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

Hideto Kaba[1,2] and Guang-Zhe Huang[1]

[1]Department of Integrative Physiology, Kochi Medical School, Nankoku, Kochi 783-8505, Japan and [2]Division of Adaptation Development, Department of Developmental Physiology, National Institute for Physiological Sciences, Okazaki 444-8585, Japan

Correspondence to be sent to: Hideto Kaba, e-mail address: kabah@med.kochi-u.ac.jp

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 vaginocervical 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 DL-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 phentolamine, 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 α-receptors of the α₂-type. This is substantiated by the fact that the α₂-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 sodium currents. EPSCs were elicited by stimulation of the LOT every 30 s. NA suppressed evoked EPSCs and this effect was mimicked by the α₂-adrenergic agonist clonidine.

Secondly, we addressed the question of whether NA acts on the pre- or postsynaptic neuron by recording miniature EPSCs (mEPSCs) from a granule cell in the presence of tetorodotoxin and picrotoxin. Picrotoxin was added to block GABAergic transmission from granule to mitral cells. NA reduced the frequency of mEPSCs, while the amplitudes of mEPSCs were little affected. This conclusion was supported by the cumulative probability distributions of amplitudes and inter-event intervals. On average, the mean frequency decreased during NA application to 50% of the control frequency, whereas the mean amplitude was not significantly affected. The effect of NA on mEPSCs was reproduced by the α₂-adrenergic agonist clonidine. Taken together, our results demonstrate that NA suppresses glutamatergic transmission from mitral to granule cells by a presynaptic mechanism downstream from calcium entry.

Thirdly, we examined the effect of NA on calcium currents in mitral cells, because calcium entry through high-voltage-activated channels is essential for transmitter release. Calcium currents were isolated by blocking sodium currents with tetorodotoxin and potassium currents by including CsCl in the patch pipette. External calcium was replaced with Ba to increase the amplitude of the current. NA suppressed high-voltage-activated calcium currents and the effect of NA was mimicked by the α₂-adrenergic agonist clonidine.

NA allows the postsynaptic granule cell to fire more action potentials during theta frequency stimulation

So far, we have looked at the effect of NA on low frequency stimulation-evoked EPSCs and spontaneous EPSCs, but the frequencies of discharge of the mitral-to-granule cell synapse during pheromonal learning may be much higher. Therefore, we finally tested the effect of NA on a postsynaptic granule cell maintained in the current clamp mode during theta frequency stimulation of the LOT. NA reduced the depolarizing plateau due to its suppressive action on EPSCs. Conversely, the postsynaptic granule cell reliably fired action potentials when subjected to a 10 Hz train of stimuli.

In summary, NA suppresses glutamate release via the presynaptic activation of α₂-adrenergic receptors. This action of NA allows the postsynaptic granule cell to fire more 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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