1 **Title: *Probing olfactory detection in space and time***

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7 **Abstract:**

8 In this issue, Gill et al. 2020 apply holographic optogenetic stimulation in the olfactory bulb to control
9 select neuronal ensembles in 3D. This approach allows them to dissociate the contribution of temporal spike
10 features and spike rate to stimulus detection.

11 **Preview:**

12 The ability to detect volatile chemicals in the environment is critical for survival in many animal species.
13 Odorant detection involves the transformation of chemical signals into spatiotemporal patterns of neural
14 activity in olfactory sensory areas. In the first step of this process, inhalation carries airborne odorant
15 molecules into the nasal cavity, where they bind olfactory receptors of the nasal epithelium. The axons from
16 each receptor cell expressing the same olfactory receptor projects to one or two olfactory glomeruli, where
17 they connect to the dendrites of excitatory mitral and tufted (M/T) cells, the major output projections of the
18 olfactory bulb (OB). In rodents, the sense of smell has evolved to be exquisitely sensitive. Mice have been
19 trained in the laboratory to reliably detect one in 1 billion molecules (Williams and Dewan, 2020). How
20 can the nervous system perform such sensitive detection and what determines perceptual detection
21 thresholds?

22 M/T cells increase their spike rates in response to olfactory stimulation. Moreover, distinct temporal spike
23 patterns, related to respiration and behavioral performance, have been observed in M/T cells (Cury and
24 Uchida, 2010; Shusterman et al., 2011), suggesting the temporal coordination of spikes is critical for
25 olfactory computations. Dissociating the contribution of spike timing and spike rates in psychophysical
26 paradigms is experimentally challenging. Even precisely timed delivery of odorant stimuli can introduce
27 signal fluctuations, which are difficult to differentiate from noise fluctuations intrinsic to the neural system.

28 In this issue of *Neuron*, Gill et al. (Gill et al., 2020) developed a powerful optical approach to independently
29 control the timing and spatial activation patterns of neurons in the OB. They use two-photon holographic
30 stimulation in mice expressing red-shifted opsin ChrimsonR and the genetically encoded calcium indicator
31 GCaMP6 to measure and stimulate neural activity with cellular resolution and millisecond precision in a
32 small three-dimensional volume. While their technical approach is broadly similar to previous reports in
33 awake mice (Yang et al. 2018), the authors also introduce an elegant sham-photostimulation technique to
34 control for non-specific experimental effects. By increasing laser pulse duration, they dramatically reduce
35 the incidence of simultaneous 2P absorption and thus 2P excitation, while all other experimental parameters
36 remain constant.

37 Mice were trained in a go/no-go paradigm to report photostimulation with a lick-response. They correctly
38 indicated activation of as few as 9 but typically <20 neurons, as long as neurons were activated
39 simultaneously within a small 10ms time-window. Increasing the activation time window deteriorated
40 behavioral detection to chance levels (Figure 1). These findings establish that spike synchrony in the OB
41 has a major influence on perceptual detection in the olfactory system. They further indicate that efferent
42 areas involved in stimulus detection have powerful coincidence detection capabilities which are robust to
43 the presence of ongoing spontaneous M/T activity.

Compared to previous studies in which mice reported the optogenetic activation of specific ensembles of neurons in the visual or somatosensory cortex (Histed and Maunsell, 2014; Huber et al., 2008), synchronous holographic OB activation required far fewer neurons and fewer action potentials to evoke behavioral detection. Similarly, in rats trained to detect the electrical stimulation of a even a single-neuron, behavioral detection involved much longer spike trains (Houweling and Brecht, 2008). Collectively, the differences between past work and the present study can be interpreted to reflect different coding schemes, i.e. detection of coincident spike timing in the OB vs. evidence accumulation in the neocortex. Alternatively, differences could at least in part also be related to different training schedules and different behavioral reporting responses (lick-no-lick vs. lever press (Histed & Maunsell 2014) and choice-task (Huber et al. 2008)).

Interestingly, the detection accuracy was unaffected by whether a mix of mitral and (inhibitory) granule cells or just mitral cells were stimulated. Behavioral performance was also unaffected by the relative latency of the stimulation to inhalation onset (Figure 1). Mice can be trained to detect photostimulation at different times during the sniff phase, which was interpreted to indicate that inhalation-locked spike timing aids in stimulus detection (Smear et al., 2011). However, the relationship between inhalation phase and spike timing seems to have been irrelevant for stimulus detection in the present work. These observations highlight the fact that animals flexibly exploit the available evidence to perform a task which is critical for their survival under laboratory conditions. Understanding olfactory coding might thus eventually require relating back findings obtained with refined reductionist methods such as holographic optogenetic stimulation, to experiments involving the activation of larger ensembles by ecologically relevant stimuli in natural environments.

Finally, some limitations of holographic optogenetic stimulation should be considered. At present, holographic optogenetic stimulation is restricted to brain areas which are within the 2P penetration depth and accessible through a cranial window. Hence, it is well suited for studies in the OB or neocortex but less amenable to investigating information processing in olfactory cortical areas.

Despite its great spatial precision, activating fibers of passage cannot be completely avoided. Yet, even subthreshold stimulation, which only slightly increases spiking probability, can strongly influence behavioral detection (Histed and Maunsell, 2014). Moreover, mitral cells have very long lateral dendrites (1000–1500 μm), which can spread activation to distant mitral cells through dendrodendritic synapses (Xiong and Chen, 2002). Many of these neurons are likely outside the field of view and would not be captured by simultaneous imaging. The spread of activity from one mitral cell to other mitral cells is also shaped by recurrent inhibition from granule cells, which synapse along the secondary dendrites (Margrie et al., 2001). A closer investigation of activity in the different cell types of the OB circuit will provide important insights into the powerful mechanism that enables behavioral detection from the simultaneous photostimulation of sparse ensembles.

In conclusion, Gill et al. (Gill et al., 2020) establish a powerful method for probing the contribution of small groups of selected OB neurons to behavior, which provides a major step forward for studying the principles of olfactory coding. Their main finding that simultaneous spiking of few neurons is sufficient for behavioral detection further demonstrates the impressive capabilities of the olfactory system and stimulates further studies into the circuit mechanism underlying odorant detection.

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Figure 1. Behavioral Performance as a Function of Synchrony and Latency

LEFT: Mice correctly indicated photostimulation (green tick mark) when neurons were photostimulated within in a 10ms time window. Inducing spikes distributed over larger time windows (30ms and 50ms) did not cause behavioral detection (red cross mark). RIGHT: Photostimulation with different latencies relative to inhalation onset always led to behavioral detection (green check mark).