auditory cortex neurons is shaped by both thalamocortical and intracortical inputs. (**a**) The putative circuitry of excitatory connections. Inhibitory inputs are not included for simplicity. Each thalamic neuron projects to multiple cortical neurons. The thalamocortical connectivity (gold lines) is strongest between neurons with matched frequency tuning and progressively becomes weaker for neighboring cortical neurons. Each cortical neuron in turn receives inputs from multiple thalamic neurons (orange lines). This projection pattern defines the range of frequency tuning for each cortical neuron. Cortical neurons also receive recurrent intracortical input from neighboring neurons (blue lines). The intracortical connectivity is strongest for the closest neurons and becomes weaker with distance. (**b**) Frequency tuning in cortical neurons. The frequency tuning of the thalamocortical input (orange curve) is broad, as shown by Liu *et al.*[3] The frequency tuning of the intracortical input (blue curve) is sharper than that of the thalamocortical input. The resulting frequency tuning of the individual cortical neuron arises from the interaction between these two sets of input (red dashed curve).

tuning frequency is commonly seen in auditory cortex[6] and has long been accepted as a means to sharpen frequency tuning in mammalian auditory cortex[7]. The mechanism revealed in this study appears to be another tool by which auditory cortex can maintain an orderly frequency organization. This precision in frequency tuning should allow A1 to analyze the frequency content of sounds more accurately, but this may not be the only consequence of converging thalamocortical inputs. One benefit to integrating inputs from multiple thalamic neurons is to allow A1 neurons to analyze spectrally complex sounds and extract the spectral contrasts, which requires many frequency channels[8]. The convergence of subcortical inputs may also help auditory cortex separate foreground signals from background noises[9].

An intriguing observation from the Liu *et al.* study[3] is that the subthreshold thalamic inputs to A1 are particularly broad in frequency content, consistent with anatomical projection patterns, and much broader than the width of cortical spiking responses. Perhaps this is because it is important for the computational aspects of auditory cortex to maintain a perspective on general activity at the end of a long ascending pathway. Therefore, competing computational needs are present in A1: maintaining frequency-tuning precision and possessing a broad spectral perspective. As an analogy, when looking at an oil painting of Yellowstone Park scenery from a distance, it would be easy to discern streams, trees and perhaps a moose wandering in the meadow, but only colorful dots and stripes would be visible on the canvas at close range. Liu *et al.*[3] demonstrate that auditory cortex can focus on the sound details amid widely divergent inputs from the thalamus.

The intracortical inputs silenced in this study are within a relatively short distance from the recorded neuron (up to ~500 μm), though auditory cortex, like other sensory cortices, has long-range horizontal connections[10]. These long-distance intracortical connections likely

have different functions than the short-range connections. The response of an A1 neuron can be facilitated or inhibited by tone inputs from as far as several octaves away from the most sensitive frequency of that neuron[11], which is probably mediated by long-range horizontal connections. The neurons studied by Liu *et al.*[3] were in the middle cortical layers, however, and these long-range horizontal connections are predominantly confined to upper cortical layers, such as layer II/III. It would be extremely interesting to apply the silencing techniques used in this study to probe the contributions of long-range horizontal connections to auditory neuron response properties in these other layers. Another issue not addressed by Liu *et al.*[3] involves the contribution of a corticothalamic feedback loop in sharpening A1 frequency tuning. In the visual system, corticothalamic feedback can sharpen orientation tuning of visual cortex neurons[12]. Such feedback circuits can effectively narrow frequency tuning in auditory cortex as well[13]. Thus, the actual frequency tuning of A1 neurons, when observed under intact conditions, is shaped by inhibition, recurrent intracortical connections and efferent feedback circuits.

Finally, these experiments were carried out in anesthetized rats. Most types of anesthesia (barbiturates, ketamine and so on) severely alter response properties in auditory cortex, primarily causing the production of transient responses[14]. Auditory cortex can show both transient and sustained responses in awake animals[15]. The extent to which the function of the recurrent intracortical connections revealed by Liu *et al.*[3] is affected by the anesthesia and whether intracortical inputs are more susceptible to anesthesia than thalamocortical inputs is not clear. These issues should be examined in future studies. An even more intriguing question is how the interactions between thalamocortical and intracortical inputs operate in a behavioral context. After all, when a rat runs around listening for the scurrying of prey and the rustle of approaching predators, or when we sit in a concert hall paying particular attention to one instrument on the stage, the auditory cortex must be functioning unimpaired.

1. Woolsey, C.N. & Walzl, E.M. *Bull. Johns Hopkins Hosp.* **71**, 315–344 (1942).
2. Merzenich, M.M., Knight, P.L. & Roth, G.L. *J. Neurophysiol.* **38**, 231–249 (1975).
3. Liu, B., Wu, G.K., Arbuckle, R., Tao, H.W. & Zhang, L.I. *Nat. Neurosci.* **10**, 1594–1600 (2007).
4. Winer, J.A. & Lee, C.C. *Hear. Res.* **229**, 3–13 (2007).
5. Yamauchi, T., Hori, T. & Takahashi, T. *Eur. J. Neurosci.* **12**, 3433–3436 (2000).
6. Schreiner, C.E., Read, H.L. & Sutter, M.L. *Annu. Rev. Neurosci.* **23**, 501–529 (2000).
7. Suga, N. *Neurosci. Res.* **21**, 287–299 (1995).
8. Barbour, D.L. & Wang, X. *Science* **299**, 1073–1075 (2003).
9. Bar-Yosef, O., Rotman, Y. & Nelken, I. *J. Neurosci.* **22**, 8619–8632 (2002).
10. Read, H.L., Winer, J.A. & Schreiner, C.E. *Proc. Natl. Acad. Sci. USA* **98**, 8042–8047 (2001).
11. Kadia, S.C. & Wang, X. *J. Neurophysiol.* **89**, 1603–1622 (2003).
12. Cudeiro, J. & Sillito, A.M. *Trends Neurosci.* **29**, 298–306 (2006).
13. Suga, N. & Ma, X. *Nat. Rev. Neurosci.* **4**, 783–794 (2003).
14. Ter-Mikaelian, M., Sanes, D.H. & Semple, M.N. *J. Neurosci.* **27**, 6091–6102 (2007).
15. Wang, X., Lu, T., Snider, R.K. & Liang, L. *Nature* **435**, 341–346 (2005).

The main olfactory bulb and innate behavior: different perspectives on an olfactory scene

Zachary F Mainen

A recent study in *Nature* now shows that the main olfactory bulb contains a particular zone required for innate odor aversion, indicating that olfactory receptors have behavioral as well as chemical specificity.

There are about a thousand unique olfactory receptor genes in the mouse[1], and the receptors are organized into a stereotyped spatial map of glomeruli in the main olfactory bulb (MOB)[2]. Why are there so many different receptor types, and what is the functional significance of the map? These properties are commonly thought to relate to the need to make fine discriminations between many odorants. Although there has been some experimental support for this idea[3], the great abundance and notable spatial organization of the olfactory receptors remain puzzling. This may be partly due to our relative ignorance of the chemical ecology of animals that rely heavily on olfaction; we still have very little idea of what constitutes a natural olfactory 'scene' for a mouse. However, a recent study by Kobayakawa *et al.* in *Nature*[4] suggests that answers may come from considering not only the chemicals that drive olfactory receptors, but also the behaviors that the receptors drive.

Kobayakawa *et al.*[4] used targeted expression of the diphtheria toxin gene to create two lines of mice that lack glomeruli in specific zones of the MOB, corresponding to specific subsets of receptor types. The first mouse line lacked several hundred glomeruli of a total of about 1,800, all in the dorsal zone of the olfactory bulb. These 'ΔD' mice have a very interesting deficit. Normal mice tend to avoid chemicals associated with predators and spoiled foods, even without prior experience[5], but the ΔD mice failed to show these innate aversive responses, spending about as much time investigating normally aversive odors as other, nonaversive odors.

Notably, the failure of the ΔD mice to avoid these chemicals was not to the result of an inability to detect them. Through standard conditioning procedures, the same mice could readily learn aversive or appetitive responses to the odors to which they had no innate aversion. Indeed, even the detection thresholds for these odors were only weakly increased in these mice. Optical imaging experiments showed that both food- and predator-related aversive odors activated receptors both inside and outside of the dorsal zone in intact mice. The ΔD mice showed responses to these odorants only in the ventral domain; the dorsal glomeruli were entirely absent and therefore showed no activation. Presumably, the retention of odor responses in the ventral zone is what underlies the ability to sense this class of odors and to learn to associate them with behavioral

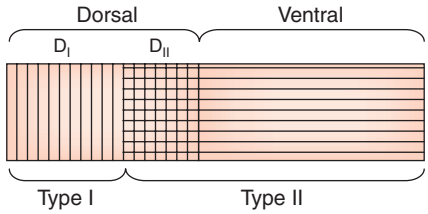

Figure 1 Schematic of the organization of the olfactory bulb into dorsal and ventral zones and class I (D_I) and class II (D_{II}) receptors.

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