Two Intra-Amygdaloid Pathways to the Central Amygdala Exhibit Different Mechanisms of Long-Term Potentiation

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Fu, Yu and Patricia Shinnick-Gallagher. Two intra-amygdaloid pathways to the central amygdala exhibit different mechanisms of long-term potentiation. J Neurophysiol 93: 3012–3015, 2005. First published December 22, 2004; doi:10.1152/jn.00871.2004. Synaptic plasticity in the amygdala is thought to underlie aversive or rewarding learning and emotional memories. In this study, different mechanisms were found to underlie synaptic plasticity in lateral (LA) and basolateral (BLA) amygdala pathways to the primary output nucleus of the amygdala, the central amygdala (CeA). Specifically, 1) long-term potentiation (LTP) at the BLA-CeA synapses was independent of inhibition and mediated through N-methyl-D-aspartate receptors (NMDARs) and L-type voltage-gated calcium channels (VGCCs), and 2) LTP in the LA-CeA pathway was gated by inhibition and mediated through VGCCs but not NMDARs.

INTRODUCTION

The amygdala plays a role in learning and memory, especially related to cues for fear and addictive drug-taking (See et al. 2003). Long-term potentiation (LTP) is a putative cellular mechanism for aversive learning (LeDoux 2000). Repetitive stimulation of cortical and thalamic afferents, important in associative processing of emotional information (LeDoux 2000), induces long-term plasticity in the LA in vivo (Doyere et al. 2003) or in vitro (Chapman et al. 2003). The cortical afferents, which carry contextual, visual, or auditory information, also generate LTP in the basolateral amygdala (BLA) (Aroniadou-Anderjaska et al. 2001; Chapman et al. 2003). Little is known about LTP in another crucial part of the amygdala complex, the central amygdala (CeA), which is downstream from the sites of associativity in the LA and BLA and serves as a primary output nucleus for information processing (Davis 1986; LeDoux 2000).

The BLA and lateral amygdala (LA) project primarily to the capsular (CeAc) division of the rat CeA (Pitkanen 2000; Pitkanen et al. 1995; Savander and Et al. 1997), where they activate AMPA and N-methyl-D-aspartate (NMDA) receptors (Lopez de Armentia and Sah 2001; Sah et al. 2003). Inhibitory synaptic responses mediated through GABA_A are also recorded in the CeA (Delaney and Sah 2001), and the majority of projection neurons are GABAergic (Swanson and Petrovich 1998). The two afferent pathways are thought to process different information, the LA-CeA, tone conditioned stimuli, and the BLA-CeA, contextual stimuli (LeDoux 2000). Other evidence suggests that the CeA itself may support fear learning (Killacress et al. 1997). A recent hypothesis posits that the LA input to the medial CeA via intercalated neurons is the main circuitry implicated in fear learning (Pare et al. 2004). The rat CeA is comprised of two primary cell types (Sah et al. 2003; Schiess et al. 1993), but there is no apparent correlation between electrophysiological and morphological characteristics (Sah et al. 2003). The purpose of this study was to compare synaptic plasticity in two pathways to the CeA, the BLA to CeA, and the LA to CeA.

METHODS

Male Sprague-Dawley (Harlan) albino rats (90–180 g) were rapidly decapitated, and slices were prepared in ice-cold (0–6°C) aerated solution (95% O_2-5% CO_2) of the following composition (in mM): 119 NaCl, 3.0 KCl, 1.2 NaHPO_4, 1.2 MgSO_4, 2.5 CaCl_2, 25 NaHCO_3, and 11.5 glucose. Coronal slices (500 μm) were kept at room temperature for 1 h. The slice was subsequently submerged in a tissue bath and incubated at 30 ± 1°C for another hour before recording. Stimulating LA or BLA (cell bodies including some fibers of passage) with concentric tungsten electrodes (150 μs, 7–21 V, 0.05 Hz) evoked field excitatory postsynaptic potentials (fEPSPs; Fig. 1, A and C) recorded in the CeAc with glass Ag/AgCl microelectrodes filled with 2 M NaCl (4–5 MΩ). BLA/CeAc slices were more rostral (2.5–3.0 mm from Bregma) than LA/CeAc slices (3.0–3.5 mm) to reduce current spread from adjacent nuclei. Recording and stimulating electrode locations were carefully noted in each experiment. For LTP, fEPSP amplitudes (adjusted to 30% of maximal response) were recorded for 30 min (0.05 Hz) prior to high-frequency stimulation (HFS, 5 trains, 1-s duration at 100 Hz, 3-min intervals). Subsequent fEPSPs (0.05 Hz) were recorded for 1 h. Six consecutive traces were averaged, and the fEPSP slope was measured and plotted versus time; data were normalized to baseline responses analyzed with a two-tailed t-test or one-way ANOVA.

RESULTS

GABAergic transmission influenced LTP in the LA more than in the BLA pathway. Although single LA stimuli generated robust fEPSPs, HFS of the LA failed to generate LTP in the CeAc (100.1 ± 14.2% of baseline, P > 0.05, Fig. 1B, n = 9). Rather, HFS evoked a short-term potentiation (STP) that endured no more than 8–10 min and increasing the stimulus strength did not result in LTP. In contrast, immediately after HFS was applied to the BLA, the fEPSP amplitude and slope were enhanced, and remained elevated (Fig. 1D) within a range of 120–150% of control values (139.3 ± 14.3% of baseline, P < 0.01, n = 10). In 16% of the cases (2/12), the fEPSP

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increase was transient and returned to baseline within 10 min (data not shown). Picrotoxin (PTX, 10 μM), a GABA_A channel blocker, was tested since CeA inputs may be affected by a powerful inhibitory influence mediated by GABA_A receptors (Pare et al. 2003). In PTX, HFS of the LA consistently generated LTP (150.7 ± 34.9% of baseline, P < 0.01, Fig. 1B, n = 12). In contrast, PTX had no significant effect on LTP in the BLA to CeAc pathway (PTX: 149.1 ± 18.7%; control: 139.3 ± 14.3%, Fig. 1D, n = 10) but consistently induced BLA-CeA LTP in all slices tested. PTX also had no significant effect on fEPSP amplitude (control: 0.28 ± 0.02 mV; PTX: 0.32 ± 0.05 mV), duration (control: 11.9 ± 0.5 ms; PTX: 12.6 ± 1.1 ms), and latency (control: 2.4 ± 0.2 ms; PTX: 1.6 ± 0.5 ms).

LTP pharmacology also differed in the LA and BLA to CeAc pathways (Fig. 2A). In the presence of the NMDA receptor antagonist, n-aminophosphonovaleric acid (APV, 50 μM), LTP could still be induced in the LA-CeAc pathway (before: 150.7 ± 34.9%, n = 12; after: 137.1 ± 12.2%, n = 6, P > 0.05, Fig. 2A); however, LTP was abolished in the CeAc when stimulating the BLA (109.8 ± 6.1% of baseline, n = 6, Fig. 2B).

Further experiments examined the contribution of L-type voltage-gated calcium channels (VGCCs) to LTP in the two pathways. In PTX (150.7 ± 34.9%, 10 μM), the L-type VGCC, nimodipine (10 μM), reduced LTP in LA-CeAc pathway to 120.1 ± 29.6% (n = 5, Fig. 2C), suggesting a requirement for L-type VGCC in the induction of LTP in this pathway. Addition of nimodipine (10 μM) to the PTX solution completely blocked the HFS-induced LTP (PTX: 149.1 ± 18.7%; PTX/NIM: 104.4 ± 8.7%, n = 5) in the BLA-CeAc pathway. When both APV (50 μM) and nimodipine (10 μM) were added to the PTX solution, LTP induction was completely blocked in LA (n = 8)- and BLA (n = 3)-CeAc pathways (Fig. 3A).

Subsequent experiments compared paired-pulse ratios (PPRs) in the two pathways as a monitor of presynaptic release mechanisms (Fig. 3B). In this paradigm, the first response, fEPSP1, and the second, fEPSP2, were elicited in LA/BLA afferents at intervals of 35–200 ms. The ratios were expressed as the slopes of fEPSP2/fEPSP1. In both pathways paired pulse facilitation was recorded at all interstimulus intervals. After HFS, the PPRs decreased, suggesting presynaptic mechanisms may contribute to LTP induction in both pathways. At the 50-ms interstimulus interval, PPRs were 1.40 ± 0.05 and 1.45 ± 0.08 (n = 8) before and 1.17 ± 0.04 and 1.18 ± 0.07 (n = 8, P < 0.05) after HFS in the LA-CeAc and BLA-CeAc pathways, respectively.
DISCUSSION

The principal finding of this study was that different mechanisms underlie the induction of LTP in pathways from the LA and BLA to the CeA, specifically 1) LTP at the BLA to CeAc synapse was independent of inhibition and mediated through NMDARs and L-type VGCCs, and 2) LTP in the LA to CeAc pathway was gated by GABA and was mediated primarily through L-type VGCCs.

Inhibition in the LA-CeA pathway may reduce or restrain activity-dependent facilitation since LTP was induced only in its absence. Interposed between the LA and BLA and the CeA is a ribbon of intercalated cell masses (Millhouse 1986), which may serve as an inhibitory gate (Pare et al. 2003; Royer et al. 1999). Additionally, axon collaterals of GABAergic neurons within the CeA itself may generate feedback inhibition that could result in inhibition of the fEPSP (but see Pare et al. 2003; Royer et al. 1999). In contrast, in the BLA-CeAc pathway, inhibiting GABA transmission had little overall effect on LTP induction (Figs. 1D and 3A). This difference in PTX effect may be related to topography within the amygdala and the physiology of the intervening intercalated cells (Pare et al. 2003; Royer et al. 1999). The intercalated cells have lateromedial correspondence between their position and input to the CeA and the afferent source within the LA/BLA complex (Pare et al. 2003; Royer et al. 1999). The more lateral intercalated cell clusters inhibit more medial clusters, which results in disinhibition (Royer et al. 1999) and would allow LTP induction in the BLA pathway. Disinhibition is less likely with LA stimulation, which would engage more laterally positioned intercalated cell masses and result in an inhibitory brake on afferent information. Intercalated neurons also possess a potassium current activated at subthreshold membrane potentials that is inactivated in response to suprathreshold depolarization, after which inactivation is slowly removed; the resultant effect is a self-sustaining state of heightened excitability after suprathreshold activity (Royer et al. 2000). This current would sustain inhib-

FIG. 2. N-methyl-D-aspartate receptor (NMDAR) antagonists significantly blocked induction of LTP in the BLA-CeAc (B) but not the LA-CeAc (A) pathway, whereas LTP in both pathways was reduced by L-type voltage-gated calcium channel (VGCC) block. A and B: LTP time course in the PTX is plotted in the absence (○, same as in Fig. 1) and presence of APV (50 μM, ●) in both pathways. C and D: LTP in PTX (○, same as Fig. 1) is reduced in both pathways after addition of nimodipine (NIM, 10 μM, ●).

FIG. 3. Pharmacology of LTP but not paired-pulse ratio (PPR) was different in both pathways. A: LTP recorded 30 min after terminating HFS is expressed as a percentage of baseline fEPSP slope after drug treatments. B: PPRs were similarly reduced after HFS in LA and BLA to CeAc pathways. *: Significant difference (P < 0.05), 2-tailed t-test.
itory activity from the intercalated cells in the LA-CeA pathway and exert a persistent disinhibition of the medial intercalated neurons by the more lateral intercalated cells when the BLA pathway is activated, thus providing a physiological basis for different mechanisms underlying activity-dependent plasticity in the LA and BLA pathways to the CeA. Additionally, the monosynaptic connections between the LA and BLA to the CeA suggest that these direct pathways may play a role in fear conditioning independent of the intercalated GABA-releasing cells (Pare et al. 2004).

In the absence of GABAergic inhibition, the mechanisms underlying LTP induction were pathway specific. At the LA-CeA synapse, LTP is independent of NMDARs and dependent on VGCCs. In contrast, LTP of BLA-CeAc pathway was dependent on NMDARs and L-type VGCCs. NMDAR- and VGCC-dependent LTP can be induced separately (Bauer et al. 2002; Morgan and Teyle 2001), depending on the induction protocol (Bauer et al. 2002; Weisskopf et al. 1999). At other amygdala synapses, LTP induction involves postsynaptic VGCCs (Weisskopf et al. 1999) or pre- or postsynaptic NMDARs (Humeau et al. 2003). In the BLA-CeA pathway, complete block of LTP by either NMDARs or VGCCs antagonists, however, suggests specific roles in the induction process involving interrelated mechanisms. For LTP induction, presynaptic and postsynaptic mechanisms at BLA-CeA synapses that require VGCC at one and NMDARs at the other synaptic site may be required. Alternatively, postsynaptic activation of both NMDARs and VGCCs may be necessary to achieve intracellular Ca\(^{2+}\) levels required for induction of BLA-CeA LTP. Conversely, in both pathways, LTP was associated with a decrease in PPRs, suggesting enhanced transmitter release plays a role in the expression of CeA LTP. Finally, this modulation of associative information by subsequent downstream CeA synapses may represent pathway specific integrative properties of the amygdala and provide distinct processing of tone or contextual information in cues related to anxiety and drug-taking.

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References


