Neurobiology of a Simple Memory

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INTRODUCTION

Habituation is one of the simplest forms of memory, yet a clear understanding of its neurobiological mechanisms remains elusive. Significant strides have been made in invertebrates, where habituation of sensorimotor reflexes seems to be mediated by synaptic depression (Castellucci et al. 1970; Kandel 2001), whereas the specific mechanisms of the depression may vary depending on the nature of induction and duration of the resulting memory (Bailey and Chen 1988; Efrazeddine and Glanzman 2003). In vertebrates, habituation of spinal cord mono-synaptic reflexes may also be mediated by synaptic depression of sensory input (Farel and Thompson 1976), although precise mechanisms of the synaptic plasticity are unknown.

Recent work in mammalian olfaction has proven very fruitful in the search for mechanisms of simple habituation memory. The olfactory system is a relatively simple system, highly conserved across vertebrate evolution (Hildebrand and Shepherd 1997) and has long been implicated as having unique ties to memory processes. Importantly, odors evoke both autonomic reflexes and behavioral investigation, two behaviors that can be easily quantified to allow correlation between neural mechanism and behavioral outcome.

This review summarizes work over the past 10 yr in our and others’ laboratories investigating the neurobiology of olfactory habituation. Although much remains to be learned, this work is the most complete description of the mechanisms of a simple form of memory in the mammalian brain to date, describing both necessary and sufficient central mechanisms of behavioral response decrement with repeated stimulation.

Although habituation is classically considered a simple form of memory, it may serve as an important building block in more complex forms of cognition and attention (Fabiani et al. 2006; Miller et al. 1977; Postle 2005). Furthermore, disruptions in habituation and sensory gating are linked to disorders such as schizophrenia (Ludewig et al. 2003) and autism (Frankland et al. 2004; Ornit et al. 1993), as well as aging (Fabiani et al. 2006; O’Connor et al. 2008) and substance abuse (Hunt and Morasco 2004). Thus understanding the mechanisms of this form of memory could have far reaching implications.

BEHAVIORAL PARADIGMS

Odor stimuli can elicit a variety of responses, ranging from autonomic reflexes to behavioral arousal and investigation. Two primary behavioral paradigms have been used to study odor habituation in mammals (Fig. 1). The simplest, both in terms of underlying neural circuitry and interpretation is the odor-evoked heart-rate orienting reflex (Sokolov and Vinogradova 1975). Novel sensory stimuli evoke a bradycardia reflex, which habituates with repeated stimulation (McDonald et al. 1964). Odors activate olfactory sensory neurons within the nose, which form glutamatergic synapses on second-order neurons called mitral cells. Mitral cells project to the olfactory cortex, a major subdivision of which is called the piriform cortex. Mitral cells are glutamatergic and target pyramidal cells within piriform cortex that express N-methyl-D-aspartate (NMDA) and non-NMDA receptors, including metabotropic glutamate receptors (Shipley and Ennis 1996). Presynaptic terminals of mitral cell axons also express metabotropic glutamate receptors (Wada et al. 1998). Piriform cortical pyramidal neurons project to the basolateral amygdala, which in turn projects to the central amygdala. The central amygdala is a major amygdaloid output nucleus, with projections to hypothalamus and brain stem. Its projection to the brain stem dorsal motor nucleus of the vagus leads to a parasympathetic input to the cardiac pacemaker cells. Thus novel odor stimulation can produce a polysynaptic heart-rate deceleration reflex.

The second major behavioral paradigm for investigating odor habituation in mammals is monitoring investigation of scented objects (Cleland et al. 2002; Hunter and Murray 1989). An object scented with a novel odor is presented, and the duration of investigation is monitored and compared with investigation time on subsequent presentations of the same scent. Over repeated trials, investigation decreases.

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There can be multiple motivating factors contributing to, or limiting, scented object investigation, and the circuitry from nose to motor output in this case is phenomenally complex. Nonetheless, important insights into mechanisms of habituation have been obtained with this paradigm, and mechanisms identified in the reflex paradigm have been cross-validated in the investigation paradigm.

Both odor-evoked heart-rate orienting reflexes and odor investigation habituate with repeated stimulus presentations and spontaneously recover. Habituation in both paradigms is odor specific. Although not investigated in the heart-rate reflex paradigm, habituation of odor investigation can show both short- and long-term forms, with important behavioral and neurobiological differences as described below. When combined with electrophysiological and pharmacological techniques, these two behavioral assays have provided excellent insight into the mechanisms of odor habituation.

**FIG. 1.** Experimental paradigms. A: schematic representation of the electrophysiological experiments. Intra- and extracellular recordings were made from pyramidal cells in anterior piriform cortex (PCx). Odor responsiveness of individual cells was first tested with a 2-s odor stimulus. Habituation of the odor response was achieved by either presenting the same odorant for 50 continuous s or presenting ten 2-s odor stimuli separated by 30 s. A 2-s odor pulse tested the posthabituation response to the same odorant. Typically, pyramidal cell responses were significantly reduced by the prolonged or intermittent stimulation. B: bradycardia autonomic reflex habituation paradigm for adult rats. Odor-evoked heartrate orienting responses to 4-s odor stimuli were monitored with telemetry devices (inset) before and after stimulus repetition with 10- to 30-s interstimulus intervals. These orienting responses showed pronounced habituation after 30–60 trials. C: behavioral habituation paradigm for adult rats and mice. Odors are presented to the mouse in their home cage, either by hanging a tea ball containing an odor-saturated swab from the cage roof or by presenting odor-saturated filter paper on the roof of the cage. Investigation of the odorant is measured during odor presentation. Animals are typically first presented with a blank (mineral only) stimulus, followed by 4 presentations of the habituation odor. A different test odor is presented during the 6th trial. In the short-term paradigm, odors are presented for 20 s separated by 10-s intertrial intervals, whereas in the long-term paradigm, odors are presented for 50 s separated by 5-min intertrial intervals.

Although olfactory sensory neurons can adapt to repeated or prolonged odor stimulation (Kurahashi and Menini 1997; Zufall and Leinders-Zufall 2000), behavioral odor habituation is a central phenomenon. As is the case in other sensory systems, central olfactory neurons show greater response decrement than peripheral receptors, with piriform cortical neurons showing rapid, nearly complete response adaptation within seconds or minutes of stimulation in both rodents (Wilson 1998a) and humans (Sobel et al. 2000). In a direct comparison of receptor adaptation and perceptual habituation in humans, Hummel et al. (1996) showed that changes in the periphery could not account for the magnitude of decrease in perceptual intensity estimates. Disruption of sensory neuron adaptation in transgenic mice can impair odor perception (Kellhner et al. 2003); however, as described below, these effects are most...
likely caused by aberrant patterns of central circuit activation rather than a direct effect on habituation per se.

CORTICAL ADAPTATION AND SHORT-TERM HABITUATION

Simultaneous recordings of second-order mitral cells and their target piriform cortical pyramidal cells showed that the cortical neurons adapt to repeated or prolonged odor stimulation more rapidly and completely than their afferent mitral cells (Wilson 1998a). Mitral cells do adapt to odors (Gray and Skinner 1988; Scott 1977; Wilson and Sullivan 1992); however, adaptation of their downstream targets is both faster and more complete. The cortical adaptation is odor-specific, showing minimal cross-adaptation between molecularly similar or dissimilar odors and even relatively little cross-adaptation between a familiar odor mixture and its components (Wilson 2000a,b, 2003). In vivo intracellular recordings showed that this cortical adaptation is associated with depression of the glutamatergic mitral-pyramidal cell synapse and that this synaptic depression recovers with the same time course as the short-term adaptation of odor-evoked postsynaptic potentials (Wilson 1998b).

In vitro, electrical stimulation of mitral cell axons in a pattern mimicking mitral cell odor-evoked activity produces a similar synaptic depression (Best and Wilson 2004; Thompson et al. 2005). This depression is homosynaptic (Best and Wilson 2004), perhaps contributing to the odor specificity of cortical adaptation. Additional in vitro work showed that the activity-dependent cortical afferent synaptic depression was dependent on group III metabotropic glutamate receptors (Best and Wilson 2004), which have been found presynaptically on mitral cell axon terminals (Wada et al. 1998). Pharmacological blockade of these receptors in vitro, with either (RS)-a-cyclopropyl-4-phosphonophenylglycine (CPPG) (Best and Wilson 2004) or (RS)-a-methylserine-O-phosphate (MSOP) (M. Kodohisa and D. A. Wilson, unpublished observations) prevents afferent synaptic depression.

Antagonists of group III metabotropic glutamate receptors not only prevent synaptic adaptation in vitro but also prevent or reduce odor habituation at the behavioral level. Bilateral, intrapiriform cortex infusions of CPPG block habituation of the odor-evoked heart-rate orienting response (Best et al. 2005) and reduce habituation of investigation of scented objects (Yadon and Wilson 2005). Similarly, systemic injections of the metabotropic glutamate receptor antagonist LY341495 reduce short-term odor investigation habituation (McNamara et al. 2008). Intracerebral infusions distant to the piriform cortex produced no significant effect on habituation of odor object investigation (Yadon and Wilson 2005). Preliminary results (Wilson, unpublished observations) further suggest that intrapiriform infusions of the metabotropic glutamate receptor agonist L-(+)-2-amino-4-phosphonobutyric acid produce a depression of odor-evoked heart-rate orienting reflexes, comparable to those seen during habituation.

Together, these results strongly argue that depression of mitral cell afferents to the piriform cortex, via a presynaptic metabotropic glutamate receptor-mediated mechanism, leads to both short-term cortical odor adaptation and short-term behavioral odor habituation. Metabotropic glutamate receptors have been implicated in synaptic depression in several systems (Bandrowski et al. 2002; Bespalov et al. 2007; Burke and Hablitz 1994; Schoppa and Westbrook 1997; Weber et al. 2002). However, we believe the observations reported here are the most direct link between these receptors, synaptic depression, and short-term behavioral habituation, and provide the most complete mechanistic description of this form of simple memory in the mammalian brain.

LONG-TERM HABITUATION

In addition to short-term habituation described above, extended and/or spaced odor exposure can lead to long-term habituation. In humans exposed daily to odors in the home or workplace, habituation to the exposed odor (elevated detection threshold) can last weeks (Dalton 2000; Dalton and Wysocki 1996). Similarly, in rodents, depending on the parameters of habituation induction, odor habituation can last either a few minutes following rapid, short presentations or >1 h following spaced, long presentations (McNamara et al. 2008).

In invertebrates, the behavioral characteristics and neurobiological mechanisms of short- and long-term habituation can differ in several features, including the presence or absence of anatomical changes and the involvement of specific receptor or second messenger cascades (Bailey and Chen 1988; Rose et al. 2003; Stopfer et al. 1996). We have recently begun a comparison of both the behavioral characteristics and neural mechanisms of long-term habituation in the mammal with a specific emphasis on identifying differences with short-term habituation. Our early results suggest that long-term habituation induced by spaced, prolonged odor exposure differs from short-term habituation in the specific glutamatergic receptors involved, the locus of change within the olfactory pathway, and in its stimulus specificity (McNamara et al. 2008).

Short, rapidly presented (10-s interstimulus interval) odor stimuli induce a short-term habituation of odor investigation lasting a few minutes (McNamara et al. 2008; Yadon and Wilson 2005). As noted above, blockade of group III metabotropic glutamate receptors, systemically (McNamara et al. 2008) or via intrapiriform cortical infusions (Yadon and Wilson 2005) prevents or reduces this habituation. This short-term habituation is highly odor specific, with very little cross-habituation even between molecularly similar odors (Fletcher and Wilson 2002; McNamara et al. 2008), as is adaptation of the responses of piriform cortex single units to odors (Wilson 2000a,b).
In contrast, habituation induced by spaced (5-min inter-stimulus interval) odor stimuli lasts ≥1 h and is unaffected by metabotropic receptor blockade. Rather, systemic injections of the NMDA receptor antagonist MK-801 significantly block long-term habituation (McNamara et al. 2008). MK-801 injections have no effect on short-term habituation (McNamara et al. 2008). Furthermore, although the piriform cortex is a critical locus for short-term habituation, infusions of MK-801 locally into the olfactory bulb block long-term habituation (McNamara et al. 2008). Thus McNamara et al. (2008) showed a double-dissociation between short- and long-term habituation, with NMDA receptor antagonists blocking long-term habituation but having no impact on short-term habituation and metabotropic glutamate receptors blocking short-term habituation but having no effect on long-term habituation. Finally, the NMDA mechanism of long-term habituation seems to be localized within the olfactory bulb, whereas the metabotropic glutamate receptor mechanism of short-term habituation seems localized within the piriform cortex.

Precisely how NMDA receptor activation within the olfactory bulb leads to long-term odor habituation is unclear. NMDA receptors are involved in a variety of functions within olfactory bulb circuits, including response of second-order neurons or olfactory sensory neuron input (Ennis et al. 1998), mitral cell to mitral cell cross-talk and/or auto-excitation (Isaacson 1999; Salin et al. 2001; Schoppa and Urban 2003), and mitral cell interactions with inhibitory interneurons including granule cells (Isaacson and Strowbridge 1998). Furthermore, NMDA receptors are involved in olfactory bulb circuit plasticity (Ennis et al. 1998; Mandairon et al. 2006; Tyler et al. 2006).
2007; Wilson 1995) and olfactory memory formation (Kendrick et al. 1992; Lincoln et al. 1988; Mandairon et al. 2006). Further work will be required to identify the specific process of olfactory bulb NMDA receptor involvement in long-term odor habituation.

The different circuits underlying short- and long-term habituation (Fig. 2) may be expected to affect other characteristics of habituation, such as its specificity. For example, olfactory bulb mitral cells show strong cross-habituation (strong generalization: Buonviso and Chaput 2000; Buonviso et al. 1998; Wilson 2000a), whereas piriform cortical neurons show little cross-habituation (Wilson 2000a). In concert with these physiological differences, McNamara et al. (2008) showed behaviorally that cortically mediated short-term habituation of odor investigation is highly odor specific, whereas olfactory bulb-mediated long-term habituation shows much more stimulus generalization.

COMPUTATIONAL MODELING

The insights gained into mechanisms of habituation have led to predictions regarding its role in cognitive and perceptual phenomena. The most thoroughly studied to date has been an examination of the role of habituation and cortical adaptation in perceptual background segmentation. The relative simplicity of the olfactory system has allowed us to examine background segmentation with electrophysiological methods (Kadohisa and Wilson 2006), behavioral methods, and computational modeling (Linster et al. 2007).

Using a combined model of olfactory bulb and cortex, Linster et al. (2007) showed how synaptic adaptation at the olfactory bulb input to the piriform cortex can be sufficient to create odor specific adaptation in olfactory cortex neurons and that this feature can produce odor background segmentation (Fig. 3). Further modeling including short-term synaptic potentiation at piriform cortex pyramidal synapses suggested that it is the interaction between synaptic adaptation at afferent synapses and synaptic potentiation at intrinsic synapses that ensure the high odor selectivity observed in electrophysiological (Wilson 2000a,b, 2003) and behavioral (McNamara et al. 2008) short-term habituation.

SUMMARY

In summary, the olfactory system presents unique opportunities to examine the neurobiology of simple memory. The data described here show both a pharmacological and anatomical distinction between short-term and long-term memory for habituation. Furthermore, the differential locus of change underlying short- and long-term memory lead to predictable differences in their characteristics, such as specificity. These findings suggest that habituation in the mammalian brain is a rich, complex, and distributed process—not nearly as simple as generally described.

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