Brief Communication

Theta bursts in the olfactory nerve paired with β-adrenoceptor activation induce calcium elevation in mitral cells: A mechanism for odor preference learning in the neonate rat

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Odor preference learning in the neonate rat follows pairing of odor input and noradrenergic activation of β-adrenoceptors. Odor learning is hypothesized to be supported by enhanced mitral cell activation. Here a mechanism for enhanced mitral cell signaling is described. Theta bursts in the olfactory nerve (ON) produce long-term potentiation (LTP) of glomerular excitatory postsynaptic potentials (EPSPs) and of excitatory postsynaptic currents (EPSCs) in the periglomerular (PG) and external tufted (ET) cells. Theta bursts paired with β-adrenoceptor activation significantly elevate mitral cell (MC) calcium. Juxtaglomerular inhibitory network depression by β-adrenoceptor activation appears to increase calcium in MCs in response to theta burst stimulation.

Early odor preference learning provides us with a model in which the necessary and sufficient inputs for learning can be localized to a relatively simple cortical structure, the olfactory bulb (Sullivan et al. 2000). The critical changes for this natural form of learning occur in the olfactory bulb network (Coopersmith and Leon 1986, 1987, 1995; Wilson et al. 1987; Woo et al. 1987; Wilson and Leon 1988; Wilson and Leon 1991; Guthrie et al. 1993; Johnson et al. 1995; Yuan et al. 2002). Odor preference learning is induced by the pairing of odor with activation of the locus coeruleus noradrenergic system, a component of our arousal circuitry (Harley 1987; Berridge and Waterhouse 2003; Berridge 2008), which is critically involved in other forms of memory (Harley 1987; Berridge and Waterhouse 2003) and compromised in diseases of memory, such as Alzheimer’s (Palmer and Dekosky 1993; Weinshenker 2008).

The unconditioned stimulus for early odor preference learning is mediated by β-adrenoceptor activation in the olfactory bulb (Sullivan et al. 1991, 1992; Wilson and Sullivan 1991, 1994; Wilson et al. 1994; Harley et al. 2006). β-Adrenoceptor agonists and antagonists infused in the olfactory bulb can induce or block learning, respectively (Sullivan et al. 1989, 2000).

Despite the fact that the behavioral model for early odor preference learning has been established for decades (Sullivan et al. 1988, 1989), and despite the fact that the olfactory bulb circuit contains synapses that are essential for the formation of a localizable long-term memory that is easily indexed in behavior (Coopersmith and Leon 1986; Wilson et al. 1987; Woo et al. 1987; Wilson and Leon 1988; Woo and Leon 1991; Johnson et al. 1995; McLean et al. 1999; Yuan et al. 2002), the circuitry and the synapses in the olfactory bulb that mediate learning are not well understood. Long-term potentiation (LTP), the putative synaptic model for associative learning in other brain regions (Bliss and Lomo 1973; Brown et al. 1988; Barnes 1995; Malenka 1994), has not been demonstrated compellingly in the rat olfactory bulb. The lack of evidence for a synaptic locus and a mechanism to support odor preference learning is partly due to the dissociation of the neural changes previously observed following early odor learning (seen at the glomerular input level) (Coopersmith and Leon 1986; Wilson et al. 1987; Woo et al. 1987; Wilson and Leon 1988; Woo and Leon 1991; Johnson et al. 1995; Yuan et al. 2002) and the innervation pattern of noradrenergic fibers in the olfactory bulb (seen mostly in the deep layers of the olfactory bulb, but sparse in the glomerular layer) (McLean et al. 1989; McLean and Shipley 1991).

McLean and colleagues have proposed, based on physiological and anatomical evidence, that early odor preference learning leads to a long-term facilitation of the olfactory nerve (ON) inputs to mitral cells (MCs, the main output cell of the olfactory bulb) (McLean et al. 1999; Yuan et al. 2003b; McLean and Harley 2004). In the present study, odor input is mimicked in vitro by theta burst stimulation (TBS) of the ON, and the modulation of glomerular and MC responses to theta bursts alone and in conjunction with bath application of the β-adrenoceptor agonist, isoprotorenol, is assessed. The results support the McLean glomerular/MC hypothesis of early odor preference learning.

In the first set of experiments (Fig. 1A–D), the effects of theta burst ON input on the field glomerular excitatory postsynaptic potential (EPSP) were tested. The ON was stimulated by a single test stimulus (20–100 μA) every 20 sec in horizontal olfactory bulb slices from postnatal 6–14-d-old Sprague–Dawley rats (Fig. 1A). TBS (10 bursts of high frequency stimulation at 5 Hz, each burst containing five pulses at 100 Hz, same stimulation intensity as test stimuli) that mimics the sniffing cycles in the ON (Kepecs et al. 2006) was given after a baseline was taken. All the experiments were done in aCSF containing (in millimolars) 119 NaCl, 2.5 KCl, 1.3 MgSO4, 1 NaH2PO4, 26.2 NaHCO3, 22 mM glucose, and 2.5 CaCl2, equilibrated with 95% O2/5% CO2. Field recording pipettes were filled with aCSF. All recordings were acquired at 30°C–32°C. Data are presented as mean ± SEM. Student’s t-test was used to determine statistical significance.

There was on average a 14.5 ± 2.5% increase in the field EPSP peak amplitude at 30 min post-TBS induction (N = 34, t = 5.82, P < 0.001, Fig. 1B). Bath application of D-APV (50 μM), an NMDAR antagonist, did not eliminate TBS potentiation; the EPSP peak is 115.6 ± 5.4% of baseline, 30 min post-induction (N = 5, t = 2.90,
The result suggests that plasticity occurs at the first step of odor processing (Ennis et al. 1998; Mutoh et al. 2005; Tyler et al. 2007; Dong et al. 2008; Jones et al. 2008). Hence, the synapses between the ON and its postsynaptic neurons are potential targets for learning-dependent plasticity as suggested by the long-lasting metabolic and anatomical changes observed at the olfactory bulb glomerular level following early odor preference training (Coopersmith and Leon 1986; Wilson et al. 1987; Woo et al. 1987; Wilson and Leon 1988; Woo and Leon 1991; Johnson et al. 1995; Yuan et al. 2002). Paired stimuli given to the ON using

\[ P = 0.022, \text{Fig. 1D}. \) This result suggests that plasticity occurs at the first step of odor processing (Ennis et al. 1998; Mutoh et al. 2005; Tyler et al. 2007; Dong et al. 2008; Jones et al. 2008). Hence, the synapses between the ON and its postsynaptic neurons are potential targets for learning-dependent plasticity as suggested by the long-lasting metabolic and anatomical changes observed at the olfactory bulb glomerular level following early odor preference training (Coopersmith and Leon 1986; Wilson et al. 1987; Woo et al. 1987; Wilson and Leon 1988; Woo and Leon 1991; Johnson et al. 1995; Yuan et al. 2002). Paired stimuli given to the ON using

\[ \text{Theta bursts and } \beta \text{-agonist elevate mitral cell } Ca^{2+} \]
a 50-msec interval were used to test presynaptic changes to TBS. Paired-pulse ratios, an indicator of changes in presynaptic release (Murphy et al. 2004), decreased following TBS (91.5 ± 2.8% of control, N = 7, t = 3.05, P = 0.011, Fig. 1C). The decrease in paired-pulse ratio suggests TBS potentiation is presynaptically mediated. The glomerular potentiation seen here is consistent with a recent adult mouse model showing an increase in odor-specific glomeruli and olfactory sensory neurons following odor learning (Jones et al. 2008).

In the second set of experiments, glomerular excitatory postsynaptic currents (EPSCs) in postsynaptic juxtaglomerular (JG) cells were recorded in voltage-clamp mode (membrane potential held at −60 mV). The effects of TBS on JG cell EPSCs were tested. Patch pipettes were filled with an internal solution containing (in millimolars) 114 K-glucuronate, 17.5 KCl, 4 NaCl, 4 MgCl2, 10 HEPES, 0.2 EGTA, 3 Mg2ATP, and 0.3 Na2GTP. There are two main populations of JG cells in the glomeruli, periglomerular (PG) cells, and external tufted (ET) cells. PG cells are inhibitory on MCs (Hayar et al. 2004). PG cells form two functionally distinct populations: ~30% are driven by monosynaptic ON input and then excite PG interneurons (Murphy et al. 2005), while ET cells are excitatory neurons, which receive monosynaptic ON input and then excite PG interneurons (Hayar et al. 2004). PG cells form two functionally distinct populations: ~30% are driven by monosynaptic ON input and then excite PG interneurons (Hayar et al. 2004). PG cells form two functionally distinct populations: ~30% are driven by monosynaptic ON input and then excite PG interneurons (Hayar et al. 2004). PG cells form two functionally distinct populations: ~30% are driven by monosynaptic ON input and then excite PG interneurons (Hayar et al. 2004). PG cells form two functionally distinct populations: ~30% are driven by monosynaptic ON input and then excite PG interneurons (Hayar et al. 2004). PG cells form two functionally distinct populations: ~30% are driven by monosynaptic ON input and then excite PG interneurons (Hayar et al. 2004). PG cells form two functionally distinct populations: ~30% are driven by monosynaptic ON input and then excite PG interneurons (Hayar et al. 2004). PG cells form two functionally distinct populations: ~30% are driven by monosynaptic ON input and then excite PG interneurons (Hayar et al. 2004). PG cells form two functionally distinct populations: ~30% are driven by monosynaptic ON input and then excite PG interneurons (Hayar et al. 2004). PG cells form two functionally distinct populations: ~30% are driven by monosynaptic ON input and then excite PG interneurons (Hayar et al. 2004).

The glomerular potentiation seen here is consistent with a recent adult mouse model showing an increase in odor-specific glomeruli and olfactory sensory neurons following odor learning (Jones et al. 2008). These JG cells form the remaining population receive their input mainly through ET populations:

**Figure 2.** Pairing of TBS and isoproterenol (ISO) increased MC calcium responses. (A) ΔF/F calcium image (20× objective) of a slice stained with a calcium indicator dye, Oregon Green BAPTA-1 AM. The MC layer is labeled by white dashed lines. Scale bar, 50 μm. (B) An example of ΔF/F calcium imaging showing enhanced calcium responses following TBS+ISO in one MC (white asterisk). Lower traces are calcium transients recorded from the soma of the MC. (C) Averaged calcium transient changes in MCs following TBS, ISO, and TBS+ISO (normalized to control), measured at 30 min post-manipulations. **P < 0.01. (C1) Single-cell calcium transient changes before and after TBS. Dashed black line indicates no change. Cells above the dashed line show increased calcium responses, whereas those below the dashed line show decreased calcium responses. (C2) MC calcium responses to paired TBS and ISO. Note that the majority of MCs showed increased calcium responses following TBS+ISO. (C3) MC calcium responses to ISO only.
calcium responses, the combined effect of TBS and the ISO application on the JG cells (PG) significantly suppressed ON-evoked calcium transients in JG cells (\( N = 6 \)) and PG cells (\( N = 3 \)). These results suggest that isoproterenol application alone affects the JG cell activity, possibly due to a direct effect on JG cell excitability in acute rat olfactory bulb slices. Moreover, this suppression may be attributed to the odor learning observed behaviorally.

The effects of isoproterenol and TBS on the JG cell activity were examined in parallel. Previous research using slice physiology to identify a synaptic site of NE action was constrained by the known distribution of noradrenergic input to the olfactory bulb. Noradrenergic fibers project heavily to the subglomerular layers, where they terminate densely in the internal plexiform and granule cell (GC) layers, and moderately in the external plexiform and the MC layers (McLean et al. 1989). Based on this anatomical evidence, it was assumed that either GCs or MCs were the potential targets for \( \beta \)-adrenoceptor action. However, the Ennis group showed that the \( \beta \)-adrenoceptor agonist isoproterenol has no direct effect on MC excitation in acute rat olfactory bulb slices (Hayar et al. 2001). Although isoproterenol caused an inward current in MCs in voltage-clamp mode, this inward current was blocked by synaptic transmission blockers, suggesting an indirect effect of isoproterenol, possibly through interneurons. NE could inhibit MCs through 


disinhibit MCs through suppressing GC activity as reported in the turtle and dissociated rat olfactory bulb cultures (Jahr and Nicoll 1985).

Since TBS itself was not sufficient to produce significant MC calcium responses, the combined effect of TBS and the \( \beta \)-adrenoceptor agonist, isoproterenol, were examined. Isoproterenol was applied to the bath solution 5–10 min before the TBS and washed out after the TBS induction. The pairing of TBS and isoproterenol (10 μM) increased MC calcium responses in most of the cells measured (137.0 ± 11.0% of control, \( N = 55 \) from five slices, \( t = 3.27, P < 0.001, \) Fig. 2 panel C2). Five to ten min application of isoproterenol alone to the bath solution did not alter the MC responses observed 30 min after isoproterenol washout (102.5 ± 8.8% of control, \( N = 47 \) from four slices, \( t = 0.28, P = 0.781, \) Fig. 2 panel C3). Thus, the potentiated MC calcium response was only seen with paired isoproterenol and TBS. As a caveat it should be noted that MCs fire action potentials spontaneously in vivo at ~3 Hz (Cang and Isaacson 2003) and in vitro at ~15 Hz (up to 75 Hz) (Griff et al. 2008). A change in ΔF/F calcium response reflects a change in the ratio of the evoked response over the baseline spontaneous response. Changes in MC spontaneous firing, therefore, can affect the ΔF/F calcium signal measured from the MC. However, if pairing TBS with isoproterenol increased spontaneous firing, the increase in the evoked firing rate of MCs had to occur to a greater degree.

This result, that only the pairing of TBS with isoproterenol enhanced MC calcium responses, correlates with the behavioral studies (Langdon et al. 1997; Sullivan and Leon 1987; Sullivan et al. 2000) showing that only the pairing of an odor and isoproterenol produces associative learning, while either odor alone or isoproterenol alone does not lead to associative learning. It supports the view that at the level of physiological mechanism, classical conditioning is the interaction of arousal modulation and theta modulation while either alone does not create the necessary associative conditions. Consistent with previous work showing enhanced CREB phosphorylation in MCs following learning (McLean et al. 1999; Yuen et al. 2000, 2003a), the present calcium imaging results support the hypothesis that odor learning results in increased firing in odor encoding MCs. Increased firing in MCs is also consistent with recent work by Gire and Schoppa showing that the pairing of NE with TBS induces an enhancement of MC long-lasting depolarization and gamma frequency oscillation (Gire and Schoppa 2008). Interestingly, MC firing is mainly suppressed to a familiar odor in neonatal rats (Wilson et al. 1985). TBS potentiation of the inhibitory JG circuitry may contribute to the odor habituation observed behaviorally.

In the fourth set of experiments, the effects of isoproterenol on JG cell activity were examined. Previous research using slice physiology to identify a synaptic site of NE action was constrained by the known distribution of noradrenergic input to the olfactory bulb. Noradrenergic fibers project heavily to the subglomerular layers; they terminate densely in the internal plexiform and the granule cell (GC) layers, and moderately in the external plexiform and the MC layers (McLean et al. 1989). Based on this anatomical evidence, it was assumed that either GCs or MCs were the potential targets for \( \beta \)-adrenoceptor action. However, the Ennis group showed that the \( \beta \)-adrenoceptor agonist isoproterenol has no direct effect on MC excitation in acute rat olfactory bulb slices (Hayar et al. 2001). Although isoproterenol caused an inward current in MCs in voltage-clamp mode, this inward current was blocked by synaptic transmission blockers, suggesting an indirect effect of isoproterenol, possibly through interneurons. NE could inhibit MCs through suppressing GC activity as reported in the turtle and dissociated rat olfactory bulb cultures (Jahr and Nicoll 1985).
The effect of isoproterenol was reversed after 3A). Isoproterenol was applied for 5 min in the bath solution and calcium transients were suppressed in the presence of isoproterenol. Calcium transients from 8–20 cells per slice (Fig. 3B). The averaged results were found with calcium imaging using the averaged cell preference learning. While the present experiments were conducted with (IPSCs) in MCs (Gire and Schoppa 2008). Taken together these studies supported the hypothesis that isoproterenol still suppressed JG cell calcium transients (Fig. 3B, dashed circles). Given the results of both whole-cell recording and calcium imaging, it was reasonable to hypothesize that isoproterenol would decrease JG cell activity, reducing GABA release onto MCs and causing MC disinhibition. Indeed, it has been shown that NE application causes a reduced inhibition of postsynaptic currents (IPSCs) in MCs (Gire and Schoppa 2008)

The present results argue that potentiation of MC calcium responses occurs only when theta frequency activity is paired with beta-adrenoceptor activation. They also suggest that one critical role of NE activity via beta-adrenoceptors in the olfactory bulb is to suppress the inhibitory JG network, subsequently transiently disinhibiting MCs and providing the conditions for strengthening ON-MC connections in selected glomeruli. This mechanism likely operates in concert with changes promoted by patterned cAMP waves in MCs (Cui et al. 2007).

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