The role of metabotropic glutamate receptors and cortical adaptation in habituation of odor-guided behavior

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Decreases in behavioral investigation of novel stimuli over time may be mediated by a variety of factors including changes in attention, internal state, and motivation. Sensory cortical adaptation, a decrease in sensory cortical responsiveness over prolonged stimulation, may also play a role. In olfaction, metabotropic glutamate receptors on cortical afferent pre-synaptic terminals have been shown to underlie both cortical sensory adaptation and habituation of odor-evoked reflexes. The present experiment examined whether blockade of sensory cortical adaptation through bilateral infusion of the group III metabotropic glutamate receptor antagonist cyclopropyl-4-phosphonophenylglycine (CPPG) into the anterior piriform cortex could reduce habituation of a more complex odor-driven behavior such as investigation of a scented object or a conspecific. The results demonstrate that time spent investigating a scented jar, or a conspecific, decreases over the course of a continuous 10 minute trial. Acute infusion of CPPG bilaterally into the anterior piriform cortex significantly enhanced the time spent investigating the scented jar compared to investigation time in control rats, without affecting overall behavioral activity levels. Infusions into the brain outside of the piriform cortex were without effect. CPPG infusion into the piriform cortex also produced an enhancement of time spent investigating a conspecific, although this effect was not significant.

Learning to stop responding to stable or currently unimportant stimuli is an important behavioral capability. Habituation to such stimuli allows attention and central processing power to be directed toward dynamic or more biologically relevant stimuli. This simple form of learning may have a variety of underlying causes; however, sensory gating—a filtering of external stimuli by central sensory pathways—may be the most straightforward and direct. In Aplysia, for example, habituation of sensory-evoked reflexes is mediated by pre-synaptic depression of sensory input to motor output neurons (Kandel and Schwartz 1982). Similarly, we have recently found that habituation of odor-evoked cardiac orienting reflexes is mediated by pre-synaptic depression of sensory afferents to the primary olfactory cortex (Best et al. 2005). The question addressed here was what role does simple cortical sensory gating play in habituation of a more complex behavior such as investigation of a novel odor?

The rodent olfactory system has proven to be an excellent model system for identifying neural correlates of memory in a variety of behavioral paradigms (Gray et al. 1986; Sullivan et al. 1989; Saar et al. 2002; Ravel et al. 2003; Wilson et al. 2004). The primary olfactory (piriform) cortex receives direct input from second-order neurons, mitral cells of the main olfactory bulb. Mitral cells are glutamatergic and terminate on apical dendrites of piriform cortical pyramidal cells. Piriform cortical pyramidal cells respond to odors and show rapid odor adaptation in both anesthetized (Wilson 1998a) and awake (McCollum et al. 1991) rats. This cortical adaptation occurs despite relatively maintained input from mitral cells and is associated with depression of the mitral-pyramidal neuron excitatory synapse (Wilson 1998b). This activity-dependent synaptic depression is mediated by pre-synaptic group III metabotropic glutamate receptors (mGluRIII), and infusion of the mGluRIII receptor antagonist CPPG into the piriform cortex prevents the cortical afferent synaptic depression, cortical adaptation to odors, and habituation of odor-evoked cardiac orienting reflexes (Best and Wilson 2004; Best et al. 2005). Odor-evoked cardiac orienting reflexes are mediated by a circuit that passes through the piriform cortex on the way to the amygdala and ultimately to brainstem dorsal motor nucleus of the vagus, which drives the bradycardia response. In control animals, this odor-evoked bradycardia habituates with repeated stimulation (Fletcher and Wilson 2002; Hunt and Morasch 2004). By preventing sensory gating within the piriform cortex, odors continue to evoke heart-rate changes over many stimulus repetitions (Best et al. 2005). Thus, adaptation of this response is centrally mediated.

In contrast to orienting reflexes, behavioral investigation of scented objects most likely involves more complex central circuitry and is influenced by a wide range of non-olfactory, contextual, and internal-state variables. The present study examined the effects of selective disruption of sensory cortical adaptation on complex behaviors such as odor investigation. The results suggest that cortical sensory gating plays a significant role in habituation of complex, odor-guided behaviors, though other, non-sensory factors are also involved.

Results

In the within-animal design used here, each animal was tested four times in a counterbalanced manner. Each animal was tested with the scented jar while infused with artificial cerebrospinal fluid (aCSF) and again with CPPG, and each animal was also tested in the social dyad under both infusion conditions. The JWWatcher data file for one animal in the scented jar test was corrupted and could not be analyzed. In addition, cannula placements for three animals were judged to be outside of the piriform cortex and their data were excluded from the main analyses, though are presented separately. Thus, scented jar results are
based on eight implanted animals and social dyad results are based on nine implanted animals.

The time spent investigating the scented jar per minute decreased over the continuous 10 minute trial in both aCSF- and CPPG-infused animals (repeated measures ANOVA, main effect of time, $F_{(9,126)} = 4.93, P < 0.001$). As can be seen in Figure 1A, while both aCSF- and CPPG-infused animals showed this habituation, CPPG-infused animals had higher rates of investigation than aCSF-infused animals, primarily during the first five minutes of the test. The increased time of odor investigation in CPPG-infused animals was offset by a decreased amount of time spent in other behaviors (Fig. 1B). There was no difference in total behavioral activity (time spent investigating the scented jar combined with time spent on other behaviors) between animals infused with aCSF and those infused with CPPG (paired $t$-test, N.S.).

A further examination of the initial five minutes of investigation revealed a significant enhancement in cumulative scented jar investigation/decreased habituation in CPPG-infused animals compared to the time spent investigating the jar when the same animals were infused with aCSF (Fig. 1C). A repeated measures ANOVA revealed a significant behavior X infusate interaction in cumulative odor investigation over the initial five minute test ($F_{(1,14)} = 5.99, P < 0.05$). Post-hoc tests revealed a significant increase in cumulative scented jar investigation in the CPPG infusion conditions compared to investigation time in the same animals during aCSF infusion ($P < 0.05$). The corresponding decrease in non-odor investigation did not quite reach significance ($P = 0.07$).

Figure 2 shows the data from individual animals plotted as a difference (CPPG − aCSF) in jar investigation time for each animal. As can be seen, six out of eight animals demonstrated greater investigation of the scented jar during CPPG infusion into the piriform cortex than when the same animals were infused with aCSF.

Similar to the investigation of the scented jar, the time spent investigating a conspecific decreased over the continuous 10 minute trial in both aCSF- and CPPG-infused animals (repeated measures ANOVA, main effect of time, $F_{(9,144)} = 4.69, P < 0.001$) (Fig. 3). Total behavioral activity rates were much higher in the presence of a conspecific compared to when the same animals were exposed to a scented jar (cf. Fig. 1, note the difference in axis scale), but conspecific investigation showed significant habituation. Although there was a trend toward a CPPG-induced increase in conspecific investigation over the course of the 10 minute trial (Fig. 3A) and in cumulative investigation during the first five minutes (Fig. 3C), there was no significant effect of CPPG in this behavioral test (repeated measures ANOVA, behavior X infusate interaction, N.S.).

As noted in the Materials and Methods, behaviors directed toward the conspecific included both direct “sniffing” behaviors as well as other contact behaviors not specifically including a sniffing component. A separate post-hoc analysis selective to sniffing behaviors directed at the conspecific showed a similar trend but still no statistically significant effect of CPPG infusion.

The data described here were from animals confirmed to have both cannulas placed within the piriform cortex (Fig. 4). Three animals had at least one of the bilateral cannulas placed well outside of the piriform and were excluded from the analysis above. An examination of the effect of CPPG on investigation of the scented jar showed no consistent effect in these three animals (Fig. 5), with a mean difference between five minutes cumulative investigation time under CPPG versus under aCSF of $−3.8$ s. This negative value corresponds to a mean $3.8$ s greater investigation during aCSF infusion than during CPPG infusion, the opposite direction of animals with properly placed cannulas.
Discussion

The present results demonstrate that infusion of the mGluRIII antagonist CPPG bilaterally into the anterior piriform cortex disrupts habituation of odor investigation behaviors. CPPG infusion had no effect on total activity levels in this context; the increased time spent investigating the scented jar was compensated for by a decrease in other behaviors. Infusion of CPPG outside of the piriform did not interfere with habituation of odor investigation. Furthermore, the mGluRIII mechanism is most likely limited to short-term effects of continuous exposure (Best and Wilson 2004). In situations where an animal has behavioral control of stimulus exposure through changes in sniffing and head position, depression of cortical afferents may be mitigated, and other, more long-term mechanisms not examined here may become involved in controlling stimulus responsiveness.

Nonetheless, the present results suggest that a relatively simple mechanism such as cortical afferent synaptic depression mediated by mGluRIII's can modulate a complex behavior such as odor-guided object investigation. Disruption of sensory gating is associated with a number of disorders including autism spectrum disorders and schizophrenia (Ornitz et al. 1993; McAlonan et al. 2002; Frith and Hill 2003; Ludewig et al. 2003). Of particular interest is the possible cascade of neural and behavioral consequences that could occur if sensory gating were disrupted developmentally. The mGluRIII-mediated synaptic depression believed to be mediating the effect described here is present and functional early in development (Thompson et al. 2005). Disruption of normal sensory gating, especially during early development, could result in (1) abnormal neurocircuitry (e.g., modified activity-dependent dendritic pruning and/or cell survival), (2) abnormal circuit function (e.g., modified synaptic plasticity and/or...
or sensory coding), and (3) disruptions in higher order information processing (e.g., impaired selective attention and/or habituation). Each of these issues is currently being explored.

Materials and Methods

Subjects

Thirty-six adult male Long-Evans hooded rats were obtained from Harlan Bioproducts for Science. Of these, 12 animals were implanted with cannula's and used for data collection, while 24 animals were used as conspecific targets in social dyad interaction tests but were otherwise not manipulated. Rats were fed standard rodent pellets and water ad libitum. Rats were housed in polypropylene cages with lights maintained on a 12-h light/12-h dark cycle; behavioral tests occurred during the morning or afternoon. Rats were implanted with cannulas when they were approximately 300 g. 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.

Surgery/cannula implantation

Rats were anesthetized with isoflurane and placed in a stereotaxic apparatus. Guide cannulas (26 gauge, Plastics One) were placed +1.0 mm anterior and ±4.5 mm lateral to bregma and were lowered 7.0 mm ventral, reaching the anterior piriform cortex. The bilateral cannulas, as well as three anchoring screws, were affixed into the skull using dental cement. Rats were allowed to recover at least one week before the first behavioral test.

Testing apparatus and behavioral analysis

Each rat participated in two social dyad and two scented jar investigation tests in a within-subjects design with order of testing and drug infusion counterbalanced. The social dyad (experimental rat with another rat) and scented jar (experimental rat with a novel odor inside a jar) tests were identical with the exception of the different stimulus.

A 26 cm × 50 cm × 30 cm high, glass aquarium was used for testing. Clean wood shavings were placed in the bottom of the aquarium before each test. All tests (10 minutes in duration) were videotaped with a digital camcorder (Sony Handycam) and recorded to DVD-R discs.

Ethograph software was used to score behaviors (JWatcher, Macquarie University, http://galiform.bhs.mq.edu.au/jwatcher/). Behavioral scoring occurred live and also by an observer blind to infusion condition from the recorded video. Both methods produced similar results. Behaviors were divided into two broad classes: Those directed toward the scented jar or other rat, and those not directed toward the jar or other rat. Total time spent in each behavioral class was determined per minute.

After the 10-minute test was complete, animals were removed from the test chamber, dummy cannulas were reinserted, and the animals were returned to their home cages. Additional testing occurred with a minimum of four days between tests.

Behavioral testing—social dyad

An additional, non-implanted rat was necessary in the social dyad test. These “partner” rats weighed within 50 g of the experimental rat’s weight. These rats were housed separately from the experimental rats and were only used in one behavioral test. Therefore, the experimental rat was exposed to a unique rat for each test.

After the infusion of the experimental animal was complete, the partner rat was placed into the testing chamber, immediately followed by the experimental rat. During the test, data was only recorded for the experimental rat. Monitored behaviors directed at the partner rat included: Aggressive behavior, crawling over the other rat, sniffing tail, sniffing genitals, sniffing body, sniffing head, crawling under, and grooming the other rat. Behaviors not directed toward the partner rat included: Grooming self, scratching, digging, rearing, sitting side by side with the partner rat but otherwise not interacting, and smelling feces. After the 10-minute test, both rats were returned to their home cages.

Behavioral testing—scented jar

Peppermint (McCormick) was selected as the novel odor. Three holes were drilled into the lid of a 5 cm diameter × 5 cm tall clear glass jar, which was used to hold the peppermint odor. Ten minutes before each test a cotton square was placed inside the jar and moistened with 100 µL of peppermint extract and the holes sealed with plastic tape until the time of test.

Procedures for the odor condition were identical to those of the social dyad condition except the scented jar was lowered into the cage before the test instead of another rat. Behaviors directed toward the scented jar were: Sniffing the jar and crawling over the jar; nonsocial behaviors recorded were the same as in the social dyad condition. After each 10-minute test, the cotton square was discarded and the jar was wiped clean with deionized water.

Drug infusion

Cyclopropyl-4-phosphonophenylglycine (CPPG, 2.5 mM; Tocris) dissolved in artificial cerebrospinal fluid (aCSF) or aCSF alone were infused in the dyad and odor conditions in a counterbalanced design. This concentration was chosen based on dose-response curves determined in a previous examination of the effects of piriform cortex CPPG infusions on odor-evoked reflex habituation (Best et al. 2005). Twenty minutes prior to behavioral testing, rats were briefly sedated with isoflurane, the dummy cannulas removed from the guide cannulas and replaced with internal cannulas (33 ga). Animals recovered and were attached via tubing to an infusion pump, which delivered a flow of CPPG or aCSF. Infusions were bilateral. Rats were infused at a rate of 0.15 µL/min for 20 min for a total infusion of 3 µL/side. Immediately following infusion, rats were placed in the test chamber.
Histology
After each rat’s fourth and final test, they were overdosed with urethane and transcardially perfused with saline and 4% paraformaldehyde. Brains were sectioned coronally at 40 for verification of cannula tip locations in cresyl violet stained sections.

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