Brief Communication

Cortical Metabotropic Glutamate Receptors Contribute to Habituation of a Simple Odor-Evoked Behavior

Aaron R. Best, Jason V. Thompson, Max L. Fletcher, and Donald A. Wilson
Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019

Defining the circuits that are involved in production and cessation of specific behaviors is an ultimate goal of neuroscience. Short-term behavioral habituation is the response decrement observed in many behaviors that occurs during repeated presentation of nonreinforced stimuli. Within a number of invertebrate models of short-term behavioral habituation, depression of a defined synapse has been implicated as the mechanism. However, the synaptic mechanisms of short-term behavioral habituation have not been identified within mammals. We have shown previously that a presynaptic metabotropic glutamate receptor (mGluR)-dependent depression of synapses formed by olfactory bulb afferents to the piriform (olfactory) cortex significantly contributes to adaptation of cortical odor responses. Here we show that blockade of mGluRs within the olfactory cortex of awake, behaving rats diminishes habituation of a simple odor-induced behavior, strongly implicating a central mechanism for sensory gating in olfaction.

Key words: **sensory habituation; memory; odor coding; metabotropic glutamate receptor; piriform cortex; adaptation**

Introduction

An animal's CNS is inundated with a flood of sensory inputs, some of which are critical for survival and some of which are redundant and/or currently irrelevant for ongoing behavior. To gain control over this potential information overload, several processes exist to gate sensory input and devote processing resources to the most important or relevant stimuli. Disruptions in these sensory gating and attention mechanisms have been implicated in disorders such as schizophrenia and autism (Ornitz et al., 1993; McAlonan et al., 2002; Ludewig et al., 2003). Adaptation to sensory stimulation and resulting behavioral habituation is one such mechanism that allows filtering of more static, behaviorally less relevant stimuli (Thompson and Spencer, 1966; Christoffersen, 1997). The neural mechanisms of sensory habituation have been determined in several invertebrate models (Zucker, 1972; Kandel and Schwartz, 1982; Christoffersen, 1997) but have been more elusive in the mammalian brain.

In this study, we took advantage of the relative simplicity of the mammalian olfactory system, and the known circuitry underlying simple olfactory orienting responses, to test a proposed cortical synaptic mechanism of short-term habituation to odors. The mammalian olfactory pathway begins within the nose at olfactory receptor neurons. Olfactory receptors, as receptors in most sensory systems, can adapt to prolonged stimulation, although olfactory receptor adaptation is slow in freely breathing animals (Adrian, 1950; Chaput, 2000) and is hypothesized to be more important for maintaining receptor activity within an optimal dynamic range rather than for behavioral adaptation (Moore, 1994; Kurahashi and Menini, 1997). The second-order olfactory neurons, mitral/tufted cells, receive direct input from receptor neurons and, in turn, form the lateral olfactory tract (LOT), which projects caudally to olfactory cortical structures, including the anterior piriform cortex (aPCX), without an intervening thalamic connection. Both mitral/tufted cells and piriform cortical neurons project to amygdaloid nuclei, which in turn control sensory-evoked autonomic responses, including heart-rate orienting responses (HRORs) (see Fig. 2*B*) (Kapp et al., 1992). HRORs can be evoked in response to novel sensory stimuli of any modality and reflect a sensory-evoked change in behavioral and autonomic tone (Sokolov, 1975).

Prolonged odor exposure results in a decrement in odor responsiveness within aPCX in both rats (Wilson, 1998) and humans (Sobel et al., 2000). Furthermore, it has been shown, within rats, that main olfactory bulb activity is relatively sustained during this adaptation (Wilson, 1998). We demonstrated recently that prolonged activation at the glutamatergic mitral/tufted–piriform cortex pyramidal cell synapse *in vitro*, in a manner consistent with prolonged odor-evoked activity, results in a synaptic depression very similar in recovery time course and magnitude to odor-induced adaptation at this synapse *in vivo* (Best and Wilson, 2004). In addition, we found that this synaptic depression requires group II and/or III metabotropic glutamate receptor (mGluR) activation for full expression. Group II/III mGluRs are specifically expressed in a manner consistent with a role as presynaptic autoreceptors at the mitral-to-cortical pyramidal cell synapse, although group II mGluR expression is limited if present at all in the rat (Wada et al., 1998). Here, we further examined the specific contributions of group II and group III mGluRs at this synapse with *in vitro* pharmacology and then examined the role of

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Correspondence should be addressed to Donald A. Wilson, Department of Zoology, University of Oklahoma, Norman, OK 73019. E-mail: dwilson@ou.edu.

A. R. Best's present address: Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

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mGluRs in habituation of a simple odor-mediated behavioral response. The results suggest that a simple cortical mechanism underlies sensory gating in olfaction.

Materials and Methods

Subjects. Long–Evans Hooded rats obtained from Harlan Bioproducts for Science (Indianapolis, IN) were used. Rats were housed in polypropylene cages with food and water available *ad libitum*. Lights were maintained on a 12 h light/dark cycle with all testing during the light hour cycle. Animal care and protocols were approved by the University of Oklahoma Institutional Animal Care and Use Committee in accordance with National Institutes of Health guidelines.

In vitro *electrophysiology.* Standard *in vitro* slice techniques were used with superfused 400-μm-thick coronal slices of the aPCX (Best and Wilson, 2004). Pharmacological manipulations were performed with a within-slice design, and the effects of 100 μM L-AP-4, a group III mGluR agonist, and 50 μM (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate [(2*R*,4*R*)-APDC], a group II mGluR agonist, on lateral olfactory tract-evoked field potentials were assessed. After 10 min of baseline response measurements, drugs were infused for 10 min, followed by a washout period between and after drug manipulations to assess recovery.

Chronic electrophysiology. Monopolar Teflon-coated 0.127-mm-diameter stainless steel wire electrodes (A-M Systems, Carlsborg, WA) were implanted via the dorsal cranial surface into layer III of aPCX of three rats based on stereotaxic and physiological markers under 50 mg/kg bodyweight pentobarbital. Odor-evoked local field potential (LFP) responses were recorded at 2.1 kHz, and 1048 bin Hanning window fast Fourier transforms were analyzed off-line using Spike2 software (Cambridge Electronic Design, Cambridge, UK) on a personal computer. Odor-evoked beta frequency (15–35 Hz) responses were summed and normalized relative to pre-odor levels for correlation between animals. The odors used were taken from a panel including benzyl acetate, ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl propionate, ethyl valerate, heptanal, isoamyl acetate, methyl butyrate, and propyl butyrate.

Cannula and telemetry implantation. Thirty adult rats were anesthetized with a dose of 50 mg/kg bodyweight pentobarbital and isoflurane as necessary. Guide cannulas (33 gauge; Plastics One, Roanoake, VA) were placed bilaterally 4 mm lateral and 0.5 mm anterior to bregma and were inserted 6.5 mm ventral to the dorsal brain surface. Guide cannulas were then stabilized with acrylic cement. Heart-rate telemetry devices (Data Sciences International, St. Paul, MN) were implanted under the dorsal surface of the skin for subsequent monitoring of odor-evoked heart-rate orienting responses. Rats were given 0.4 ml of microcillin (Pacific Animal Health, Irwindale, CA) and 2 ml of saline postoperatively. Rats were allowed at least 1 week to recover before testing.

Behavioral testing. Rats were placed in the testing chamber and allowed 15 min to habituate to their environment. Then six different odors (4 s odor duration) were presented with a 30 s interstimulus interval to get baseline odor-evoked heart-rate orienting responses (Fletcher and Wilson, 2002). There is between-animal variation in HROR magnitude and in which odors are most effective at driving HRORs. Thus, for each animal, the two odors evoking the most robust HRORs were determined, and their data were used for analyses of exposure and non-exposure effects. Subsequently, artificial CSF (ACSF), 1.25 mM cyclopropyl-4-phosphonophenylyglycine (CPPG) (in ACSF), or 2.5 mM CPPG (in ACSF) was infused at 0.15 μl/min for 20 min. Near the end of infusion, rats were again presented with the odors as described previously to obtain postinfusion baseline responses. After a 5 min rest, the rats were exposed to a habituating series of odor presentations consisting of 60 4-s-duration presentations at 0.1 Hz of one of the odors. This is the habituation protocol used throughout this report. HRORs were again determined for both the exposed and non-exposed odor to gauge the effect of the infusion on habituation. Each rat underwent both drug and control condition with 1 week between testing in a counterbalanced design. Approximately 65% of Long–Evans rats demonstrate robust, reliable odor-evoked HRORs with the odors and concentrations used here (Fletcher and Wilson, 2001). Only rats that showed a heart-rate drop of 10 beats/min to at least two of the odors presented were used in the experiment.

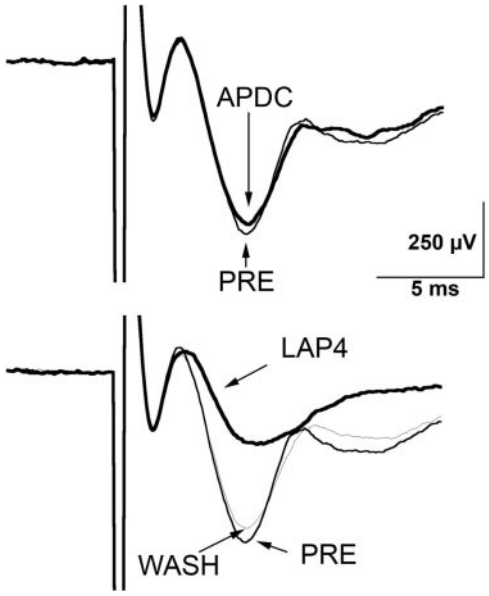

Figure 1. Representative examples of the effect of the group II mGluR agonist (2*R*,4*R*)-APDC (***A***) and the group III mGluR agonist L-AP-4 (***B***) on LOT-evoked potentials recorded in layer I of the aPCX *in vitro*. L-AP-4 induced a marked depression that essentially recovered within 10–15 min, whereas (2*R*,4*R*)-APDC was without detectable effect. Pre, Preexposure.

Data from one rat was thrown out because of damage at the site of the cannula tip.

Histological analysis. Cannula tracts were reconstructed from 40 μm sections after transcardial perfusion. The cannulas were histologically confirmed as being between 1 mm anterior and 0.26 mm posterior to bregma [0.199 ± 0.058 mm (mean ± SEM) anterior]. The mean relative location of the cannula tips was 0.306 ± .040 mm (mean ± SEM) from layer I of aPCX (see Fig. 3*A*).

Results

The mGluR antagonist CPPG shows 20-fold selectively for group III over group II mGluRs, and, as noted above, immunohistochemical studies suggest very limited group II mGluR expression in rat piriform. However, to confirm which mGluR group was predominantly responsible for the synaptic depression believed to underlie the behavioral effects studied here, we examined the effects of a group II and a group III mGluR agonist on piriform cortical synaptic efficacy. Whereas the selective group III mGluR agonist L-AP-4 (100 μM; $n = 6$) produced a significant depression of the piriform cortical afferent synapse within 60 s of bath application (Fig. 1), *in vitro* (mean postdrug response, 50.2 ± 1.5% of predrug baseline), the group II mGluR agonist (2*R*,4*R*)-APDC (50 μM; $n = 6$) did not [mean postdrug response, 92.5 ± 2.5% of predrug baseline, repeated-measures ANOVA; main effect of drug, $F_{(3,15)} = 77.67$, $p < 0.001$, *post hoc* comparisons showed a significant depression induced by L-AP-4 and no significant change induced by (2*R*,4*R*)-APDC]. The L-AP-4-induced depression had recovered to 84.9 ± 4.4% of baseline within 15 min of washout. Thus, together with previous data (Wada et al., 1998), these findings suggest that the results described below reflect primarily selective group III mGluR mechanisms.

To test whether habituation of odor-evoked activity within aPCX is correlated with habituation of the odor-evoked HROR, single electrodes were chronically implanted unilaterally into aPCX layer III to record LFP oscillations. Representative examples of simultaneously recorded HRORs and LFPs before and after odor habituation are shown in Figure 2*A*. Cortical LFP responses to five 4 s odor presentations before and five 4 s odor

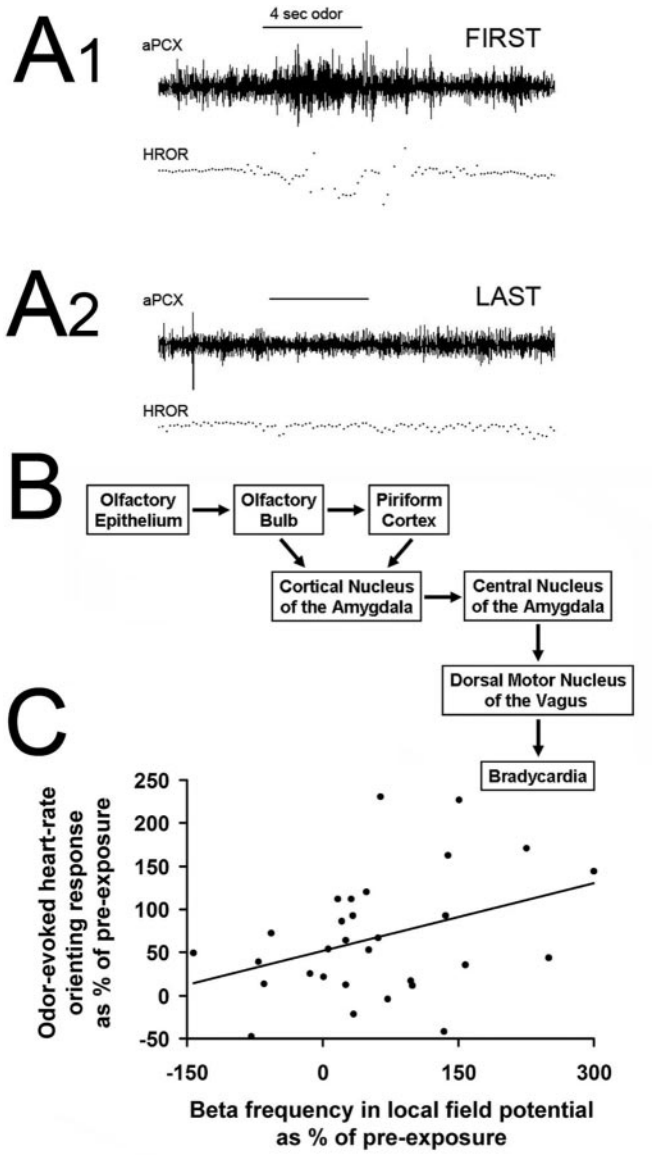

Figure 2. The amplitude of the odor-evoked HROR is correlated with odor-evoked oscillatory activity within aPCX. ***A***, Examples of odor-evoked HROR and simultaneously recorded piriform cortex odor-evoked LFP responses to odors before (***A1***) and after (***A2***) habituation. LFP records are filtered for beta and gamma frequency (15–90 Hz) activity. Four second odor stimulus presented at the horizontal bar. ***B***, Schematic diagram of the circuitry underlying HRORs. ***C***, Although HROR amplitude is mediated by a variety of factors (Sokolov, 1975), there was a significant correlation between odor-evoked HROR amplitude and the power of beta frequency oscillations recorded in aPCX in awake rats. Data points are simultaneously recorded HROR and LFP responses to 4 s odor stimuli before and after habituation.

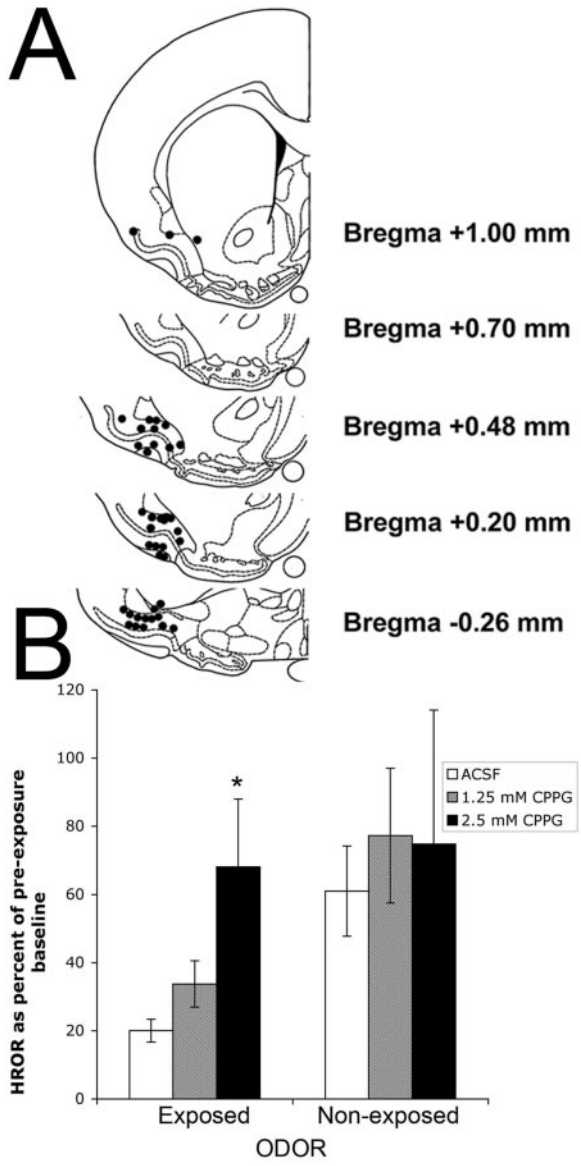

Figure 3. ***A***, Location of infusion cannula tips (bilateral implants depicted unilaterally). Stereotaxic images are adapted from the atlas of Paxinos and Watson (1998). ***B***, Blockade of mGluRs in olfactory cortex decreases habituation of the odor-evoked HROR to the exposed odor. Odor-evoked HRORs were normalized to preexposure responses. Data are presented for the odor used for extended exposure and for the non-exposed odor not used for extended exposure (data presented as mean ± SEM). The asterisk represents a significant difference from the response to the exposed odor in ACSF-infused animals ($p < 0.05$).

presentations after habituation exposure were recorded and compared with their simultaneously recorded odor-induced HROR. Both beta frequency oscillations in the cortical LFP records (mean posthabituation response as percentage of baseline, 34.2 ± 4.3%; $n = 3$; paired one-tailed t test; $t_{(2)} = 17.43$; $p < 0.01$) and averaged HRORs (mean posthabituation response as percentage of baseline, 17.8 ± 5.1%; $n = 3$; paired one-tailed t test; $t_{(2)} = 17.03$; $p < 0.01$) are depressed significantly and to a similar level after habituation. Additionally, the prehabituation and posthabituation magnitude of individual odor-evoked cortical LFP beta oscillations and simultaneously recorded HRORs responses are significantly, positively correlated ($r = 0.37$; $p < 0.05$) (Fig. 2*C*).

Next, we tested whether blockade of cortical mGluR autoreceptors within the olfactory cortex, which had been shown to reduce afferent synaptic depression and cortical odor adaptation (Best and Wilson, 2004), would reduce behavioral habituation of the odor-evoked HRORs. Although infusions were selectively directed at the aPCX (Fig. 3*A*), given the geometry of the piriform cortex and neighboring regions of the olfactory cortical complex, some diffusion into other regions was inevitable. In two animals, a saturated solution of fast green dye was infused (0.15 μl/min for 20 min) through the cannulas before perfusion to visualize diffusion. Dye extensively labeled the aPCX, with some staining of overlying orbitofrontal cortex and underlying olfactory tubercle, and was thus limited to olfactory cortical regions. Staining did not extend into the anterior olfactory nucleus (also known as anterior olfactory cortex) or main olfactory bulb.

The amplitude of odor-evoked HRORs were compared before and after bilateral infusion of the group II/III mGluR antagonist CPPG into the olfactory cortex. The amplitude of odor-evoked HRORs were determined for several odors in each animal before and after onset of infusion of either CPPG (1.25 or 2.5 mM) or ACSF. There was no significant effect of the CPPG infusion itself on the amplitude of baseline odor-evoked HRORs compared with ACSF infusions (paired *t* test; $t_{(9)} = 0.78$; NS).

One of the odors was then selected as the habituation odor, and a habituation exposure with that odor (4 s odor pulses, 10 s interstimulus interval, 60 repeats) was initiated. Immediately after the habituation exposure, the amplitude of odor-evoked HRORs to the exposed and non-exposed odors were again assessed in random order. CPPG (2.5 mM) infusion dramatically and significantly decreased the extent of habituation of the odor-evoked HROR amplitude compared with ACSF-infused animals ($n = 10$ in each group except the 2.5 mM CPPG group, in which data for the recording of the response to the control odor in one animal was corrupted; main effect of condition; ANOVA and Fisher's *post hoc* tests; $F_{(1,17)} = 7.31$; $p < 0.05$) (Fig. 3*B*). The residual decrease in odor-evoked HROR amplitude after habituation exposure in 2.5 mM CPPG-infused animals was similar to the generalized decrease in HROR amplitude averaged across the nonhabituated odors (Fig. 3*B*). The effects of the 1.25 mM CPPG infusion on HROR amplitude habituation were intermediate between that of the ACSF control and 2.5 mM CPPG infusion.

Discussion

These results suggest that synaptic depression of the primary cortical afferent synapse in the olfactory cortex underlies habituation of a simple olfactory behavior in rats. The 2.5 mM dose of CPPG essentially completely blocked habituation, leaving HROR responses at the same level as responses to the non-exposed odor. The nonselective cross-habituation observed to the non-exposed odor and residual habituation to the exposure odor in CPPG-infused animals may represent adaptation of olfactory bulb mitral/tufted cells, olfactory receptor neurons, unidentified cortical or limbic mechanisms, and/or non-olfactory changes in internal state (Sokolov, 1975; Potter and Chorover, 1976; Wilson, 2000; Zufall and Leinders-Zufall, 2000). However, these results clearly suggest that peripheral receptor adaptation is not the primary mechanism for short-term odor-specific decreases in behavioral responses after exposure.

These results also strongly argue for a role of the olfactory cortex in mediating simple behavioral and autonomic responses to odors. Gating of sensory responses at the cortical level, rather than more peripherally, may allow for rapid dishabituation if attention or stimulus contingencies change. The mGluR and its associated intracellular signaling cascade itself may in fact be a site of dishabituation, because several mechanisms exist for modulation of mGluR activity (Cai et al., 2001), including noradrenergic input. We are currently investigating this possibility.

In addition to contributing to habituation, selective cortical adaptation may also play an important role in analysis of complex, often overlapping, patterns of odors encountered in real-world situations (Wilson and Stevenson, 2003). For example, selective cortical filtering of background odors with maintained responsiveness to novel or changing stimuli may facilitate figure–ground separation and analysis of complex spatial and temporal patterns of odors in an animal's environment. Rapid adaptation could allow the cortex to function as a high-pass filter, responding selectively to stimulus change, in either quality or intensity, and suppress static or background inputs (Kadohisa and Wilson, 2004).

Although infusion cannulas were localized within the aPCX, some CPPG diffusion to neighboring olfactory cortical areas was inevitable. Thus, conservatively, these results suggest a role for olfactory cortex in odor habituation, not necessarily solely the aPCX. However, the circuitry underlying odor-evoked HRORs (Fig. 2) involves an olfactory bulb and piriform cortical projection to the cortical nucleus of the amygdala. The present infusion did not extend to the olfactory bulb, thus a role for piriform cortex is suggested. Neurons in the olfactory tubercle, for example, are not known to project to the amygdala (Shipley and Ennis, 1996). Together with the observed CPPG blockade of aPCX synaptic depression and LFPs (Best and Wilson, 2004), these data strongly suggest an aPCX mediation, although additional work may be required for precise determination. Nonetheless, the results clearly demonstrate a central, cortical origin for olfactory sensory gating.

Finally, the mechanisms identified here may have relevance not only to olfaction but also to adaptation in sensory systems involving thalamocortical pathways. Similar forms of synaptic depression have been described in thalamocortical sensory systems, although their role in behavioral sensory perception have not been directly assessed (Chung et al., 2002; Weber et al., 2002). The relatively simple architecture of the olfactory system may represent an ideal system for additional examination of the mechanisms and consequence of this form of sensory gating.

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