PROJECT SUMMARY (See instructions):

Reciprocal interactions between mitral and granule cells in the olfactory bulb external plexiform layer (EPL) modify the timing of mitral cell action potentials and thereby influence the information that the olfactory bulb exports to its multiple targets. Theoretical and experimental results have indicated that this powerful and plastic EPL network is not responsible for the simple “contrast-enhancement” function often attributed to it (instead, this largely occurs in the glomerular layer). Rather, EPL interactions perform additional, subsequent operations on odor representations that mediate changes in odor perception and representation based in part on an individual animal’s history of odor learning. Specifically, we here propose that computations in the EPL serve to render mitral cell output patterns selective for certain higher-order features of odors in the same sense that neurons in primary visual cortex are selective for higher-order visual features such as edge and orientation – that is, they reflect the co-activation of certain spatiotemporal combinations of receptors that together are characteristic of a meaningful odor.

We here propose to develop detailed theoretical models of this hypothesis and its implications and to test its main critical predictions experimentally. The model in its present form predicts (1) that whereas granule cells can be excited by mitral cell lateral dendrites irrespective of their physical proximity, spike timing in mitral cells is affected only by inhibition from physically neighboring granule cells, and (2) that granule cells require the simultaneous activation of specific sets of afferent inputs in order to evoke a whole-cell regenerative response and thereby evoke lateral inhibition. This architecture has substantial implications for the processing of odor representations. To test this model, we will determine whether granule cell effects on mitral cell activity depend on physical proximity, measure the form and specificity of the afferent activity patterns required to evoke spikes in granule cells us, and test the model’s assumptions regarding the structure of olfactory bulb plasticity by measuring the perceptual effects of competing odor representations.

RELEVANCE (See instructions):

Dementias such as Alzheimer's disease arise from failures of learning and memory mechanisms. In most cases, the underlying causes of dementia are not clear, as they result from subtle failures of complex interacting neuronal systems. By studying how learning is used to modify the structure and content of perceptual representations, we seek to understand how these systems can be protected from such failures.

PROJECT/PERFORMANCE SITE(S) (if additional space is needed, use Project/Performance Site Format Page)

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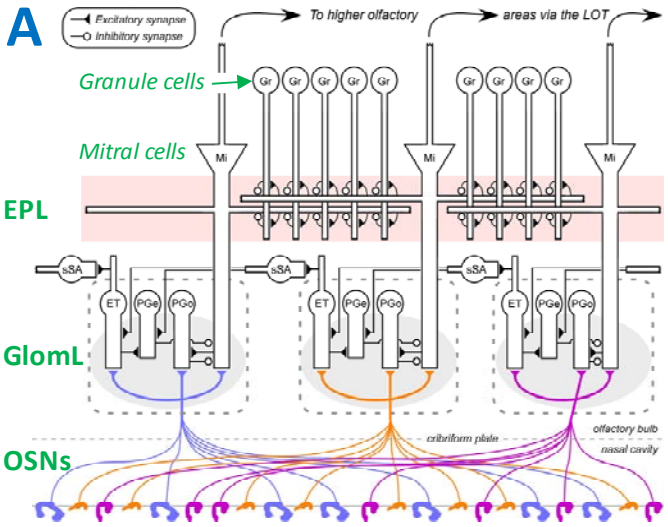
1. Introduction and Specific Aims

The dense synaptic connectivity of the external plexiform layer (EPL; Figure 1A), is one of the most compelling architectural features of the mammalian main olfactory bulb (MOB). As the EPL comprises a functionally *lateral inhibitory network* – that is, the lateral dendrites of mitral cells (principal neurons) excite granule cells (inhibitory interneurons), which reciprocally inhibit the same mitral cell dendrite as well as those of other mitral cells (Figure 1B) – the EPL often has been modeled as directly analogous to the retina. However, theoretical work by PI Cleland (Cleland et al 2007; Cleland & Sethupathy 2006), since experimentally supported by work from several groups (Fantana et al 2008; Gire & Schoppa 2009; Mandairon et al 2006; Soucy et al 2009) including that of PI Schaefer (see *Background and Significance*), has challenged this assumption, proposing instead that glomerular-layer circuitry is responsible for most of the operations traditionally attributed to EPL. The question then arises: what is the function of EPL circuitry? **We here propose an experimentally falsifiable theory for the operation and function of the MOB EPL network.** Dr. Thomas Cleland will be responsible for the theoretical and computational analyses of the mitral-granule network of EPL as well as the biophysical modeling of charge spread and interspinal communication within granule cells (Aim 1), including participating in the training of students/postdocs from the Schaefer lab. He will also be responsible for the behavioral/perceptual studies of Aim 4. Dr. Andreas Schaefer will be responsible for the electrophysiological and optical imaging studies described in Aim 2 and Aim 3, including participating in the training of students/postdocs from the Cleland lab. This new collaboration will enable a stronger and more coordinated investigation of EPL function than either lab would be capable of individually.

Specific Aim #1. To construct a testable, predictive functional model of mitral-granule interactions in the external plexiform layer (EPL) of olfactory bulb. This model will be based on our existing dynamical model of EPL circuitry expanded to incorporate the following principles of operation: (1) while the *recurrent* inhibition of mitral cells by granule cells requires only graded inhibition, *lateral* inhibition between mitral cells requires regenerative activation in granule cells (*spikes,* referring both to sodium action potentials and calcium T current-based low-threshold spikes), (2) granule cell spikes can be selectively evoked by the simultaneous activation of specific groups of mitral cells, (3) granule cells' output synapses must be physically close to mitral cell somata/apical dendrites in order to meaningfully affect the timing of mitral cell action potentials via shunting inhibition, and (4) that these principles can combine to render mitral cell output patterns selective for particular characteristic *patterns* of receptor activation (*higher-order receptive fields*), such as would be diagnostic of whole, natural odors. This constitutes an olfactory analogue of the *binding problem* as articulated in the visual system.

Specific Aim #2. To test the spatial extent of inhibition of mitral cells by granule cells. It is known that mitral cell action potentials are propagated broadly across the EPL via their lateral dendrites. In contrast, inhibition does not propagate, suggesting that direct effects on mitral cell soma/apical dendrite activity (and hence on mitral cell spike timing) arise only from granule cells that are physically adjacent to the mitral cell. To assess the extent of such distance-dependence, whole-cell recordings will be made from mitral cells in MOB slices while granule cells (expressing channelrhodopsin-2) are optically stimulated in spatially selective patterns, varying in breadth and in proximity to the recorded mitral cell. Additionally, lateral inhibition will be evoked and recorded from mitral cells by electrical and optical stimulation of glomerular inputs, while granule cells expressing halorhodopsin (NPhR3) are optically silenced in patterns that will vary in extent, position, and intensity to assess which granule cells are mediating the inhibition.

Specific Aim #3. To determine the range and specificity of afferent activity patterns required to evoke regenerative spikes in granule cells. Intracellular recordings from granule cells in combination with calcium imaging and whole-cell recordings of their dendrites will be made in MOB slices from Chr2-OMP transgenic mice (in which channelrhodopsin-2 is expressed in the axonal arbors of olfactory sensory neurons, which comprise the bulk of MOB glomeruli). "Artificial odor" stimuli will be delivered via optical activation of groups of glomeruli in characteristic spatiotemporal patterns that mimic naturalistic odor input. Patterns varying in breadth, intensity, and spatial specificity will be delivered in order to establish the conditions under which granule cells discharge spikes and thus deliver lateral inhibition.



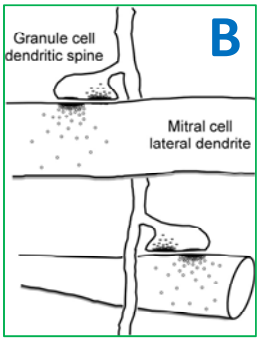


Figure 1. Architecture of main olfactory bulb (MOB). **(A)** Network diagram of the olfactory bulb. OSNs: primary olfactory sensory neurons. GlomL: glomerular layer. EPL: external plexiform layer. Adapted from Cleland (2010). **(B)** Diagram of mitral-granule synaptic interactions. Mitral cell dendrites excite granule cells spines, which in turn inhibit mitral cell dendrites. *Recurrent* inhibition involves a single granule cell spine and is graded, whereas *lateral* inhibition requires the granule cell to spike (sodium or LTS) so that activity propagates between spines.

Specific Aim #4. To elucidate the rules governing competing interactions among learned odor representations. The theoretical model described in Aim 1 proposes mechanisms by which structurally and perceptually similar odorants that become associated with different outcomes can be efficiently discriminated from one another via EPL operations. Using established behavioral/psychophysical studies in mice that rely specifically upon olfactory bulb circuitry, we will assess the principles by which these "competing" odor representations are resolved.

2. Background and Significance

Computational models serve to efficiently direct experimental efforts, reveal the less-obvious implications of experimental results, and define specific testable predictions. Consequently, they are essential tools for understanding and interpreting complex data sets drawn from diverse sources. The theoretical models that frame this proposal and comprise Aim 1 are constrained by both existing experimental results and first principles of neurophysiology, and descend from previous theoretical models of olfactory bulb function by PI Cleland. Those earlier models made testable predictions that were subsequently confirmed by multiple laboratories, most importantly and recently by PI Schaefer. We first review this theory briefly as it establishes the basis for the hypothesis of EPL function that we outline in this proposal.

The tuning curves of primary olfactory sensory neurons (OSNs) directly reflect the broad receptive fields of olfactory receptors (Araneda et al 2000). Axons from OSNs that express the same olfactory receptor converge together upon the surface layer of the MOB to form *glomeruli* – each a cluster of OSN axonal arbors, the collective activity of which reflects the activation of one specific type of olfactory receptor (Figure 1A). Mitral cells – which in the mammalian MOB receive direct afferent input from exactly one glomerulus – generally inherit the chemical receptive field of these convergent OSNs, but also exhibit a clear inhibitory surround – i.e., they are inhibited by odorants that are on the edges of the range of structurally similar odorants that excite them (Fletcher & Wilson 2003; Yokoi et al 1995). That is to say, mitral cells' receptive fields, and their surrounds, are defined (appropriately) with respect to *odorant chemical similarities*, which also correlate with perceptual similarities (Abraham et al 2010; Abraham et al 2004; Cleland et al 2002; Linster & Hasselmo 1999). The implication is that odorants on the edges of an OSN's chemical receptive field – i.e., that are relatively weak agonists for the olfactory receptor – end up *inhibiting* the corresponding mitral cells, directly analogous to how the edges of visual objects or auditory tone bands evoke an edge-enhancing inhibitory surround. However, owing to the great diversity of primary odor qualities (*high dimensionality*), the physical proximity of MOB neurons (and glomeruli) cannot reliably reflect the similarity of their receptive fields as it does in the retina and cochlea, and lateral inhibition cannot underlie the inhibitory surround observed in mitral cells (Cleland 2010; Cleland & Sethupathy 2006). It has since been experimentally confirmed that the physical proximity of glomeruli indeed does not correlate with the similarity of their chemical receptive fields (Soucy et al 2009), and

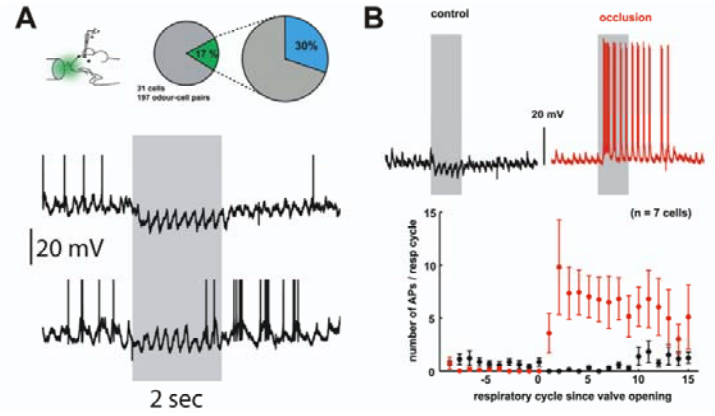


Figure 2. (A) Whole-cell recordings from mitral cells in the mouse main olfactory bulb *in vivo* show odor-evoked phasic inhibition in 30% of all cases where significant odour-evoked responses could be observed (17% of all odor-cell pairs). Two examples of phasic inhibition are shown below. **(B)** Topical application of gabazine + muscimol did not affect spontaneous firing rate or input resistance but significantly occluded synaptic inhibition (not shown). Such occlusion of inhibition reliably converted odor-evoked phasic inhibition into strong odor-evoked excitation (top panel), resulting in substantial AP discharge in response to odors (bottom panel). Gray shading denotes 2 sec odor presentations.

that lateral inhibitory weights in MOB do not correlate with either physical proximity or receptive field similarity (Fantana et al 2008). This theory challenged the classical model in which the lateral inhibitory network of the EPL was believed to mediate the "surround" inhibition observed in mitral cells (Shepherd & Greer 2004; Yokoi et al 1995). Instead, it proposed a mechanism based upon intraglomerular feed-forward inhibition coupled with a normalizing circuit in the deep glomerular layer (Cleland et al 2007; Cleland & Sethupathy 2006), and predicted that ON-driven periglomerular cells (PGo; Figure 1A) – a class of local inhibitory interneuron that receives direct afferent input alongside mitral cells (Shao et al 2009) – should be more strongly activated by afferent input than their corresponding mitral cells, and also be able to gate mitral cell activation by shunting inhibition upon the mitral cell primary dendrite. Both of these predictions have since been experimentally confirmed (Gire & Schoppa 2009). Finally, the theory suggested that the suppression of mitral cell spiking activity observed by Yokoi *et al.* (1995) would not substantially depend upon granule cell activity – a prediction since confirmed by Fukunaga, Herb, and Schaefer (2010*, Soc. Neurosci. Abstr.* 369.15) and outlined here.

Direct, phasic inhibition is a dominant response of mitral cells to odor presentations *in vivo* (Figure 2A). Occlusion of inhibitory synaptic activity (without epileptogenesis) by the simultaneous, balanced bath application of the $GABA_A$ antagonist gabazine and the $GABA_A$ agonist muscimol abolished odor-evoked phasic inhibition and nearly always resulted in strong phasic excitation (Figure 2B), consistent with our predicted mechanism of feed-forward glomerular-layer inhibition. Virus-mediated expression of the optogenetic silencer *Natronomonas pharaonis* Halorhodopsin (eNPhR 3) in granule cells was used to assess the contribution of granule cells to this phasic inhibition (Figure 3A). In preliminary experiments, mitral cell firing rates increased in response to the optical silencing of granule cell activity, indicating effective suppression of granule cell activity. Odor-evoked phasic inhibition, however, was completely unaffected (Figure 3B). These results support the hypothesis that contrast enhancement by mitral cell spike suppression depends on feed-forward inhibitory interactions in the glomerular layer, provoking the question of what types of computations then might be mediated by the extensive mitral-granule cell network within the EPL.

3. Theoretical Foundation

The architecture of the EPL network is compelling. The lateral dendrites of mitral cells extend for many hundreds of microns in the mouse MOB, practically the entire breadth of the functional network. Action potentials in mitral cells propagate regeneratively down these lateral dendrites, exciting many different granule cells across the MOB. Whereas electrical measurements of laterally-propagating action potentials show that spike amplitude decreases with distance (Lowe 2002; Margrie et al 2001), perhaps owing to the progressively shrinking dendritic diameter, imaging experiments indicate that the calcium transient evoked by this propagating signal does not decline in amplitude with distance from the soma (Charpak et al 2001; Debarbieux et al 2003; Xiong & Chen 2002). Consequently, the signaling strengths along mitral cell lateral dendrites can be considered largely independent of physical proximity to the soma. These lateral dendrites release glutamate onto granule cell dendritic spines, most of which release GABA right back onto the mitral cell dendrite in a graded manner (***recurrent inhibition***) (Abraham et al 2010; Isaacson & Strowbridge 1998; Lagier et al 2004). Spiking granule

cells additionally can release GABA onto the lateral dendrites of other mitral cells, thereby effecting functional ***lateral inhibition*** between mitral cells (Egger et al 2005) (Figure 1B). As described above, the weight of lateral inhibition between any two mitral cells does not correlate with their physical proximity (Fantana et al 2008). Rather, it has been suggested that these weights are plastic and depend on olfactory learning, continuing to shape odor representations subsequent to glomerular-layer computations such as contrast enhancement (Cleland 2010). Indeed, it is clear that plasticity within MOB circuitry underlies some aspects of odor learning. Behavioral studies reveal learning-dependent changes in perceptual generalization (Cleland et al 2009) that can be blocked, impaired, or replicated by drug infusions directly into MOB (Chaudhury et al 2010; Mandairon et al 2006). The consolidation of this learning depends on protein synthesis within the MOB (Kermen et al 2010) and requires the integration of newly differentiated granule cells into EPL circuitry in an odor-dependent pattern (Lazarini & Lledo 2011; Moreno et al 2009). The implication is that acquired distinctions among arbitrary odor representations can be embedded into MOB circuitry via the lateral inhibitory circuitry of EPL, and that the pattern of these distinctions depends on learning (rather than on the similarity of chemoreceptive fields). Critically, these acquired distinctions may serve to distinguish the representations of *whole odors*, rather than simply the collective activity of individual glomerular columns. Such a mechanism would resolve a critical component of the olfactory binding problem, enabling computations to be performed with respect to multiglomerular, whole odor-specific patterns rather than reducing these patterns to the sums of their elements. This hypothesis rests upon several theoretical principles of MOB function that are closely derived from existing data and first principles of biophysics but have not been directly tested. We here propose to test these principles and quantitatively evaluate their implications.

(1) *Granule cell inhibition affects mitral cell spike timing rather than suppressing spikes*. As the excitation of mitral cells arises from afferent inputs in the distant glomerular layer (Figure 1A), and spikes can be generated within the mitral cell primary dendrite (Chen et al 2002; Djurisic et al 2004; Shen et al 1999), granule cell inhibition of mitral cell lateral dendrites is poorly located to silence mitral cell spiking in the way that periglomerular cell inhibition does (as described above). Rather, granule cell inhibitory inputs are more likely to simply delay the propagation of spikes, as suggested by recent biophysical models of shunt inhibition on lateral dendrites (David et al 2008; Figure 4A). The delay of spikes in a synchronous oscillatory network such as the MOB has the effect of phase-constraining (quasi-synchronizing) mitral cell action potentials (Figure 4B,C), an effect that has been widely observed in recordings from MOB (David et al 2009; Kashiwadani et al 1999; Lagier

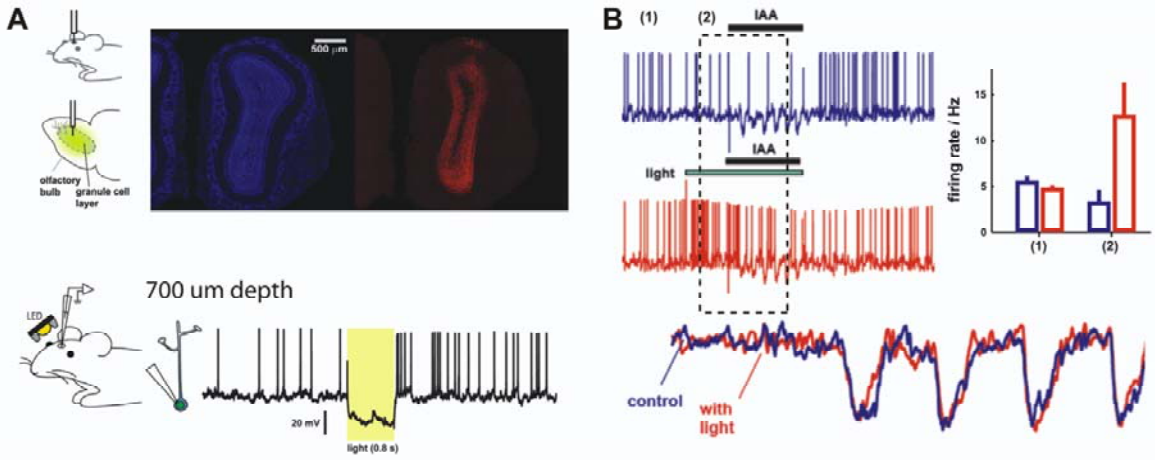


Figure 3. (A) Targeted genetic manipulation of approximately 80% of all granule cells can be achieved by stereotactic injection of high-titer adeno-associated viruses (AAV) into the core of the olfactory bulb. *Top:* Coronal sections through the MOB 2 wk post-infection with a Cre-expressing AAV are shown. *Red*: antibody stain for virus-mediated expression of nuclear marker Cre recombinase; *blue*: DAPI staining of all cell bodies. Expression efficiency was verified by blind electrophysiological characterisation of randomly selected granule cells. Expression is largely restricted to granule cells through usage of glutamic acid decarboxylase (GAD) promoter fragments. *Bottom*: Expression of the chloride pump NPhR in granule cells enables light-mediated silencing of their activity. Whole-cell recordings were performed from granule cells in animals with virus-mediated expression of NPhR in approximately 80% of all granule cells. Upon exposure to yellow light through a surface mounted LED, granule cells hyperpolarized by 10-30 mV at depth up to 1 mm from the surface (700 um depth traces shown). **(B)** Whole-cell recording from a MC in an animal with NPhR-expressing granule cells. Light exposure increased the spontaneous mitral cell firing rate. Odor presentation resulted in phasic inhibition that was unaffected by light-mediated silencing of granule cell activity (*bottom traces*: magnification of dotted box above; APs clipped).

et al 2004; Schaefer et al 2006; Schaefer & Margrie 2007; Schoppa 2006). Moreover, pyramidal cells in piriform cortex are strongly preferentially activated by synchronized spikes among mitral cells (Luna & Schoppa 2008). The representation of sensory information by spike timing in oscillatory networks has been widely debated (Colgin & Moser 2010; Engel et al 2001; Fries et al 2007; Paik & Glaser 2010) and will be elaborated for the MOB specifically in Aim 1.

(2) *Mitral cell effects are broad; granule cell effects are local.* Mitral cell action potentials propagate down their lateral dendrites, exciting many granule cells via reciprocal synapses. However, inhibition does not propagate as does excitation. As the effect of shunting inhibition on cellular activity is mediated by the reduction of input resistance, inhibition delivered at larger electrotonic distances should have reduced or negligible impact (Figure 4A). Consequently, granule cell inputs that are physically adjacent to a mitral cell's soma/apical dendrite should be effective at influencing its spike timing, while those that are relatively distal along its lateral dendrite should be correspondingly ineffective (David et al 2008). This does not imply that these distal reciprocal synapses have no role to play in the overall system – e.g., dense reciprocal interactions across the MOB are likely to render coherent field potential oscillations considerably more stable than they otherwise would be (Bazhenov et al 2008) – but simply that they are unlikely to mediate direct effects on the activity pattern of the mitral cells that they inhibit. The network topology implied by this principle is that granule cells can receive input from potentially large numbers of mitral cells irrespective of their location, but can in turn directly modify the spike timing/activity of only a small number of mitral cells within a restricted local area. This hypothesis will be experimentally tested in Aim 2, and its computational implications explored in Aim 1.

(3) *Different physiological mechanisms underlie recurrent and lateral inhibition*. Most mitral-granule interactions occur at reciprocal synapses between mitral cell lateral dendrites and granule cell spines, and it has been established that granule cell spines release GABA back onto mitral cells via graded inhibition at these synapses (Isaacson & Strowbridge 1998; Lagier et al 2004). However, lateral inhibition, unlike recurrent inhibition, requires that the excitation received by one spine be propagated to another spine along the granule cell dendrite (Figure 1B). In rodent granule cells, calcium imaging experiments have shown that propagation of activity from one spine to another only occurs when regenerative activation spreads throughout the entire granule cell dendritic tree; that is, responses to low-amplitude inputs remain confined to individual spines, whereas larger inputs – possibly requiring depolarization of multiple spines – evoke regenerative processes that reliably invade spines along the entire dendritic tree (Egger et al 2005). Both sodium action potentials and T-type calcium current low-threshold spikes (LTSs) can mediate these regenerative effects in granule cells, though the LTS mechanism appears more reliable in terms of generating whole-cell regenerative responses in response to afferent inputs (Egger et al 2003; 2005); sodium spikes seem more reliably characteristic of granule cell activation by ascending piriform cortical projections (Strowbridge 2009). Both sodium spikes and LTSs (henceforth collectively referred to as *spikes*) have quantitatively similar effects upon calcium transients within granule cell dendrites and spines (Egger et al 2005). Computationally, these data have two important implications. First, they suggest that effective lateral inhibition can be regulated quasi-independently from effective recurrent inhibition, and possibly even separately regulated physiologically (e.g., by modifying the excitability of granule cells with acetylcholine (Pressler et al 2007) or depolarizing them via ascending

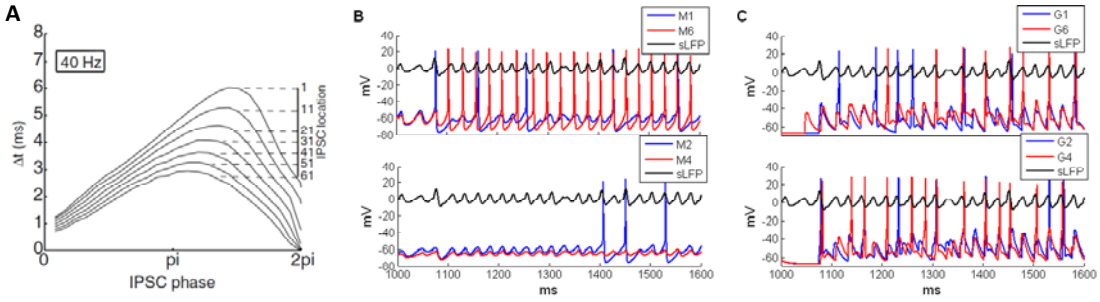


Figure 4. Mitral-granule network model properties. **(A)** Effect of IPSCs on the timing of mitral cell action potentials in our computational model. The IPSPs' effects on mitral cell spike timing depend on the distance of the synapse from the mitral cell soma and on the phase of the ongoing gamma oscillations in the model. The latter effect facilitates phase compression of spike times, increasing spike synchronization in mitral cells. **(B)** Network interactions in the later model evoke field potential oscillations (black) and constrain mitral cell spike times (colors). Four arbitrary mitral cells are depicted in two plots. **(C)** The same process constrains granule cell spike times; four spiking granule cells are depicted in two plots.

projections (Strowbridge 2009)). Second, it indicates that lateral inhibition is thresholded; that is, a given granule cell will not deliver lateral inhibition unless it is sufficiently depolarized to spike, and then will deliver strong, reliable IPSCs onto its target mitral cell(s). How many, *and which*, mitral cells must be activated in order to initiate a spike in a given granule cell then become questions that depend on the regulation of granule cell thresholds and of specific patterns of mitral-to-granule synaptic weights.

The functional implication of this architecture is exciting; it suggests that granule cells may exhibit complex, "higher-order" receptive fields selective for specific *combinations* of mitral cell activity patterns across the MOB – perhaps corresponding to the principal features of a complete and behaviorally relevant odor. (This process is analogous to how the activation of edge-selective neurons in primary visual cortex depends upon the coactivation of correctly oriented "rows" of retinal ganglion cells). Specifically, given that granule cells require a certain cumulative level of input from mitral cells in order to fire a spike (Egger et al 2005), and given that each granule cell may have relatively few synaptic inputs (estimated at ~100 by Egger & Urban 2006), and further that these inputs may have different synaptic weights (dependent on prior odor learning), then each granule cell can be predicted to fire regeneratively only in response to the simultaneous activation of specific sets of mitral cells (a multiglomerular, "higher-order" receptive field; Figure 5). This hypothesis will be directly tested in Aim 3, whereas its implications for the information exported from olfactory bulb (via mitral cell activation patterns) will be explored in Aim 1.

4. Experimental Design and Techniques

Specific Aim #1. To construct a testable, predictive functional model of mitral-granule interactions in the external plexiform layer (EPL) of olfactory bulb.

Our theoretical and computational models of olfactory bulb operations anchor the selection and design of experiments in this proposal, and by implementing ongoing experimental results will serve to guide further studies. The particular strengths of the model for present purposes include (1) its biophysical basis, such that electrophysiological measurements of membrane properties can be directly implemented, (2) its 'reduced compartmental' design, such that free parameters are minimized while essential compartmentalization is retained, and (3) its inclusion of dynamical properties. This is particularly important for the present application, given that EPL interactions are believed to have a strong dynamical component, regulating the timing rather than the existence of mitral cell spikes. Weaknesses of this model include its slow development time; owing to its strong dependence on quantitative data and the interactions of multiple dynamical elements, it must be constructed gradually, beginning with the periphery and initially building important individual cellular and microcircuit models in isolation. The model presently incorporates glomerular layer computations including dynamically accurate mitral and assorted juxtaglomerular cells (Cleland et al 2007; Cleland & Sethupathy 2006; Rubin & Cleland 2006) and an oscillatory EPL network of mitral and granule cells (Figure 4), primarily implemented in the simulator NEURON (Hines & Carnevale 1997). In the present proposal, we propose to increase the complexity of the granule cell model to study the mechanism of summation among different mitral cell inputs, and to expand our EPL network into a two-dimensional map-based model capable of addressing the questions regarding the topology of EPL projections and interactions described herein.

Aim 1a. Implement a biophysically-based model of summation and signal spread in granule cell dendrites. Calcium influx in dendritic spines comprises contributions from NMDA receptors, calcium-dependent calcium release from thapsigargin-sensitive internal stores within the spine, and the activation of low-threshold T-type calcium currents (Egger et al 2005). Critically, synaptically-evoked calcium transients are contained within individual spines until an all-or-nothing activation threshold is crossed, at which point calcium influx spreads throughout the entire dendritic tree via a T-current dependent low-threshold spike (Egger et al 2005). The observed physiological isolation of granule cell spines from their adjacent dendrites is notably greater than observed in other neuronal spines (Egger et al 2005; Svoboda et al 1996), perhaps owing in part to their unusually long spine necks (~2.5 um), but in any case enabling the spines to respond quite independently to synaptic input from mitral cells (mediating recurrent inhibition) until the spike threshold is crossed (activating lateral inhibition).

Summation. Based on these data, how, and to what extent, do inputs from different spines summate to evoke a whole-cell spike in granule cells? The substantial physiological isolation of granule cell spines from their parent dendrite renders this question non-trivial. We will extend our existing granule cell model (Figure 4) to incorporate the calcium dynamics of granule cell spines along with their thresholded whole-cell spikes, and deploy multiple spines along an extended, realistic dendritic shaft in order to study the summation of inputs from

different spines. The extent to which summation across multiple spine inputs (i.e., originating from different mitral cells) occurs in granule cells will be measured in Aim 3.

Potential pitfalls. The null hypothesis is that there is no summation of inputs across different spines, such that granule cells do not summate activity arising from different mitral cells, but rather evoke thresholded global spikes solely based on input levels from each spine individually (i.e., firing whenever any one spine exceeds the threshold for spike evocation). Such a result from Aim 3 would falsify the "multiglomerular/higher-order receptive field" hypothesis posed above, and instead define granule cells (roughly) as OR operators rather than AND operators. However, the basic framework of the questions posed in this proposal would remain: what afferent activation profiles cause granule cells to globally depolarize and deliver lateral inhibition, and what computations can be performed based on their patterns of connectivity with mitral cells?

Signal spread. Interestingly, the calcium transients attributable to local synaptic and to global spike activation summate approximately linearly within granule cell spines, indicating a complexity of calcium dynamics at the reciprocal synapse greater than a simple spike-based 'integrate and fire' mechanism could represent (Egger et al 2005). Among other properties, this property enhances the quantitative independence of recurrent and lateral inhibitory weights. Moreover, it preserves the integrity of the odor-specific activity map by ensuring that the stronger recurrent inhibition evoked by more strongly activated mitral cells is preserved, rather than equalizing the strength of inhibition across all mitral cells by virtue of a global spike. The calcium dynamics enabling these partially independent yet intertwined processes will be modeled, with particular attention to the fraction dependent on calcium flux through NMDA receptors (Balu et al 2007; Halabisky et al 2000; Jahr & Nicoll 1982) and the roles that different local synaptic weights or altered calcium dynamics may play in the efficacy of lateral inhibition.

Aim 1b. Implement a two-dimensional map-based EPL network model of recurrent and lateral inhibition. We will extend our existing dynamical models of mitral-granule interactions (Figure 4; also David et al 2008; Rubin & Cleland 2006) to incorporate an explicit two-dimensional spatial map localizing mitral and granule cells with respect to one another (Bazhenov et al 2008; Rulkov et al 2004). The model will be constructed and parameterized as follows: **(1)** The numbers and ratios of neuron types and synaptic connections will be based on current estimates (e.g., Egger & Urban 2006; Schoenfeld & Cleland 2005) and deployed in explicitly spatial coordinates on a spherical surface; larger-scale versions of the model will be run on a massively parallel computational cluster (see *Facilities*). **(2)** All mitral-granule synapses will be reciprocal, supporting gamma-band network oscillations via recurrent inhibition as implemented in our current MOB dynamical model (Figure 4). **(3)** The efficacy of mitral cell excitation of granule cell spines will be proximity-independent (except for propagation delays); in contrast, the capacity of granule cell shunt inhibition to influence mitral cell spike timing will decline with the distance between the reciprocal synapse and the mitral cell soma/apical dendrite (David et al 2008; also cf. preliminary data of Figure 6C). This decay function will be parameterized to fit the data generated in Aim 2, primarily by varying relevant passive parameters within reasonable ranges (e.g., dendritic diameter, taper, axial resistivity, leak conductances). Importantly, lateral inhibition is not expected to *suppress* mitral cell spikes via shunting in the way that glomerular-layer inhibition does (Cleland & Sethupathy 2006; Gire & Schoppa 2009), but only to *delay* them, hence altering their timing (David et al 2008) and entraining them with respect to ongoing beta/gamma-band network oscillations (David et al 2009; Lagier et al 2004; Schaefer et al 2006). **(4)** In contrast to *recurrent* inhibition, *lateral* inhibition will require whole-cell spike activation in granule cells, which will be evoked by specific combinations of glomerular activity levels (*AND* gating) if supported by the data from Aim 2, or otherwise by the suprathreshold activation of any one of a small set of glomeruli (*OR* gating; cf. discussion in Aim 1a). **(5)** The effects of granule cell receptive field breadth will be simulated to measure its efficacy at differentiating highly similar odorants.

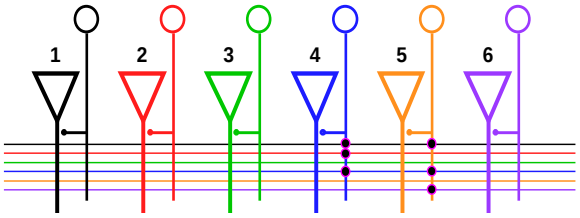


Figure 5. Conceptual model of the hypothesized construction of higher-order receptive fields in mitral cells. Each granule cell (circular soma) effectively inhibits only its neighboring mitral cell (triangular soma) but receives excitatory inputs from all mitral cells. Granule cells will spike only when receiving three simultaneous inputs. Nonzero mitral-to-granule synaptic weights are depicted with black/pink dots. Here, mitral cell #4 is inhibited only when an odorant simultaneously excites mitral cells #1, #2, and #4. If (e.g.) #1 and #4 fire alone, they generate no lateral inhibition. If #1, #4, and #6 fire together, however, they inhibit mitral cell #5 (and not #4).

Analysis and interpretation. The main goals of this modeling Aim are to better understand and integrate the emergent properties of the MOB network. First, the initial model will instantiate the hypotheses described above so as to frame the experiments described in Aims 2-4 and assemble their results into a common framework. Second, later revisions of the model (incorporating data from Aims 2-4) will enable us to better understand the complexities of mitral cell receptive fields and the different metrics (gross activity, temporal patterning) by which the elements of these receptive fields can be read out by follower cells (Linster & Cleland 2010). Specifically, as described above, mitral cell activation is inherited from the activation of a single olfactory receptor type. Granule cell-mediated lateral inhibition within the EPL, in contrast, modifies mitral cell spiking patterns according to the influence of other mitral cells activated by different receptors. Guided by the lateral inhibitory effects on mitral cell spike timing as measured in the experiments of Aim 2, this model will enable the formulation of concrete hypotheses regarding how mitral cells integrate information about the larger olfactory scene into their temporal activity patterns, and how synaptic plasticity in this layer could embed learning about meaningful odors into the network with minimal interference among learned odor patterns. Third, the model will enable quantitative assessment of what improvements in odor segmentation and recognition could be gained if granule cells are indeed higher-order feature detectors as hypothesized herein. By systematically varying the requirements for spike firing and the gating of lateral inhibition from the single-input "OR" gate extreme to a broad requirement for coactivation of several glomerular inputs ("AND" gate), we will be able to assess the degree to which granule cell selectivity may improve the differentiation of highly overlapping odor representations mediated by mitral cell population activity.

Potential pitfalls. We have already established in standalone models that GABAergic shunting inhibition from granule cells can delay mitral cell spikes but is unlikely to prevent them from propagating (David et al 2008); such periodic inhibition in an oscillating MOB network readily entrains mitral cell spike timing, consistent with the observed pattern of phase-constrained mitral cell spikes in electrophysiological recordings (David et al 2009). It is very likely that this fine regulation of action potential timing carries important sensory information, as outlined above and clearly demonstrated in the analogous honeybee system (Stopfer et al 1997). The metric by which neural ensembles encode information in quasi-synchronous spiking patterns has been widely studied (Fries et al 2007; Linster & Cleland 2010), but is certainly not known. The present experiments will not determine exactly how spike timing regulation encodes information. However, they may constrain and help frame future hypotheses.

Specific Aim #2. To test the spatial extent of inhibition of mitral cells by granule cells.

The central question of this Aim is how the proximity of granule cells (GCs) to mitral cells (MCs) corresponds to the strength of their impact on mitral cell activity. As discussed above, we predict that the efficacy of GC-mediated inhibition of MC activity should decrease rapidly with distance. Most experiments will be performed in brain slice preparations from mice selectively infected with optogenetic constructs in granule cells, enabling their activation or silencing by light. This technique also enables the activation or silencing of *groups* of cells in multiple arbitrary locations simultaneously via application of complex programmable patterns of light. *In vivo* control experiments also will be performed to identify any artifactual effects of the slicing procedure.

Aim 2a. Spatial extent of GC mediated inhibition onto MCs. We will measure the efficacy of GC-to-MC inhibition via whole-cell recording from MCs (and for control purposes from GCs as well), using mice expressing channelrhodopsin in GCs such that these cells can be optically activated in spatially selective patterns and their effect on recorded MC activity assessed. Specifically, we will infect GCs sparsely with the blue light-sensitive optically-gated cation channel channelrhodopsin-2 (ChR2) (Nagel et al 2003) through stereotactic injection of the adeno-associated virus construct AAV-ChR2 (serotype 1/2) into the granule cell layer of P28-P35 mice (Abraham et al 2010) (Figure 3). Two to three weeks after infection, horizontal brain slices will be prepared as described previously (Abraham et al 2010; Schaefer et al 2006). To assess the efficacy of our light-based activation of granule cells, whole-cell recordings from infected GCs will be made while patterns of optical stimulation are systematically varied in their intensity and breadth of field; excitatory synaptic activity will be blocked pharmacologically to isolate direct light-evoked events (cf. preliminary data in Figure 6A). Illumination will be achieved initially by wide-field LED illumination and subsequently by commercial video projectors coupled into the illumination beam path through a switchable mirror.

In a different set of experiments, whole-cell recordings will be made from mitral cells while ChR2-infected granule cells are optically stimulated with spatially restricted spots of blue light (cf. preliminary data in Figure

6B). The center of this illumination then will be systematically moved across the GC layer while recording light-evoked changes in MC activity (visible IPSCs or statistical changes in spiking activity) in order to assess the proximity-dependence of GC inhibitory efficacy (cf. preliminary data in Figure 6C). Optical stimulation of GCs also will be paired with direct depolarization of MCs to increase the visibility of GC-MC inhibition.

Potential pitfalls. (a) Infection selectivity. Preliminary data indicate that GCs can be reliably and selectively infected (Figure 3) (Abraham et al 2010). If AAV-ChR2 infection is insufficiently restricted to GCs, different GAD promoter fragments will be tested. To adjust the sparseness of GC infection, serotypes other than AAV1-2 can be used. For example, preliminary data indicate that AAV1 results in strong but sparse infection, whereas AAV9 selectively infects GCs and not MCs, and AAV8 results in strong and highly promiscuous infection in the OB. *(b) Expression strength.* Generally, ChR2 expression is high enough to enable precise optical evocation of spikes. However, if expression strength is insufficient and cannot be improved by longer expression times, different AAV serotypes and promoters again could be used, including nonspecific but powerful promoters such as EF1-A or CMV. To reduce MC infection in this case, a combined conditional approach using double-inverted ORF (DIO or "double-floxed") variants of AAV-CHR2 combined with either prior Lenti-Cre infection or with animals transgenically expressing Cre recombinase under a GAD promoter fragment (Fuchs et al 2001) could be used. *(c) Interneuron specificity.* Generally, GC-layer injection of AAV will result in infection of non-GC cells in that layer such as deep short-axon cells. These neurons, however, comprise only a tiny fraction of cells in the GC layer (Shepherd & Greer 2004). To reduce the complexity of interpretation, highly sparse infections could be produced such that relatively few cells are infected; subsequent immunohistochemical staining then would reveal the specific subtype of interneuron infected. Under present conditions, however, we do not expect any confounding effects through stimulation of non-GC interneurons. If need be, expression could be optimized using the techniques outlined in (b). *(d) Slice artifacts.* The slicing procedure will partially disrupt the EPL network. In order to assess the effect of such disruption, we will model the slicing procedure and quantify the loss of potential synaptic contacts as a function of slice thickness and the distance between GC and MC, based on detailed morphological reconstructions of MCs and GCs. Control experiments also can be performed with tangential MOB slices in which the EPL network is kept more intact at the cost of severing mitral cell apical dendrites. Furthermore, as described above (Figure 3) we will perform some *in vivo* whole-cell recordings from MCs while exciting selected regions of the GC network (Figure 2). A key issue for these *in vivo* studies will be to obtain sufficient expression and illumination strengths to excite GCs up to 1 mm below the MOB surface. Preliminary data (Figure 2) indicate that this is readily achieved using yellow light (enabling NPhR3-dependent silencing of GCs), and our measurements of light tissue penetration suggest that the blue wavelength needed for ChR2 activation also will penetrate sufficiently. To confirm this, recordings from GCs will be systematically performed *in vivo* at different depths to quantify light penetration. Strategies for increasing expression strength, if necessary, are outlined in (b). Illumination could be enhanced if necessary by using high-power commercial projectors or digital mirror devices (DMDs) and dedicated high power LEDs (Dhawale et al 2010).

Aim 2b. Spatial extent of GC mediated inhibition between glomerular columns. We also will assess the proximity-dependence of effective MC-GC-MC lateral inhibition as naturally evoked by stimulation of afferent inputs to MOB by selectively silencing granule cells in specific locations (similarly to Aim 2a) and assessing the

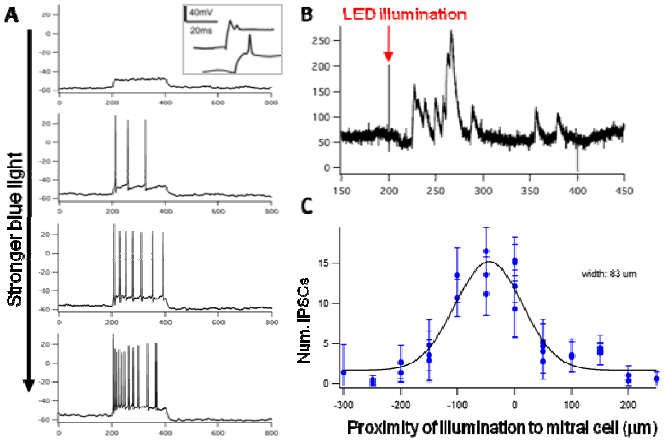


Figure 6. Preliminary data for studies proposed herein. **(A)** Increased optical stimulation intensity produces correspondingly strong responses in a ChR2-expressing granule cell. *Inset.* Dendritic patch recordings from a granule cell. **(B)** IPSCs evoked in whole-cell recordings from mitral cells upon LED optical stimulation of ChR2-expressing granule cells. IPSCs were blocked by 10 uM gabazine (not shown). **(C)** Numbers of IPSCs recorded in mitral cells in response to an 83 um spot illumination of the GC layer from a slice containing ChR2-expressing granule cells, as a function of proximity to the mitral cell.

effects on lateral inhibition. As described above (*Background and Significance*; Figure 3), GC silencing will be achieved via AAV-mediated expression of NPhR3 coupled with optical stimulation. Selective afferent stimulation of MOB glomerular inputs will be performed both electrically with bipolar theta-glass electrodes placed in individual glomeruli as well as optically using transgenic mice expressing ChR2 under the olfactory receptor neuron-specific OMP promoter. This mouse line was generated by Dr. Tom Bozza (Northwestern University, Chicago, IL, USA); a colony has recently been established in our facility at Heidelberg.

To establish a baseline for position-dependent silencing experiments, we will first prepare acute slices from OMP-ChR2 mice, establish whole-cell recording in a mitral cell, and systematically vary illumination along the glomerular layer. MC inhibition will be quantified as a function of GC-MC proximity and illumination strength. Secondly, AAV-NPhR virus will be injected into OMP-ChR2 mice to infect GCs as described above. Two to three weeks later, acute slices will be prepared and recording and stimulation conditions established as above. In addition to blue-light afferent excitation, yellow light also will be employed to selectively silence spatially-selected subpopulations of infected GCs. Again, light stimulation will be delivered via commercial video projectors or DMDs enabling efficient co-illumination with both blue and yellow light. By systematically varying the sites of glomerular stimulation and the positions and intensity of GC silencing, we will generate a detailed, quantitative description of which granule cell populations shape MC-MC inhibition. Preliminary theoretical/computational results (Aim 1) and data (Figure 6C) predict that effective GC effects on mitral cell activity arise only from GCs that are physically very close to the recorded MC.*Potential Pitfalls.* Technical pitfalls and their possible solutions are largely identical to those described under Aim 2a. If adequately independent stimulation with yellow and blue light proves difficult using commercial projectors, we will instead employ either DMDs with tailored high-power LEDs or bandwidth-restricted lasers. Finally, our preliminary data indicate efficient silencing of GCs using NPhR3 (Figure 3); however, the new proton pump silencing tool Arch (Chow et al 2010) will be tested as a possible substitute.

Specific Aim #3. To determine the range and specificity of afferent activity patterns required to evoke regenerative spikes in granule cells.

The central goal of this aim is to determine the conditions under which granule cells display either subthreshold, graded depolarizations within spines or suprathreshold, global spikes. To this end, we will both record intracellularly from granule cells in MOB slices and image calcium signals in their dendrites. Stimulation will be largely performed through optical stimulation of incoming ORN fibers within glomeruli in OMP-ChR2 mice (as described in Aim 2b), but in some studies also will include direct dendritic current injection to bypass mitral cell activation.

Aim 3a. Assess the impact of patterned glomerular stimulation on granule cells. To assess the integration properties of granule cell dendrites, we will prepare MOB slices from OMP-ChR2 transgenic mice and perform whole-cell recordings from granule cells (preliminary data in Figure 2). (i) First, inputs will be evoked by brief optical stimulation of individual glomeruli at different locations and with varying intensities and durations. GC responses will be monitored with particular attention to the parameters determining the presence or absence of spikes (LTS or sodium). Then, broader spatiotemporal patterns of optical stimulation will be played onto selected groups of glomeruli, varying the breadths and variances of stimulation patterns as well as their overall intensities and durations. The relationship of evoked responses generated by these multiglomerular stimulation patterns also will be assessed with respect to the stimulation of individual glomeruli (e.g., is their summation biased?). These multiglomerular stimulation paradigms will enable us to define granule cell responses to naturalistic odor-like stimulus patterns (odors typically generate broadly distributed activity in the glomerular layer). After enough recordings have been made to generate a statistical estimate of the properties of afferent stimulation that evoke spikes in granule cells (i.e., the typical granule cell receptive field), the theoretical model of Aim 1A will be updated and constrained accordingly. A key hypothesis of the present model is that individual granule cells will show strong selectivity for distinct and specific patterns of glomerular activation.

Potential pitfalls. The interneurons within the GC layer are not necessarily homogeneous. Visually guided recordings from somata of a given size in a specific location will reduce variability in cell type selection, but also will introduce a selection bias into our data. While it initially will be necessary to focus on classical granule cells with small somata, high input resistances, a slender apical dendrite and no axon, we will routinely fill all of the cells from which we record with biocytin and subsequently reconstruct their morphologies with the help of

undergraduate students. This will enable their *post hoc* identification and facilitate future studies by providing unambiguous comparisons of the properties of different cell types.

Aim 3b. Assess the subcellular distribution of evoked responses in granule cells. To investigate the mechanisms of dendritic integration within GCs, we will perform calcium imaging of GC dendrites in MOB slices (Abraham et al 2010; Egger et al 2003; 2005) while also recording from these dendrites. MOB slices from OMP-ChR2 animals will be prepared as described above. Whole-cell recordings will be established from GCs while including the synthetic calcium dye OGB-1 (100 uM) in the pipette. Calcium responses will be measured at different locations along the apical dendrite to determine the spatial localization of sub- and suprathreshold signals, as previously demonstrated by Egger and colleagues (2003, 2005). Specifically, we will image multiple dendritic spines while optically stimulating individual glomeruli or groups of glomeruli in order to assess whether or not inputs on multiple different spines are required for granule cells to spike and, more generally, attempt to quantitatively describe the nonlinearities arising from combinatorial glomerular stimulation as a function of the sub- or suprathreshold dendritic calcium signals observed via stimulation of individual glomeruli. Elucidating these relationships will clarify the mechanisms governing granule cell dendritic integration and substantially constrain the model of Aim 1A. Some electrophysiological recordings from granule cell dendrites (Figure 6A, *inset*) also will be made to measure dendritic depolarization as a function of subthreshold glomerular stimulation.

Potential pitfalls. (a) Calcium imaging: While we and others have previously measured dendritic calcium signals in response to glomerular stimulation (Abraham et al 2010), the characterization of input patterns that evoke granule cell spikes and the subsequent study of distributions of dendritic activity might be overly time-consuming and result in low recording yields. In this case, we could resort to sparse expression of a genetically encoded dye (e.g. GCaMP3, which we have used previously *in vivo*), thereby enabling clear dendritic calcium imaging without the need for whole-cell recording. *(b) Dendritic recording.* Dendritic recordings are not necessary for the success of this Aim but may improve the precision of some results. In particular, simultaneous somatic and dendritic recordings (Figure 6A, *inset*) are difficult to obtain due to the small diameter of granule cell dendrites. However, we have had success with the genetic labeling of granule cell dendrites, which enables the fluorescent targeting of individual granule cell dendrites and the measurement of dendritic spike activity without a somatic pipette.

Specific Aim #4. To elucidate the rules governing competing interactions among learned odor representations.

Behavioral and psychophysical studies are often the most efficient way to ask high-level questions about perceptual processing, such that the answers constrain theoretical models and determine the structure of subsequent experiments. In this proposal, we present the evidence-based hypothesis that granule cells serve as higher-order feature detectors, responding specifically to combinatorial, reasonably odor-specific patterns of glomerular activation and modifying the spike patterning of mitral cells based on this higher-order information. Notably, MOB-dependent learning takes place over a seconds to minutes timescale (Cleland et al 2009; Mandairon & Linster 2009; McNamara et al 2008), whereas consolidation of this learning into long-term memory requires the differentiation and integration of new granule cells (Kermen et al 2010; Lazarini & Lledo 2011; Moreno et al 2009). In this Aim, we will examine the interactions among the learned odor representations of similar odorants, with particular attention to the differences, if any, between odor pairs that are simultaneously

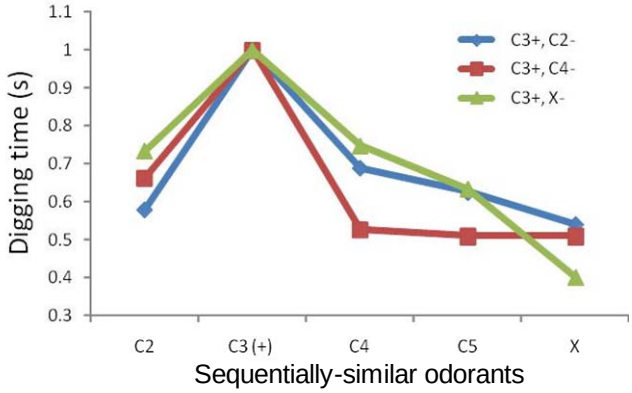


Figure 7. Behavioral generalization gradients reflect learned discriminations between odorants. All three groups of mice were equivalently rewarded for digging in dishes scented with a three-carbon aliphatic odorant *C3(+).* Each group was also taught that they would receive no reward for digging in response to a different odorant – either the similar 2-carbon odorant *C2* (blue), the similar 4-carbon odorant *C4* (red), or a dissimilar control odorant *X* (green). The generalization gradients sharpen preferentially to distinguish C3(+) from the known-unrewarded similar odorant.

conditioned (Figure 7), versus odor pairs in which one of the odors' representations has been previously consolidated (i.e., requiring the differentiation of new granule cells). In the latter case, for example, will the generalization signature of the consolidated odor change to "make room" for the representation of the new odor in the same way as if both odors were learned together? How plastic are the "boundaries" of an odor representation after it has been consolidated, and what are the parameters that regulate this plasticity (e.g., time, salience, extent of learning, relative importance; cf. Cleland et al 2009)? Answers to these questions will strongly shape evolving hypotheses of the extent to which long-term odor memory persists without reducing the capacity to learn new odors, compatible with the hypothesized *AND* gates of granule cells.

Natural odor stimuli vary (over time, temperature, ripeness, cultivar, etc.) in their precise chemical composition; the degree of tolerable variance is an integral part of learned odor representations and is measured using generalization gradients. Specifically, olfactory generalization gradients measure how much variance in the quality of a conditioned odorant stimulus is tolerated before the animal interprets it as a different odor with different implications (Cleland et al 2009). Animals' appetitive responses to reward-paired odors generalize to structurally and perceptually similar odorants; the resulting generalization gradients are plastic, progressively sharpen with learning (Cleland et al 2002; Cleland et al 2009), and depend upon computations within MOB specifically (Mandairon & Linster 2009; McNamara et al 2008). In preliminary studies, we have shown that the simultaneous differential learning of two similar odors (one rewarded, one not) shapes their generalization gradients such that information about the nonrewarded odorant modifies the learned boundaries of the rewarded odor representation narrowly and specifically to reflect this information, enabling more effective discrimination of the two odors (Figure 7). Similar effects have been observed in insect models (Daly et al 2001; Fernandez et al 2009). It is not yet known to what extent this interference phenomenon will extend to odors that have been previously learned and consolidated, thereby incorporating new granule cells into the EPL network (Kermen et al 2010; Moreno et al 2009). Specific experiments will be designed based upon initial results, but will begin with this protocol: Mice will be associatively conditioned to a conditioned stimulus (CS1) odor using an established multi-day paradigm resulting in a consolidated long-term memory for that odorant and its association with reward (*data not shown*; compare to Kermen et al 2010). A control group will be similarly conditioned to a dissimilar odorant. Mice will subsequently be normally trained with a different unconditioned stimulus (e.g., different reward level; Cleland et al 2009) on an odorant *CS2* that is highly similar to CS1, such that its generalization gradient in the control group extends to incorporate CS1 at low learning levels (i.e., the representations may *compete* for some of the same odor qualities, notably those between CS1 and CS2). (In contrast, CS1 would be overtrained to the extent that its gradient no longer extends to include CS2; Cleland et al 2009). The question is: does this prior learning of CS1 in the experimental group alter the generalization gradient of CS2 compared to controls? By a strict "odor space" metaphor, one would predict that it would. If it does not, or if *significant changes in non-olfactory context* between CS1 training and CS2 training enable CS2 to generalize across CS1 to a degree similar to the control group (in the new context), then this long-standing metaphor may not hold, suggesting that *the patterns of mitral-granule lateral inhibitory efficacy across olfactory bulb may be dynamically reconfigured by non-olfactory contextual cues* and substantially changing the underlying principles of our present theoretical model (Aim 1). If warranted, this hypothesis will be developed in future studies.

Conclusion

The great strength of the olfactory bulb as a preparation is that both bottom-up afferent sensory information (responsive to changes in physical stimulus properties) and top-down information about the animal's motivation, learning, and physiological-behavioral state converge in a compact, experimentally isolatable, and reasonably well-characterized neural circuit. Notably, all influences – afferent and efferent – converge to influence the activity and spike timing of second-order sensory neurons (mitral cells), the axons of which export olfactory information from the olfactory bulb. Our understanding of olfactory bulb circuitry has progressed to the point where specific experimental questions can be posed regarding this construction of odor representations from the cellular and circuit levels to their implications for perception and behavior. The present application is an effort to further tie the computational properties of the MOB neural network to its capacities for the regulation of perception. Thank you for your consideration of this application.

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