exciting step forward in disentangling the genetic basis of acoustic variation in distress calls and establishes *Peromyscus* as a promising new model species[16] for studying vocal communication.

DECLARATION OF INTERESTS

The author declares no competing interests.

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Neuroscience: Seq-ing maps in the olfactory cortex

Carolyn Diaz, Kevin M. Franks*, and Robin M. Blazing
Department of Neurobiology, Duke University Medical School, Durham, NC 27705, USA
*Correspondence: franks@neuro.duke.edu
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Many cortical brain regions are spatially organized to optimize sensory representation. Such topographic maps have so far been elusive in the olfactory cortex. A high-throughput tracing study reveals that the neural circuits connecting olfactory regions are indeed topographically organized.

A central pursuit of neuroscience has been to determine the functional organization of the mammalian cerebral cortex. Most sensory cortical areas form 'topographic maps', highly organized maps of features of the external world. For example, the visual cortex is organized retinotopically and the auditory cortex contains a tonotopic map in which the spatial arrangement of neurons corresponds to their preferred sound frequency. These maps preserve the spatial arrangement of the receptors that first process sensory information and are thought to promote efficient processing of sensory information and to ensure appropriate connectivity between different brain regions.

Somewhat perplexingly, however, this does not seem to be conserved in the mapping from odorant receptors to the olfactory system. In this system, olfactory sensory neurons expressing the same type of odorant receptor converge onto a unique pair of spatially discrete bundles of neurites in the olfactory bulb called 'glomeruli', where they form excitatory synaptic connections with mitral and tufted cells, the output neurons of the olfactory bulb. Importantly, mitral and tufted cell dendrites innervate just one glomerulus and therefore receive input from a single type of odorant receptor. Thus, the olfactory bulb exhibits a stunning topographic organization with ~1800 distinct information channels each of which corresponds to the activity of a single odorant receptor type. The mitral and tufted cells then relay this information to olfactory cortical areas, including the piriform cortex, but these projections seem unstructured, suggesting that the olfactory system discards these dedicated processing channels. The next olfactory processing center, the piriform cortex, however, appears to discard these dedicated processing channels. Neurons in the piriform cortex receive input from multiple different glomeruli[1], and previous tracing studies from small numbers of projection neurons have failed to reveal any clear organization in the

structure of projections from the olfactory bulb to the piriform cortex[2–4]. Consistent with these anatomical observations, odors activate ensembles of neurons which are distributed across the piriform cortex with no apparent topography[5–7]. These findings have inspired a *tabula rasa* model for cortical olfactory processing. According to this model, the mind is a 'blank slate' at birth, and ideas and attributes can only be acquired through experience. Olfactory neuroscientists have drawn inspiration from this ancient philosophical framework to describe a model in which odor representations in the piriform cortex only achieve behavioral significance through learning and neural plasticity[8–10].

In a compelling new study, Yushu Chen and colleagues[11] leveraged the power of two novel sequencing-based neuroanatomical tracing techniques, Multiplexed Analysis of Projections by Sequencing (MAPseq) and Barcoded Anatomy Resolved by Sequencing (BARseq)[12,13], to assess connectivity in the olfactory system. Using these high-throughput approaches, the authors revealed that neural projections to and from piriform cortex do exhibit spatially structured connectivity, calling the *tabula rasa* model of the olfactory cortex into question.

MAPseq uses a virally delivered barcode mRNA library to uniquely label hundreds to thousands of neurons within a given region. These barcodes are trafficked throughout the axonal processes of each cell, and so the projection targets of individual neurons can be identified by dissecting and sequencing the tissue in connected brain regions. The number of transcripts of each barcode can then be used to quantify the relative projection strength of the source neurons to any given region. Because the tissue must be dissected and homogenized, the spatial resolution of MAPseq is limited to the size of the tissue sample. BARseq improves this resolution by combining MAPseq with *in situ* sequencing, allowing for precise localization of the barcoded somata within a given brain region.

First, Chen and colleagues[11] used BARseq to trace the projection patterns of mitral and tufted cells, which they could distinguish based on their laminar position in the bulb. While BARseq is more spatially precise, this approach labels far fewer cells than MAPseq. Therefore, the authors identified the projection targets of thousands of olfactory bulb neurons using MAPseq, and then trained a simple machine learning algorithm using the BARseq data to distinguish between putative mitral and tufted cell axons in the MAPseq dataset. These data were consistent with several previous findings: that putative mitral cells largely project to piriform and putative tufted cells to olfactory tubercle[14,15]; that intra-piriform output decreases as a function of distance[16]; and that the cortical amygdala receives more input from the dorsal aspect of the bulb[2].

Now with their validated large-scale dataset, the authors were better able to assess the degree of topography in olfactory bulb projections. Indeed, they revealed structured correlations in the distribution of olfactory bulb projections to the piriform cortex and four other higher order olfactory regions: accessory olfactory nucleus, olfactory tubercle, cortical amygdala, and lateral entorhinal cortex. For example, individual mitral cells that project to anterior piriform cortex were more likely to also project to the anterior olfactory nucleus, while mitral cells that project to posterior piriform cortex were more likely to also project to lateral entorhinal cortex and cortical amygdala (Figure 1A). The authors additionally identified two distinct populations of mitral cells: "narrowly projecting" mitral cells with relatively focal cortical projections, primarily in anterior piriform cortex and the anterior olfactory nucleus, and "broadly projecting" mitral cells whose axonal arbors were much more diffuse. Thus, this study is the first to

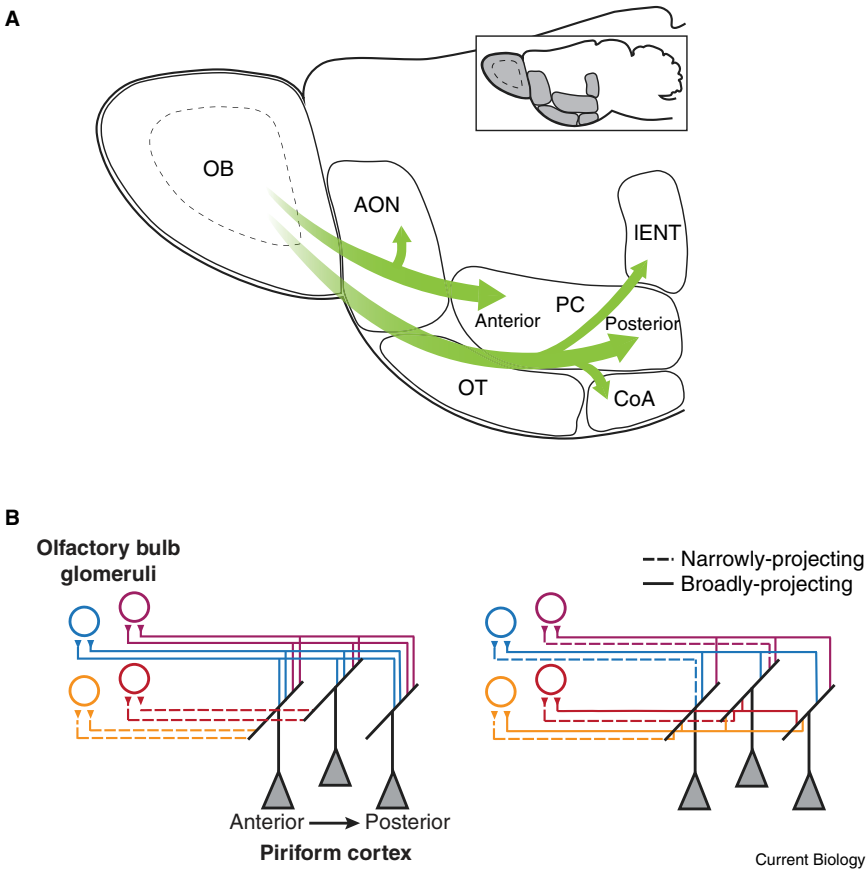

Figure 1. Olfactory bulb projections co-innervate piriform and extra-piriform regions.
(A) Olfactory bulb projections to anterior piriform cortex are more likely to also innervate AON, and projections to posterior piriform cortex are more likely to also innervate lENT and COA. (B) Left: Sister MCs could be either narrowly- or broadly-projecting, segregating the information that piriform neurons receive. Right: Sister MCs could be both narrowly- and broadly-projecting, relaying olfactory information throughout piriform cortex. Abbreviations: AON: accessory olfactory nucleus; CoA: cortical amygdala; lENT: lateral entorhinal cortex; OB: olfactory bulb; OT: olfactory tubercle; PC: piriform cortex.

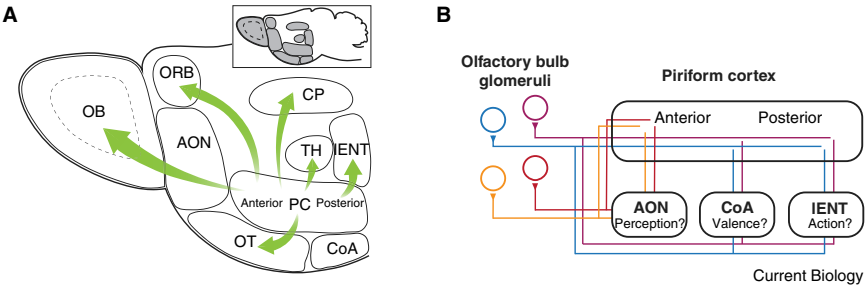

Figure 2. Piriform projections to other regions show structure.
(A) Piriform contains six clusters of structured projections which vary both in their targets and location along the anterior–posterior axis. (B) Co-innervation of high-order olfactory regions by olfactory bulb and piriform projections results in "triads" of olfactory information. Abbreviations: AON: accessory olfactory nucleus; CP: caudate putamen; CoA: cortical amygdala; IENT: lateral entorhinal cortex; TH: mediodorsal nucleus of the thalamus; OB: olfactory bulb; ORB: orbitofrontal cortex; OT: olfactory tubercle; PC: piriform cortex.

show structure in the projections from the olfactory bulb to the piriform cortex.

However, one limitation to this high-throughput technique is that the parent glomeruli of the labeled mitral cells cannot be identified. Thus, for example, the authors cannot determine whether all mitral cells innervated by a given glomerulus — so-called sister mitral cells — are either narrowly or broadly projecting to piriform or whether each set of sister mitral cells is composed of both mitral cell types (Figure 1B). If some glomeruli only contain narrowly-projecting mitral cells, and these all project to a similar region within piriform, then the glomerular map may be partially conserved. If glomeruli contain a combination of narrowly- and broadly-projecting mitral cells, then information from each glomerulus would be distributed in a more unbiased manner throughout the piriform cortex. A high-throughput tracing method that preserves the glomerular identity of the mitral cell axons would resolve this issue.

Next, Chen and colleagues[11] examined the projection patterns from piriform cortex neurons and found that neurons clustered into six groups based on their projection targets. Within a group, individual neurons predominantly projected to one or just a few neighboring regions. Moreover, the proportion of neurons belonging to each group differed from the anterior to the posterior piriform cortex (Figure 2A). Intriguingly, areas of the piriform cortex that project to various other higher olfactory areas receive bulb inputs that also project to those areas (Figure 2B). For example, the region of the piriform cortex that projects primarily to lateral entorhinal cortex receives inputs from mitral cells that also project to this region. This connectivity motif allows higher order olfactory areas to receive the same information via direct and indirect pathways, forming multiple parallel streams for processing of olfactory information. These "triads" may be genetically specified. Alternatively, two cortical areas that receive the same type of mitral cell input (i.e. inputs from common glomeruli) are more likely to be co-active than two areas receiving input from mitral cells that don't share glomeruli. Thus, their preferential interconnectivity could simply result from the strengthening of synaptic connections between coactive cells. Future experiments examining the developmental trajectories of these motifs could help distinguish these possibilities.

The findings of Chen and colleagues[11] suggest that each projection motif may extract and then relay distinct types of odor-related information to the appropriate downstream area. A similar phenomenon has been observed in the primary visual cortex, where intermixed populations of cells are tuned to different spatial and temporal frequencies of visual stimuli. While these visual stimulus features are not topographically organized, the axons innervating each higher visual area exhibit similar spatial and temporal frequency tuning, forming separate processing streams for different types of stimuli[17]. The anatomical data of Chen and colleagues[11] suggest that the olfactory bulb and piriform cortex may also transmit distinct forms of sensory information via parallel streams.

In support of this model, sister mitral cells have different biophysical properties[18] and can respond differently to the same odor stimulus[19,20]. Given this heterogeneity, sister mitral cells could extract different features of the odor stimulus and form distinct projection motifs which relay this information to the appropriate downstream region. Therefore, rather than a topographic map of receptor space, odor information in piriform may be spatially organized according to these extracted features (Figure 2B).

If the olfactory system does indeed form separate processing streams for stimulus features computed in the olfactory bulb, what might these features be? Chen and colleagues[11] suggest that the three major circuit motifs revealed in their experiments could correspond to separate odor perception, valence, and action pathways (Figure 2B). These parallel pathways would allow for features, such as odor identity and intensity, to be computed in one brain region, while simultaneously, odor salience and the appropriate behavioral response to the stimulus would be computed in others. Bidirectional connectivity between areas involved in each of these pathways would ensure that the appropriate contextual information is provided to perform each of these parallel operations. While this model is intriguing, a host of different behavioral and physiological studies will be necessary to understand the true purpose of these parallel projection pathways.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Ferroptosis: Under pressure!

Adam Wahida[1,2,*] and Marcus Conrad[1]
[1]Institute of Metabolism and Cell Death, Helmholtz Center Munich, Neuherberg, Germany
[2]Division of Gynecological Oncology, National Center of Tumor Diseases (NCT), and German Cancer Research Center (DKFZ), Heidelberg, Germany
*Correspondence: adamwahida@gmail.com
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Ferroptosis is a disease-relevant and pervasive form of cell death triggered by iron-dependent lipid peroxidation and resulting in membrane rupture. A new study addresses how tension-sensing channels can balance and modulate membrane tension in the context of ferroptotic cell death.

> *Good stories are driven by conflict, tension, and high stakes*. – William Landay
>
> *Pressure pushing down on me*
> *Pressing down on you no man ask for*
> *Under pressure — that burns a building down* – Queen and David Bowie

Ferroptosis constitutes a form of regulated cell death triggered by aberrant lipid peroxidation with wide implications for health and disease. Since the term was coined in 2012[1], ferroptosis has rapidly become a well-recognized cell death modality with broad implications for various diseases[2]. Hence, cellular surveillance mechanisms for ferroptotic cell death constitute essential features of homeostasis, with potentially far-reaching consequences for therapeutic intervention[3]. To date, only a few of these systems have been characterized[4–6], with glutathione peroxidase 4 (GPX4) being the most central one, owing to its unique ability to directly reduce hydroperoxides in lipid bilayers, thereby preventing the deleterious lipid peroxidation chain reaction. Since protection mechanisms against ferroptosis are critical for homeostasis, it is astonishing that life has evolved to rely principally on just one enzyme. Thus, it is most likely that a plethora of as-yet unidentified mechanisms contribute to modulating ferroptotic cell death and its physiological functions.

At the core of ferroptosis lies the enzymatic function of GPX4, which perpetually impedes the unrestrained oxidation of unsaturated acyl chains in phospholipids and thereby averts the spontaneous rupture of the plasma membrane (Figure 1)[3,7,8]. Even before the term ferroptosis was introduced in the literature, cellular demise due to genetic loss of GPX4 had been reported in murine models of GPX4 deficiency[6] and in the