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Research Report

Distribution of NMDA and AMPA receptor subunits at thalamo-amygdaloid dendritic spines

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ABSTRACT

Synapses onto dendritic spines in the lateral amygdala formed by afferents from the auditory thalamus represent a site of plasticity in Pavlovian fear conditioning. Previous work has demonstrated that thalamic afferents synapse onto LA spines expressing glutamate receptor (GluR) subunits, but the GluR subunit distribution at the synapse and within the cytoplasm has not been characterized. Therefore, we performed a quantitative analysis for α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor subunits GluR2 and GluR3 and *N*-methyl-D-aspartate (NMDA) receptor subunits NR1 and NR2B by combining anterograde labeling of thalamo-amygdaloid afferents with postembedding immunoelectron microscopy for the GluRs in adult rats. A high percentage of thalamo-amygdaloid spines was immunoreactive for GluR2 (80%), GluR3 (83%), and NR1 (83%), while a smaller proportion of spines expressed NR2B (59%). To compare across the various subunits, the cytoplasmic to synaptic ratios of GluRs were measured within thalamo-amygdaloid spines. Analyses revealed that the cytoplasmic pool of GluR2 receptors was twice as large compared to the GluR3, NR1, and NR2B subunits. Our data also show that in the adult brain, the NR2B subunit is expressed in the majority of in thalamo-amygdaloid spines and that within these spines, the various GluRs are differentially distributed between synaptic and non-synaptic sites. The prevalence of the NR2B subunit in thalamo-amygdaloid spines provides morphological evidence supporting its role in the fear conditioning circuit while the differential distribution of the GluR subtypes may reflect distinct roles for their involvement in this circuitry and synaptic plasticity.

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Abbreviations: B, basal nucleus of the amygdala; Ce, central nucleus of the amygdala; ec, external capsule; LA, lateral nucleus of the amygdala; MGd, medial geniculate, dorsal division; MGm, medial geniculate, medial division; MGv, medial geniculate, ventral division; PIN, posterior intralaminar nucleus; SG, supragenicular body

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1. Introduction

In recent years, considerable progress has been made in elucidating the neural circuits that underlie Pavlovian fear conditioning, a behavioral task in which a neutral conditioned stimulus (CS), often an auditory tone, acquires the ability to elicit fear responses following its association with an aversive unconditioned stimulus (US), typically footshock. Evidence from different kinds of studies points to the lateral amygdala (LA) as an important site of plasticity in auditory fear conditioning (LeDoux, 2000; Maren, 2000; Sah et al., 2003; Tsvetkov et al., 2004). The LA is the primary recipient of convergent inputs from auditory thalamic and neocortical areas that process the CS. Lesions of the LA, or pharmacological disruption of neural activity in this region, prevent fear conditioning (LeDoux et al., 1990; Nader et al., 2001; Wilensky et al., 1999, 2000; Bailey et al., 1999). Neurons in LA are responsive to both CS and US (Romanski et al., 1993) and their responses to the CS changes after CS–US pairing (Quirk et al., 1995; Rogan et al., 1997; McKernan and Shinnick-Gallagher, 1997; Repa et al., 2001; Pare and Collins, 2000; Goosens and Maren, 2002). Moreover, induction of long-term potentiation by electrical stimulation of the auditory thalamus enhances CS-evoked responses in LA (Rogan and LeDoux, 1995). Together, these findings suggest that thalamo-amygdaloid synapses are facilitated in response to Pavlovian fear conditioning (LeDoux, 2000; Maren, 2001).

Auditory thalamic inputs into LA exert their effects through the postsynaptic glutamate *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors (Li et al., 1995; Farb and LeDoux, 1997; Humeau et al., 2003). Blockade of NMDA receptors in LA prevents fear conditioning (Miserendino et al., 1990; Walker and Davis, 2000; Rodrigues et al., 2001), while facilitation of AMPA receptors, and in turn NMDA receptors, enhances fear conditioning (Rogan et al., 1997).

Increasing evidence suggests that NMDA and AMPA receptors are capable of modulating synaptic plasticity through shifts in their receptor subunit stoichiometry (Shi et al., 2001; Rumpel et al., 2005; Barria and Malinow, 2005). While some information has been gleaned regarding the glutamate receptor subunit composition in identified auditory pathways to LA (Farb and LeDoux, 1997, 1999; Rodrigues et al., 2001; Mead et al., 2006), the subcellular organization of glutamate receptors at thalamo-amygdaloid synapses is not known. Therefore, the aim of the present study was to characterize and quantify the synaptic and non-synaptic distribution of the GluR2, GluR3, NR1, and NR2B glutamate receptor (GluR) subunits within dendritic spines that receive synapses from the auditory thalamus. Since receptor trafficking is a candidate mechanism underlying memory formation (Shi et al., 2001; Baudry et al., 1980), differences between the cytoplasmic and synaptic distribution of these GluR subtypes may reflect distinct roles in synaptic plasticity. Thalamic afferents to the LA were identified by anterograde tracer injection of biotinylated dextran amine (BDA) into the auditory thalamus (medial subdivision of the medial geniculate nucleus and posterior intralaminar nucleus, MGm/PIN) and the GluRs were visualized using postembedding immunogold.

2. Results

2.1. *Thalamic inputs to LA*

Injections of BDA into MGm/PIN produced labeling of cell bodies, axons, and processes at the injection site and in adjacent locally projecting regions (Figs. 1A, B). A dense plexus of anterogradely labeled BDA fibers was evident throughout the LA, particularly within the dorsal LA, and in the striatum and amygdala-striatum transition areas, regions immediately dorsal and medial to the amygdaloid complex. (Fig. 1C). Higher magnification (Fig. 1D) revealed the presence of many labeled fibers and boutons. No labeled cell bodies or dendrites in the LA were observed. Electron microscopic verification of BDA-labeling was performed on ultrathin sections prior to the immunogold analysis. Thalamo-amygdaloid-labeled terminals were comprised of loosely packed clear, round vesicles, and sometimes contained dense-core vesicles. Some BDA-

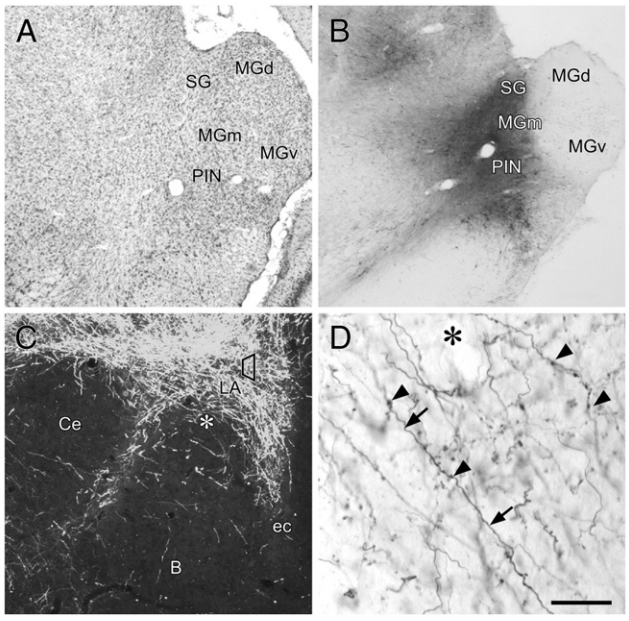

Fig. 1 – Low-power photomicrographs showing BDA injections in the auditory thalamus and corresponding anterograde transport to the amygdala. (A) Nissl-stained coronal section, approximately 6.0 mm caudal to bregma, containing the subregions of the auditory thalamus. (B) Representative BDA injection site into the MGm/PIN. Local anterograde and retrograde transport in nearby regions is also shown. (C) Dark field photomicrograph of anterograde transport to LA following a BDA injection in MGm/PIN. The trapezoid in the LA represents the approximate region sampled for electron microscopic examination. The asterisk corresponds to the region of higher magnification shown in panel D. (D) High-power magnification using Nomarski optics reveals the presence of BDA-labeled axons (arrows) and boutons (arrowheads) in the LA. Cell bodies and dendrites are not seen in the LA. Asterisk denotes region shown at lower power in panel C. Scale bar=500 μm in panels A and B, 100 μm in panel C, and 25 μm in panel D.