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Research Report

Heterogeneous electrophysiological and morphological properties of neurons in the mouse medial amygdala in vitro

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ABSTRACT

Neurons in the medial nucleus of the amygdala (MeA) play a key role in the innate maternal, reproductive, defensive, and social behaviors. However, it is unclear how activation of the vomeronasal system leads to the behavioral outputs that are associated with pheromones. Here, we characterized the electrophysiological and morphological properties of MeA neurons using whole-cell recordings in mice slice preparations. Biocytin labeling revealed that MeA neurons possessed bipolar to multipolar cell bodies and dendritic fields covering projection areas from the accessory olfactory bulb. In 70% of recorded MeA neurons, monosynaptic excitatory postsynaptic currents (EPSCs) were evoked from the accessory olfactory bulb afferent in which the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate component was dominant and was rarely followed by the *N*-methyl-D-aspartic acid component. Norepinephrine increased the frequency of spontaneous inhibitory postsynaptic currents in some neurons, whereas α-methyl-5-hydroxytryptamine increased spontaneous EPSCs in other neurons. Morphologically and physiologically, heterogeneous MeA neurons appear likely to produce multiplex outputs of instinctive behaviors.

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1. Introduction

The medial nucleus of the amygdala (MeA) is a functionally distinct structure in the amygdaloid complex and plays a key role in the innate maternal, reproductive, defensive, and social behaviors (Canteras, 2002; Meredith and Westberry, 2004; Maras and Petrulis, 2010). MeA is also called the "vomeronasal amygdala," and it receives pheromone cues via the accessory olfactory bulb (AOB) and primary olfactory inputs via the cortical nucleus of the amygdala (Winans and Scalia, 1970; Kevetter and Winans, 1981; Kang et al., 2009). Axons of mitral-tufted cells in AOB project to MeA through the lateral olfactory tract (LOT), with tertiary connections to nuclei in the thalamus, hypothalamus, and other limbic systems (Meredith, 1998; Canteras, 2002; Choi et al., 2005; Ma and Morilak, 2005; Yoon et al., 2005., Touhara and Vosshall, 2009). The AOB afferents terminate at the molecular layer of the amygdaloid cortex. Vomeronasal information passes via AOB to the vomeronasal amygdala (Meredith and Westberry, 2004). Primary olfactory information passes via the main olfactory bulb to the piriform cortex and olfactory amygdala, principally the anterior cortical amygdala, but with onward connections to the medial amygdala (Meredith, 1998; Swanson and Petrovich, 1998; Sheehan et al., 2001; Touhara and Vosshall, 2009).

Chemosignals related to the social status (pheromones) carry messages between opposite- and same-sex individuals in many species. Each individual must distinguish signals relevant to its own social behavior with conspecifics from signals used by other (heterospecific) species that are relevant to their social behavior (Karlsson and Luscher (1959); Brennan and Zufall, 2006). The first evidence for an important role of the amygdala is the discrimination of species specificity in chemosignals. Meredith and Westberry (2004) demonstrated that MeA in male hamsters responds differently to conspecific and heterospecific chemosensory stimulation. The posterior medial amygdala (MeP) is unresponsive to heterospecific stimuli and appears to be suppressed, possibly by the intercalated nucleus, which was activated by all stimuli that suppressed MeP. The main olfactory system has connections with MeA but is unnecessary for the categorization of responses in MeA. The vomeronasal system was activated by both conspecific and heterospecific chemosensory stimulation, but the AOB responses did not categorically distinguish these types of stimulation. It is believed that amygdala responses reflect a higher level of analysis from which perhaps social relevance is extracted (Samuelsen and Meredith, 2009). Most MeA neurons exhibited either tonic bursting or adapting bursting of action potentials in response to depolarizing current injections (Pitkanen et al., 1997).

How does activation of the vomeronasal system lead to behavioral outputs that are associated with pheromones? One of the major outputs of the vomeronasal system is to the medial hypothalamus (Bian et al., 2008). Projections to this area are especially prominent, and they selectively innervate parts of the three systems that control the expression of partly innate reproductive, defensive, and ingestive behaviors (Del Punta et al., 2002; Boehm et al., 2005; Yoon et al., 2005). MeA also projects to the brainstem directly, not through the cerebral cortex, i.e., pheromones trigger innate behaviors below the level of consciousness (subliminal).

To understand the integrative mechanisms in MeA, it is necessary to characterize the intrinsic properties of its constituent elements as well as cellular interactions within MeA. Here, we characterized the electrophysiological and morphological properties of MeA principal neurons in the mouse.

Activation of the amygdala norepinephrinergic (Braga et al., 2004), dopaminergic (Floresco and Tse, 2007), and serotonergic systems (Parks et al., 1998; Mo et al., 2008) during emotional behaviors plays an important integrative function in coping by modulating synaptic potentials. However, it is not known whether and to what extent activation of the ascending norepinephrinergic innervation of MeA might modulate pheromone-induced hormone secretion.

2. Results

2.1. *Heterogeneous population of MeA neurons*

A total of 266 MeA principal neurons were recorded. These cells had a resting membrane potential of less than −50 mV. MeA neurons were clearly identified as a heterogeneous class of cells. Three representative examples of the extremes and middle of this continuum are shown in Fig. 1. The neurons were classified into three types in response to a 480-ms, 280-pA depolarizing current injection—regular spiking neurons (Type I), adapting neurons (Type II), and fully accommodating neurons (Type III). Regular spiking neurons (150/266; 56%) discharged spikes at a high frequency at the start of current injection with no delay but fired, repetitively, throughout the depolarizing current step with little or no spike frequency adaptation. Adapting neurons (84/266; 32%) fired several spikes at a high frequency at the start of current injection and exhibited complete spike frequency adaptation. Finally, fully accommodating neurons (32/266; 12%) fired at most 1–3 action potentials in response to increasing amplitudes of current injection.

2.2. *Morphological characteristics of MeA neurons*

The morphology of intracellularly labeled MeA neurons was visualized by the biocytin immunostaining method in Fig. 2A. Cell bodies were measured from 10 to 30 μm along their axes. At first glance, biocytin-stained MeA neurons were categorized into two classes on the basis of the shape of their dendrites—bipolar and multipolar. Typical biocytin-stained MeA neurons are illustrated by cell type in Fig. 2B. Almost all neurons projected at least two dendrites. Dendrograms representing the branching pattern for primary dendrites of each cell are shown in Fig. 2C. Examination of all cells and their dendrograms revealed no obvious qualitative morphological differences among Type I, Type II, and Type III neurons.

Another morphological feature characteristic of MeA neurons was that, to a denser or sparser extent, almost all cells in MeA had spines (Fig. 2, top inset).