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Blockade of mitral/tufted cell habituation to odors by association with reward: a preliminary note

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Association of odor and reward during the early postnatal period modifies rat pup behavioral responses and olfactory bulb neural responses to subsequent presentations of that odor. Recent evidence has shown that olfactory bulb output neurons, mitral/tufted cells, receive convergent odor and reward inputs. The present report demonstrates that contiguous odor–reward pairings prevent mitral/tufted cell habituation to the odor that normally occurs to repeated odor-only stimulation. It is hypothesized that the maintenance of olfactory bulb responses to conditioned odors during training may allow for activation of long-term memory mechanisms.

Olfactory associative conditioning modifies subsequent olfactory bulb responses to the conditioned odor^{2,3,6,8,11,16}. Centrifugal inputs to the olfactory bulb appear to be critically involved in this neural plasticity. For example, both the neural and behavioral consequences of olfactory conditioning are dependent on noradrenergic (NE) centrifugal input to the bulb for their acquisition^{4,5,10,12,13,15}. It has been hypothesized that association of descending inputs to the olfactory bulb with primary olfactory nerve input allows changes to occur in bulb circuitry that are subsequently expressed as altered neural output to learned odors^{1,5,7,14,15}.

In the rat pup, olfactory associative conditioning modifies the subsequent spatial and temporal response patterns of olfactory bulb mitral/tufted cells to the previously learned odor^{16,17}. Using electrical stimulation of the medial forebrain bundle/lateral hypothalamus (MFB/LH) as reward, we have demonstrated functional convergence of odor and reward information on mitral/tufted cells, that is, mitral/tufted cells respond to both odor and MFB/LH stimulation^{14,15}. Subgroups of mitral/tufted cells are either suppressed, excited (disinhibited) or do not respond to MFB/LH stimulation. Importantly, the mitral/tufted cell disin-

hibitory response to MFB/LH stimulation is NE-dependent, as are the modified neural and behavioral responses to odors conditioned with this paradigm¹⁵.

Our previous work on mitral/tufted cell changes with early learning has relied on post-training analysis of single-unit response patterns and comparisons across training groups. This work has demonstrated that early associative learning shifts the excitation-suppression response ratio to the conditioned odor toward greater suppression in odor-specific regions of the bulb^{16,17}. However, how mitral/tufted cell responses change over the course of training, and how mitral/tufted cells respond during contiguous presentations of odor and reward is unknown. Observing the dynamic phase of learning-associated change in single-unit response patterns may provide critical information toward understanding the mechanisms of that change.

The present report, therefore, describes an examination of mitral/tufted cell single-unit responses to odors *during* odor–reward contiguous pairings. Odor stimuli were paired with MFB/LH stimulation at odor offset, or odor stimuli were presented alone. The results suggest that contiguous pairing of odor and reward prevents habituation that occurs to odor-only experience.

Wistar rat pups, aged postnatal day (PN) 10–12 were used as subjects. Pups were anesthetized with urethane (1.5 g/kg) and placed in a stereotaxic apparatus. Bipolar, stainless steel stimulating electrodes were placed in the lateral olfactory tract (LOT) and MFB/LH. Mitral/tufted cell single-unit activity was recorded in the ipsilateral olfactory bulb with a 2 M NaCl filled micropipette. A continuous stream of clean, humidified air was blown across the pup's snout (500 ml/min flow rate), while the pup respired naturally. Odor stimuli were delivered by adding saturated peppermint vapor to the clean airstream with a computer controlled Harvard syringe pump for a total odor concentration of 1 : 10 of saturated vapor.

Following isolation and identification of a mitral/tufted cell, responses to the odor stimulus and to the MFB/LH stimulus were determined. For odor responses, firing rates (number of spikes/250 ms bin) were compared between a 4-s pre-odor baseline and the 4-s odor stimulus with paired *t*-tests. Although the odor stimulus (and the response) generally took 1–2 s to completely dissipate after the syringe pump stopped, the response to the odor was only measured during the 4-s stimulus to avoid confounding with responses induced by MFB/LH stimulation in cells given odor–MFB/LH pairings. Responses to MFB/LH stimulation were similarly determined by comparing firing rate during a 4-s pre-MFB/LH stimulation (200 Hz, 300 ms, 1000 μ A) with post-MFB/LH firing rate. As previously reported¹⁴, mitral/tufted cells at this age respond to both odors and MFB/LH stimuli in either an excitatory or suppressive manner, or fail to change their firing rates (no response). Only cells that showed significant responses to the odor are included in the present analysis. Cells were tested regardless of their response to the MFB/LH stimulation. Odor and MFB/LH stimulation were kept to a minimum during this screening phase. The number of cells screened before training began ranged from 1 to 8. However, only 1 cell/pup was trained.

After a cell was determined suitable for training, it was randomly assigned to receive either odor-only training or paired odor–MFB/LH training. For the paired group, MFB/LH stimulation was delivered at syringe pump offset. This procedure allowed a complete 4-s odor stimulus uncontaminated by the MFB/LH stimulation, but still allowed contiguous pairing of odor and reward during the 1–2 s odor dissipation period. A 30-s inter-trial interval was used in both odor-only and paired groups. The magnitude of the response to the odor was expressed as a percentage of the response magnitude prior to training and compared across training trials and between groups using a

2-way repeated ANOVA. Response magnitude for each trial was calculated in one of two ways. In cells with spontaneous activity rates throughout training of greater than 0.5 Hz, magnitude was calculated as percent change from pre-odor baseline. Because several cells had no, or very little spontaneous activity, determining percent change using this method yield enormous and variable response magnitudes. Therefore, in cells with spontaneous activity rates of less than 0.5 Hz, the number of spikes during the stimulus were counted as the response magnitude. There was no significant difference in spontaneous activity rates between groups, and both methods yielded similar results. In some animals, electrode placements in the MFB/LH were confirmed histologically at the end of the experiment.

A total of 19 cells were tested through at least 10 training trials ($n = 10$, odor-only; $n = 9$, paired). Of the odor-only cells, 8 were excited by the odor, 2 were suppressed; 3 were excited to the MFB/LH stimulation, 2 were suppressed and 5 were non-responsive. For paired cells, 8 were excited by the odor and 1 was suppressed; 5 were excited to the MFB/LH stimulation, 1 was suppressed and 3 were non-responsive.

Odor-only cells demonstrated significant habituation to the odor within the first 5 trials and continued to habituate over the next 5 trials (Fig. 1A). In sharp contrast, cells receiving paired presentations of odor and MFB/LH stimulation did not habituate to the odor and in fact, several cells showed a marked increase in response magnitude over training (repeated ANOVA, group \times trial interaction, $F_{2,34} = 8.7$, $P < 0.001$). These effects were observed with both excitatory and suppressive odor responses.

Spontaneous activity rates did not differ between groups, nor did they significantly vary over the course of training (Fig. 1B; repeated ANOVA, group \times trial interaction, $F_{2,34} = 1.59$, n.s.). This suggests that any differences between the groups in odor response magnitude were not due to changes in pre-odor background activity.

Following training, 3 paired cells were then trained in the odor-only conditioning procedure. All 3 cells rapidly habituated, suggesting that the failure to habituate during the paired trials was not due to unusual properties of those cells (Fig. 1C).

MFB/LH stimulation (reward) is known to produce a variety of central and peripheral effects, directly or indirectly activating multiple brain sites¹⁸. These preliminary results suggest that at least one of the neurophysiological consequences of MFB/LH stimulation is attenuation of habituation to odors contiguously paired with that stimulation. Centrifugal modulation of olfactory bulb habituation has been previously shown in

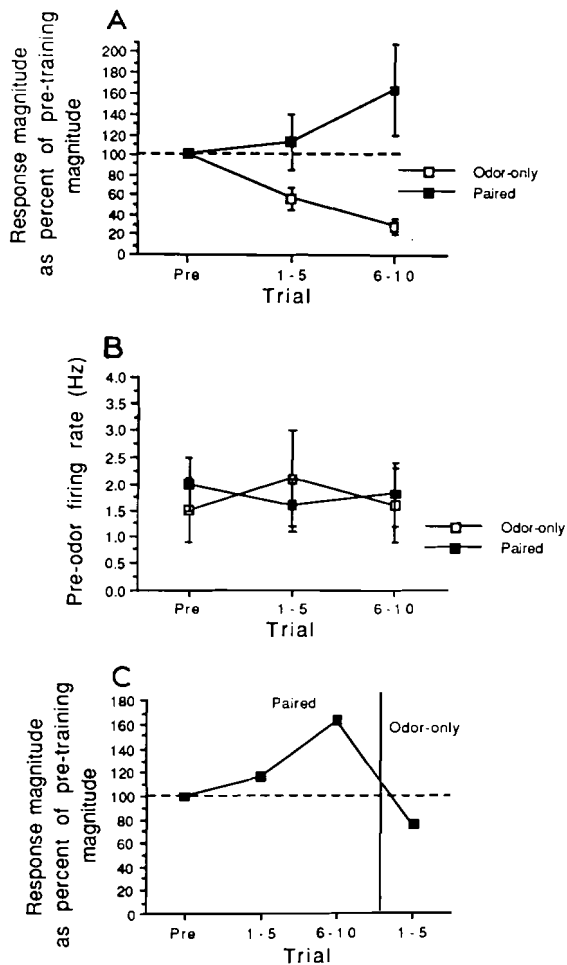


Fig. 1. A: mitral/tufted cell single unit response magnitude to the 4-s conditioned odor stimulus as a percent of pre-training response magnitude. B: pre-odor, baseline activity rate did not change as a function of conditioning group or trial number. C: cells given odor-only trials after being trained in the paired condition showed rapid habituation ($n = 3$).

mature hamsters. Mitral/tufted cells habituate more rapidly after transection of descending fibers to the bulb⁹. Together with the present results, these findings suggest that centrifugal inputs to the bulb may help maintain odor responsiveness, perhaps particularly when these inputs signal reward or have other biological significance.

Maintenance of mitral/tufted cell responsiveness during odor-reward pairings could enhance the probability that activity-dependent changes in synaptic strength could occur in the bulb or its afferents^{1,14}. This neural plasticity could then play a critical role in memory for olfactory events, and modified behavioral responses to subsequent presentations of learned odors.

These preliminary results raise several important questions which can now be addressed. (1) Are these effects odor specific? (2) Given the role of NE in early learning and olfactory bulb response to MFB/LH

stimulation^{13,15}, what role does NE play in olfactory bulb habituation? (3) How do descending inputs modulate olfactory habituation? (4) Does mitral/tufted cell habituation correlate with behavioral habituation? (5) Finally, what is the importance of reduced habituation during learning in pups?

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