Long-term Potentiation in the Accessory Olfactory Bulb: A Mechanism for Olfactory Learning

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Introduction

One context of olfactory learning that has been investigated in some detail concerns the memory, established at mating, and formed by the female mouse to the odours (pheromones) of the mating male. This olfactory memory is vital for mitigating pregnancy block that might otherwise be induced by his pheromones (Keverne and Rosser, 1986). Pheromones from an unfamiliar male, for which no memory has been formed, activate the vomeronasal system with their receptors in the vomeronasal organ, thereby initiating a neuroendocrine reflex that suppresses prolactin secretion from the pituitary (Keverne, 1983). The removal of luteotrophic support results in a fall in progesterone levels and a return to oestrus. Therefore, it has been hypothesized that the pheromonal memory functions as a gate to suppress or modulate the specific pheromonal signal (Brennan et al., 1990; Kaba and Nakanishi, 1995; Brennan and Keverne, 1997; Brennan, 2001).

The pheromonal memory is acquired with one trial learning, depends upon mating and lasts for several weeks (Keverne and de la Riva, 1982; Kaba et al., 1988). The neural changes underlying memory formation occur in the accessory olfactory bulb (AOB), the first relay in the vomeronasal system, independently of the main olfactory system and the hippocampus (Brennan et al., 1990; Kaba and Nakanishi, 1995; Brennan and Keverne, 1997). Microcircuits in the AOB include the prominent reciprocal dendrodendritic synapse between mitral cells, a single class of projection neurons and granule cell interneurons. Glutamate released from mitral cell dendrites activates the dendrites of granule cells, which in turn mediate GABAergic dendrodendritic inhibition back onto mitral cell dendrites (Jia et al., 1999; Taniguchi and Kaba, 2001). This feedback inhibition at the reciprocal synapses regulates mitral cell activity (Jia et al., 1999; Taniguchi and Kaba, 2001). The formation of the pheromonal memory requires the association of the pheromonal and the mating signals in the AOB. The mating signal is conveyed by noradrenergic projections from the locus coeruleus. Artificial vagal stimulation (Rosser and Keverne, 1985) or mating (Brennan et al., 1995) promotes the release of noradrenaline (NA) in the AOB. Blockade of α-adrenergic receptors in the AOB immediately after mating prevents the formation of the pheromonal memory (Kaba and Keverne, 1988), as does removal of noradrenergic innervation of the AOB prior to mating (Rosser and Keverne, 1985). Furthermore, memory formation is associated with neurochemical and morphological changes at the mitral–granule cell reciprocal synapses (Brennan et al., 1995; Matsuoka et al., 1997, 2004).

Despite advances such as these, however, an important void in our knowledge of electrophysiological aspects has remained: a long-lasting increase in synaptic strength, known as long-term potentiation (LTP), has been little investigated. Moreover, the cellular and synaptic mechanisms underlying noradrenergic modulation of pheromonal learning are also unknown. To address these questions, we have carried out a series of experiments in AOB slices.

Induction of LTP at the mitral-to-granule cell synapse in the AOB

We have initially analyzed synaptic transmission and its plasticity in coronal slice preparations of the mouse AOB using the field potential recording technique. A recording electrode was placed in the external plexiform layer where reciprocal dendrodendritic synapses between mitral and granule cells are formed. A stimulation electrode was placed on the lateral olfactory tract (LOT) to stimulate mitral cell axons antidromically. Stimulation of mitral cell axons evoked two negative field potentials. The first potential was not blocked by the non-N-methyl-D-aspartate (NMDA) receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), but it was abolished by the sodium channel blocker tetrodotoxin. The second potential was blocked by CNQX. Therefore, the first potential represents the antidromic activation of mitral cells and the second potential reflects monosynaptic activation of granule cell dendrites via non-NMDA receptors. We measured the maximal initial slope of this second field potential to monitor the strength of glutamatergic transmission from mitral to granule cells.

In the hippocampus, high frequency stimulation at 100 Hz effectively induces LTP of synaptic transmission. However, such high frequency stimulation of mitral cell axons was not effective in triggering LTP at the mitral-to-granule cell synapse. By contrast, theta frequency stimulation effectively induced LTP. The stimulation consisted of a 10 Hz, 20 pulse train applied 5, 10 or 20 times at 3 min intervals. Five or 10 trains of theta frequency stimulation induced only short-term potentiation that decayed back to its control value, whereas 20 trains induced LTP that maintained potentiated for at least 3 h. Theta frequency stimulation-induced LTP was blocked by bath application of the NMDA receptor antagonist D-2-amino-5-phosphonopentanoic acid (AP5), indicating that this form of LTP depends on NMDA receptor activation.

NA gates the induction of LTP via the activation of α²-adrenergic receptors

If LTP at the mitral-to-granule cell synapse underlies pheromonal learning, then NA should gate the induction of LTP. We tested this possibility by pairing NA with theta frequency stimulation that is subthreshold for LTP induction. Ten trains of theta frequency stimulation were not sufficient to induce LTP. However, when the 10 trains was paired with NA application, robust LTP was induced. These results indicate that NA can gate the induction of LTP. NA-enhanced LTP was also blocked by the NMDA receptor antagonist AP5.

We determined which type of adrenergic receptors was responsible for NA-induced LTP. NA-induced LTP was blocked by the α-adrenergic receptor antagonist phenotamine, but not by the β-adrenergic receptor antagonist propranolol. Furthermore, NA-induced LTP was blocked by the α²-adrenergic receptor antagonist idazoxan, but not by the α₁-adrenergic receptor antagonist prazosin.
clearly demonstrating that NA enhances LTP induction via $\alpha$-receptors of the $\alpha_2$-type. This is substantiated by the fact that the $\alpha_2$-adrenergic agonist clonidine mimics the effect of NA on LTP induction.

**NA suppresses glutamate release from mitral cell dendrites**

How does NA gate LTP induction at the mitral-to-granule cell synapse? We addressed this question using whole-cell patch-clamp techniques. First, we examined the effect of NA on stimulus-evoked excitatory postsynaptic currents (EPSCs) recorded from a granule cell. QX-314 was added to the pipette solution to block regenerative action potentials when subjected to a 10 Hz train of stimuli.

Conversely, the postsynaptic granule cell reliably fired action potentials during theta frequency stimulation, thereby enhancing the induction of LTP at the mitral-to-granule cell synapse.

Our studies indicate that LTP in the AOB shares essential features with pheromonal memory consolidation and therefore pheromonal memory might be understood at the cellular level through studies of LTP.

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**References**


