NMDA-dependent facilitation of corticostriatal plasticity by the amygdala

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Emotions generally improve memory, and the basolateral amygdala (BLA) is believed to mediate this effect. After emotional arousal, BLA neurons increase their firing rate, facilitating memory consolidation in BLA targets. The enhancing effects of BLA activity extend to various types of memories, including motor learning, which is thought to involve activity-dependent plasticity at corticostriatal synapses. However, the underlying mechanisms are unknown. Here we show that the NMDA-to-AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) ratio is nearly twice as high at BLA as compared with cortical synapses onto principal striatal neurons and that activation of BLA inputs greatly facilitates long-term potentiation induction at corticostriatal synapses. This facilitation was NMDA-dependent, but it occurred even when BLA and cortical stimuli were 0.5 s apart during long-term potentiation induction. Overall, these results suggest that BLA activity opens long time windows during which the induction of corticostriatal plasticity is facilitated.

basolateral | emotion | memory | synaptic plasticity

Memory formation is influenced by stress and emotions (1). However, this effect depends on the nature, timing, and intensity of the experience. For instance, stress can impair recall via the activation of glucocorticoid receptors (2, 3). In contrast, moderate amounts of stress delivered right after training can enhance memory tested days later. However, there is an inverted-U relationship between the levels of stress hormones and retention (4, 5).

Generally, it is accepted that the basolateral amygdala (BLA) is responsible for the facilitation of memory consolidation produced by moderate emotional arousal (6). After an emotionally arousing event, the firing rate of BLA neurons increases for several hours (7). Reducing BLA activity during this period decreases memory for events that took place <2 h before, in a variety of learning paradigms (8–11). Conversely, drugs that enhance BLA activity enhance recall when injected within 2 h after training (12–15).

Although much evidence indicates that the amygdala is a critical site of plasticity for the acquisition of classically conditioned fear responses (16), the effects of the manipulations mentioned above seem to result from alterations of memory storage in other neuronal structures (8). For instance, it was shown that immediate postlearning injection of amphetamines in the BLA increases hippocampal-dependent storage of spatial information and striatal-dependent storage of response information. Yet, intraamygdala injections of lidocaine just before testing retention days later had no effect on either task (17).

Collectively, these results suggest that, in emotionally arousing conditions, the amygdala can facilitate memory consolidation in other brain structures. However, the underlying mechanisms remain unknown. The BLA could facilitate activity-dependent synaptic plasticity in its various projection sites (18, 19). Consistent with this possibility, it was reported that electrical stimulation of the BLA facilitates the induction or stabilization of long-term potentiation (LTP) in the entorhino-hippocampal (20–23) and thalamocortical systems (24). However, the physiological substrates of the BLA facilitation of striatal-dependent memories have not been investigated so far.

Activity-dependent changes in the efficacy of corticostriatal synapses are thought to contribute to motor learning (25). Consistent with this finding, corticostriatal synapses exhibit various forms of short- and long-term plasticity. However, because of developmental changes and regional differences, stimulation paradigms that readily induce LTP in the cortex and hippocampus can evoke long-term depression, LTP, or no change in the corticostriatal pathway (26–32). Yet, manipulations that increase NMDA currents (e.g., removal of extracellular Mg^{2+} or postsynaptic depolarization) can facilitate LTP induction (26). Thus, the present study tested whether amygdalostriatal axons (33, 34) can facilitate plasticity at cortical inputs to projection cells of the striatum (35), the medium spiny neurons (MSNs). We show that BLA synapses can facilitate induction of corticostriatal LTP, in part because they have a high NMDA-to-AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) ratio.

Results

Database. Whole-cell recordings of MSNs ($n = 105$) were obtained under visual guidance in brain slices kept *in vitro*. MSNs were identified by their characteristic electrophysiological properties (36), including low input resistance (69.8 ± 3.1 MΩ), extremely negative resting potential (−90.0 ± 0.5 mV), and inward rectification in the hyperpolarizing direction (Fig. 1*A Inset*). In a subset of cells, we added neurobiotin to the intracellular solution for post hoc morphological identification of recorded cells. All recovered cells that had been classified as MSNs on the basis of these electrophysiological criteria ($n = 8$) had the typical morphology of principal striatal neurons (multiple primary dendrites that branch extensively and bear a high density of spines; Fig. 1*B*).

Effects of BLA Stimulation on Corticostriatal LTP. BLA and cortical axons converge in the ventral striatum; however, their functional interaction at this level is largely unknown (37). Thus, stimulating electrodes were inserted in the BLA as well as in the cortex at two different sites (Fig. 1*C1*), one of which served to control for input specificity in LTP experiments. BLA and cortical stimuli evoked synaptic responses in all tested MSNs (latency of 5.3 ± 0.1 and 4.3 ± 0.1 ms, respectively). To determine whether these responses were mediated by distinct sets of inputs, we used the occlusion test (Fig. 1*C2*). In this test, the actual and predicted

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Abbreviations: BLA, basolateral amygdala; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; LTP, long-term potentiation; MSN, medium spiny neuron; EPSC, excitatory postsynaptic current; AP5, 2-amino-5-phosphonopentanoic acid; aCSF, artificial cerebrospinal fluid; MK-801, dizocipline; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; AP, action potential.

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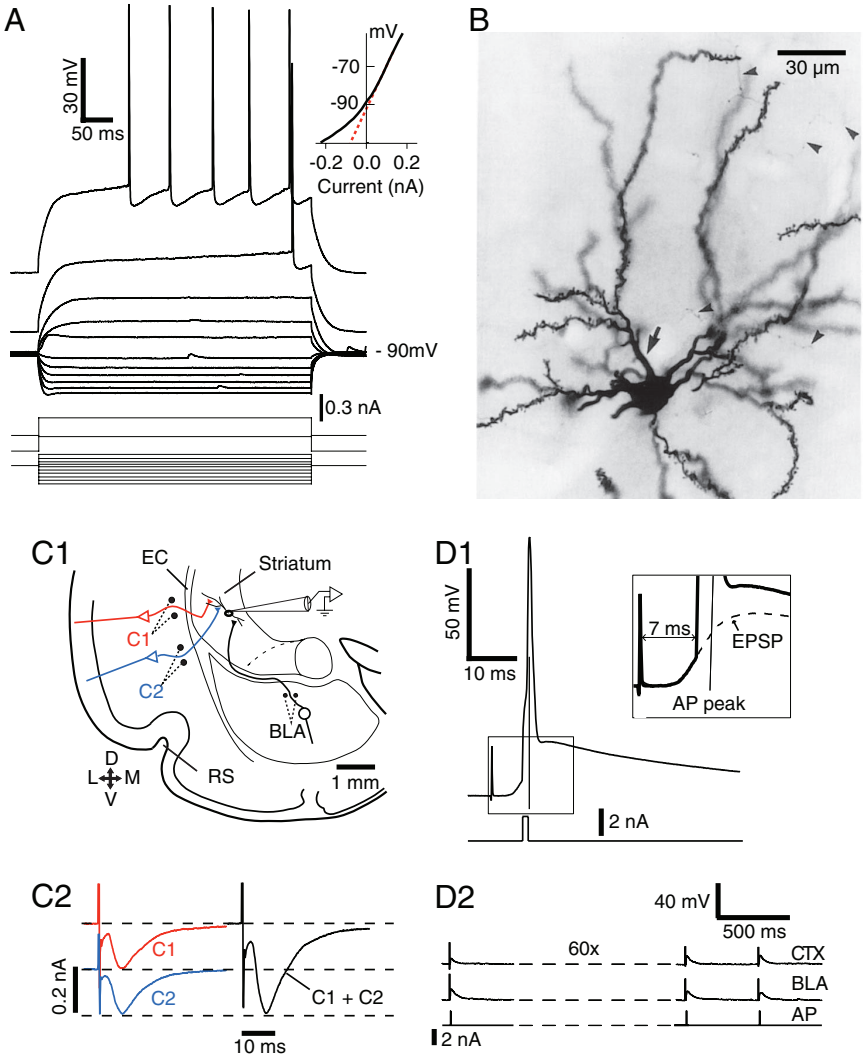

Fig. 1. Identification of MSNs and experimental approach. (*A*) Voltage responses (*Upper*) to current pulses of gradually increasing amplitudes (*Lower*). *Inset* at upper right shows current–voltage relationship. (*B*) Morphological properties of an MSN as revealed by intracellular injection of neurobiotin. (*C1*) Position of recording (pipette) and stimulation sites (dots) on a coronal section of the guinea pig brain. (*C2*) Occlusion test. EPSCs evoked by the two cortical stimulation sites are shown activated independently (*Left*) or simultaneously (*Right*). Amplitude of EPSCs evoked by the paired stimuli (*Right*) was virtually identical to the algebraic sum of EPSCs elicited by stimulating each site independently (*Left*, dashed lines). (*D1* and *D2*) Paradigm used to induce corticostriatal LTP. Spikes evoked by intracellular current injection (*D1*) were paired to synaptic responses evoked from cortical and/or BLA stimulation sites (*D2*). The timing of the stimuli was adjusted so that the spike occurred during the rising phase of the EPSPs (*D1 Inset*). CTX, cortical stimulus.

sums of excitatory postsynaptic currents (EPSCs) evoked by two stimuli are compared. When the response evoked by paired stimuli is smaller than the algebraic sum of individual responses, this finding constitutes evidence that the two stimulating electrodes activate a partially overlapping set of inputs. This test was carried out for a BLA and a cortical stimulation site ($n = 7$) as well as for the two cortical stimulation sites ($n = 8$; Fig. 1*C2*). The response evoked by the paired cortical stimuli (Fig. 1*C2 Right*) was 100.7 ± 1.4% of the summed EPSCs. When the same test was carried out with the BLA and a cortical stimulation site, the response to the paired stimuli was 96.6 ± 2.0% of the summed stimuli. In fact, the actual and predicted responses were not significantly different from each other in both cases (paired *t* tests, $P > 0.05$), which suggests that the various stimulating electrodes activated largely nonoverlapping sets of inputs converging onto MSNs.

To induce corticostriatal LTP, we used a spike-timing-dependent protocol (Fig. 1*D*) involving the repeated pairing of cortical stimuli to a current-evoked postsynaptic action potential (AP), with or without coincident BLA stimulation. These pairings were performed 60 times at 2 Hz, close to the spontaneous firing rate of principal BLA neurons (7, 38). LTP experiments began with a 10-min control epoch during which cortical and BLA stimuli (2 s apart) were activated at a low frequency (one shock per min at each site). It should be noted that stimulation intensity was adjusted to evoke control responses of ≈10 mV. Alterations in synaptic responsiveness were assessed by monitoring the first half of the rising slope of evoked EPSPs (≈3 ms from onset) and comparing the prepairing responses to those acquired 20 to 30 min after pairing.

After the pairing of cortical and BLA stimuli to postsynaptic spikes, we observed a long-lasting increase in the slope of cortically evoked EPSPs (160.9 ± 13.2%, $P = 0.0012$; $n = 11$; Fig. 2*A*), whereas BLA-evoked responses remained unchanged (115.1 ± 9.8%, $P = 0.097$; $n = 11$). Note that in this and subsequent experiments, all tested cells were included in the average. To determine whether this corticostriatal LTP was input-specific, we monitored the responses evoked from a sec-

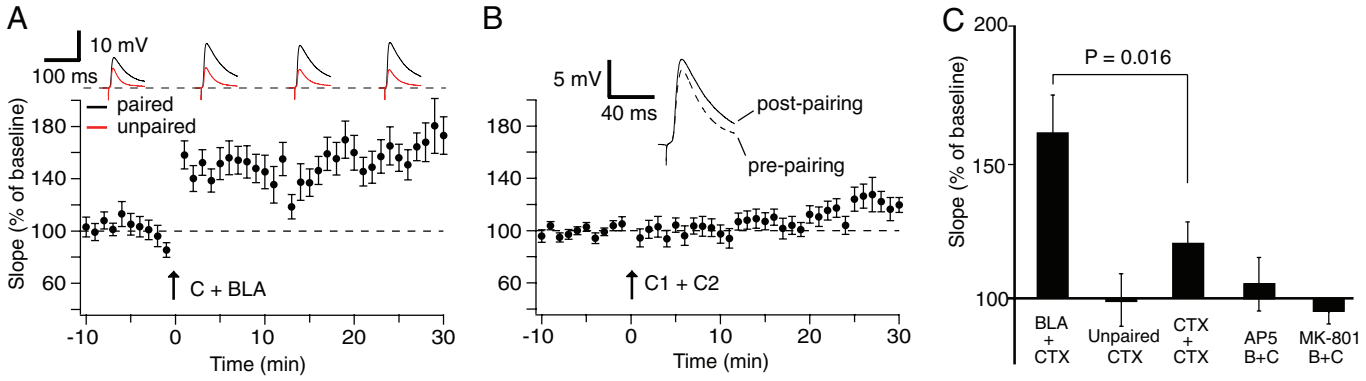

Fig. 2. BLA stimulation produces an NMDA-dependent facilitation of corticostriatal LTP. (*A Upper*) Pairing of BLA and cortical stimuli produces a marked potentiation of responses evoked by the paired cortical stimulus (black lines) but not of those evoked from an unpaired cortical stimulation site (red lines). (*A Lower*) Results are shown as normalized slope of cortically evoked EPSPs (*y* axis) as a function of time (*x* axis). An average of 11 experiments is shown. (*B*) Pairing of two cortical stimulation sites produces LTP but of significantly lower magnitude than that seen with BLA stimuli. (*B Upper*) Superimposed averages of 10 cortically evoked responses obtained before (dashed line) or 20–30 min after (solid line) LTP induction in one representative experiment (*B Lower*) Results are shown as normalized slope of cortically evoked EPSPs (*y* axis, $n = 16$) as a function of time (*x* axis). (*C*) Amount of corticostriatal LTP seen, from left to right, (*i*) with paired BLA–cortex stimuli ($n = 11$), (*ii*) at an unpaired cortical site ($n = 5$), (*iii*) with pairing of two different cortical sites ($n = 16$), (*iv*) with paired BLA–cortex stimuli in the presence of AP5 ($n = 5$), and (*v*) with paired BLA–cortex stimuli but with MK-801 in the pipette solution ($n = 5$). CTX, cortical stimulus.

ond cortical site that was not stimulated during the pairing protocol. No significant change was observed in the unpaired cortical responses (98.9 ± 9.5%, $P = 0.918$; $n = 5$; Fig. 2*A*, red lines), suggesting that only synapses activated during the pairing are potentiated.

To test whether our results were attributable to an increased level of depolarization resulting from the summation of BLA-evoked and cortically evoked responses, we replaced the BLA stimuli with a second, nonoverlapping cortical site that evoked responses of equal amplitude. Although the pairing of two cortical sites produced a significant response potentiation (119.9 ± 8.0%; $P = 0.023$; $n = 16$; Fig. 2*B*), it was significantly smaller than with BLA–cortex pairings ($P = 0.016$; Fig. 2*C*).

Moreover, the proportion of cells showing corticostriatal LTP was significantly different between the two groups (χ^2 test, $P = 0.04$). Indeed, with BLA–cortex pairings ($n = 11$), 91% of cells showed significant corticostriatal LTP, and 9% showed no change. In contrast, when we paired two cortical sites ($n = 16$), 37% showed no change, 44% showed LTP, and 19% showed long-term depression of corticostriatal inputs. These data suggest that indeed, BLA stimuli can facilitate and enhance plasticity at converging, coactive cortical inputs.

Contribution of NMDA Receptors. In many types of neurons, Ca^{2+} entry through NMDA receptors is a critical step for LTP induction (39). Consistent with this observation, we found that addition of the NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (AP5; 100 μM) to the artificial cerebrospinal fluid (aCSF) shortly before and during BLA–cortex pairings prevented LTP induction (114.6 ± 11.2%, $P = 0.27$; $n = 5$; Fig. 2*C*). In keeping with these results, when MSNs were dialyzed with the calcium chelator BAPTA [1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; 50 mM], no corticostriatal LTP was induced by BLA–cortex pairings ($n = 5$).

Because the NMDA receptors critical for LTP induction sometimes are located presynaptically (40, 41), we tested the effect of NMDA blockade by intracellular dialysis of MSNs with the NMDA antagonist dizocipline (MK-801, 1 mM). In support of the idea that the NMDA receptors required for LTP induction are located postsynaptically, no LTP was induced by BLA–cortex pairings when postsynaptic NMDA receptors were blocked by intracellular dialysis with MK-801 (95.5 ± 4.1%, $P = 0.2$; $n = 5$; Fig. 2*C*), resulting in a significant difference from control conditions (160.9 ± 13.2%, $P < 0.001$).

To test the possibility that the BLA synapses can facilitate corticostriatal LTP because of a higher contribution of NMDA receptors at BLA versus cortical inputs (19), we compared the NMDA-to-AMPA ratio at both inputs converging onto the same MSN ($n = 15$, Fig. 3*A*). To this end, slices were perfused with a Mg^{2+}-free aCSF to unmask the NMDA component, and responses were recorded in current clamp at rest, while consecutively adding to the aCSF 100 μM picrotoxin (to block $GABA_A$

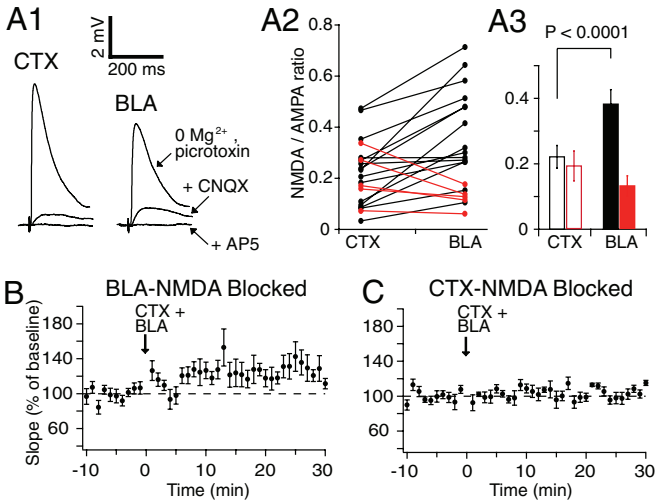

Fig. 3. Input-specific blockade of NMDA receptors with MK-801. (*A*) The NMDA-to-AMPA ratio is significantly higher at BLA than cortical inputs to MSNs. (*A1*) Test used to estimate the NMDA-to-AMPA ratio. In a Mg^{2+}-free aCSF at −90 mV, picrotoxin, CNQX, and AP5 were added sequentially to the perfusate while stimulating cortical (*Left*) or BLA (*Right*) inputs converging on the same MSN. A ratio of the pharmacologically isolated components then was computed. Red lines in *A2* and red bars in *A3* show the NMDA-to-AMPA ratio in experiments where MK-801 (5 μM) was used to selectively block NMDA receptors at BLA synapses. (*B* and *C*) Results are shown as normalized slope of cortically evoked responses (*y* axis) as a function of time (*x* axis) after input-specific blockade of NMDA receptors at BLA (*B*) or cortical (*C*) inputs. The graphs in *B* and *C* plot the average of six and four experiments, respectively. CTX, cortical stimulus.

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responses), 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (to block AMPA responses), and 100 μM AP5 (to ascertain that the residual response was mediated by NMDA receptors) (Fig. 3*A1*). As shown by the black lines in Fig. 3*A2*, we consistently found higher NMDA-to-AMPA ratios at BLA compared with cortical inputs ($n = 15$, $P < 0.0001$, paired t test). On average, the ratio was 0.38 ± 0.04 for BLA, compared with 0.22 ± 0.03 for cortical inputs (Fig. 3*A3*, black bars).

Having found a higher NMDA-to-AMPA ratio at BLA synapses, we investigated whether the NMDA receptors critical for corticostriatal LTP induction are located at BLA and/or cortical inputs. Therefore, we used a previously described protocol (42) that allows input-specific blockade of NMDA receptors by taking advantage of the fact that MK-801 is a noncompetitive, irreversible open-channel blocker. First, we tested whether we could selectively block NMDA receptors at BLA synapses by using this method. Thus, we stimulated the BLA site (15 min at 1 Hz) in the presence of 5 μM extracellular MK-801, followed by a stimulation-free 60-min washing period. At this stage, an MSN was patched and the NMDA-to-AMPA ratio was measured as above. This method produced a clear and selective reduction of the NMDA component at BLA inputs (Fig. 3*A2*, red lines). In fact, after the application of extracellular MK-801 during BLA stimulation, the NMDA-to-AMPA ratio at cortical inputs (Fig. 3*A3*, empty red bar; 0.19 ± 0.05, $n = 5$) was not significantly different ($P = 0.67$) from control (Fig. 3*A3*, empty black bar; 0.22 ± 0.03, $n = 15$). This finding was in contrast to the marked reduction ($P < 0.001$) observed at BLA inputs following the MK-801 protocol (Fig. 3*A3*, filled red bar; 0.13 ± 0.03, $n = 5$) compared with control (Fig. 3*A3*, filled black bar; 0.38 ± 0.04, $n = 15$).

Having established the selectivity of this method, we then tested whether NMDA block at BLA or cortical inputs interferes with LTP induction. After NMDA blockade at BLA synapses, BLA–cortical pairings produced a slight increase in the slope of cortically evoked responses (126.6 ± 14.5%, $P = 0.12$; $n = 6$; Fig. 3*B*) that was comparable to that seen with the pairing of two cortical sites in control aCSF (119.9 ± 8.0%, $P = 0.699$). The amount of LTP seen with MK-801 blockade of NMDA receptors postsynaptic to BLA inputs was significantly different from control (160.9 ± 13.2%, $P = 0.025$). When the same protocol was used to block NMDA receptors at cortical synapses, BLA–cortex pairings failed to induce LTP at cortical inputs (102.6 ± 3.6%, $P = 0.526$; $n = 4$; Fig. 3*C*). Together, these results suggest that although NMDA receptors at cortical synapses are required for LTP, the induction of high levels of LTP depends on NMDA receptors at BLA inputs.

Temporal Requirements. To test whether the BLA-mediated facilitation of corticostriatal LTP required precise coactivation of BLA and cortical synapses, as is typically the case for spike-timing-dependent LTP (43), we modified the pairing protocol, delaying the cortical stimuli by various intervals relative to BLA stimuli. However, to rule out the possibility that the lower pairing frequency required for these experiments modified the magnitude of the LTP facilitation, we first verified whether simultaneously applying BLA–cortex stimuli at 1 Hz rather than 2 Hz affected the amount of LTP. These two induction protocols produced nearly identical amounts of corticostriatal LTP (1 Hz, 164.3 ± 15.5%, $n = 4$, Fig. 4*B*, data-point at 0 s; 2 Hz, 160.9 ± 13.2%, $n = 11$, Fig. 2*A*; t test, $P = 0.88$).

Next, using a stimulation frequency of 1 Hz, we tested various BLA–cortex intervals (0.25, 0.5, or 0.8 s) while still pairing each of them with an AP and keeping unchanged the number of stimuli delivered at each site (Fig. 4*A1*). Despite the lack of coincidence between BLA and cortical stimuli, separating the two inputs by as much as 500 ms during induction still produced a significant facilitation of corticostriatal LTP (500 ms, 141.7 ± 10.6% of baseline, $P = 0.017$; $n = 5$; Fig. 4*A2*), whereas no significant LTP was observed with longer interstimulus intervals (800 ms, 98.8 ± 16.25%, $n = 7$; Fig. 4*C*).

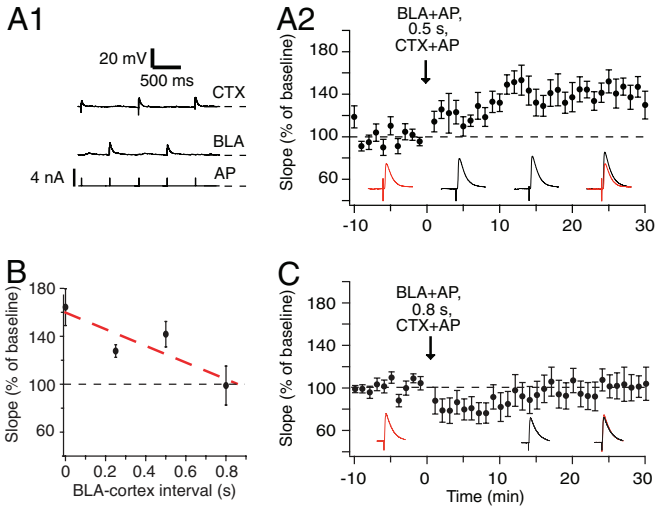

Fig. 4. BLA stimulation opens long windows of facilitated plasticity at corticostriatal synapses. (*A1*) An example of stimulation paradigm. Cortical stimuli follow BLA stimuli with a 500-ms delay. Both sites (BLA and cortex) are stimulated 60 times at a frequency of 1 Hz and paired with somatic APs. (*A2*) Corticostriatal LTP produced when BLA and cortical stimuli are separated by 0.5 s. Results are shown as normalized slope of cortically evoked EPSPs (*y* axis) as a function of time (*x* axis). Representative examples of cortically evoked EPSPs (average of 10 consecutive responses) before (red lines) and after (black lines) the induction protocol are provided at the bottom of the graph. An average of five experiments is shown. (*B*) Amount of corticostriatal LTP as a function of the BLA–cortex interstimulus interval. A linear fit to the data points is shown in red. (*C*) Corticostriatal LTP produced when BLA stimuli preceded cortical ones by 0.8 s. Results are shown as normalized slope of cortically evoked EPSPs (*y* axis) as a function of time (*x* axis). An average of 7 experiments is shown. CTX, cortical stimulus.

Finally, control experiments revealed that even when the BLA and cortical stimuli are not coincident, the pairing of both with somatic APs is still required because no facilitation of corticostriatal LTP was observed when we used unpaired cortical or BLA stimuli ($n = 5$ and $P > 0.05$ in both groups). Based on these results, it seems that the ability of BLA inputs to facilitate corticostriatal LTP spans over a period of several hundred milliseconds after the activation of NMDA receptors at BLA synapses (Fig. 4*B*).

Discussion

This study was undertaken to test whether BLA inputs can facilitate activity-dependent plasticity at cortical synapses onto principal striatal neurons. The interest of this issue stems from pharmaco-behavioral studies implicating BLA activity in the facilitation of striatal-dependent memories by emotional arousal. In keeping with this finding, our results indicate that BLA activity can facilitate induction of LTP at converging corticostriatal inputs. This effect was unusual in that it required NMDA receptor activation at both inputs, yet it could be seen even when BLA and cortex were activated 0.5 s apart during LTP induction.

BLA Inputs Facilitate Induction of Corticostriatal LTP. Activity-dependent modifications in the efficacy of corticostriatal synapses are believed to contribute to motor learning (25). In keeping with this view, posttraining AP5 injections in the striatum impair memory formation in striatal-dependent tasks (44). Moreover, the corticostriatal pathway exhibits various

forms of activity-dependent plasticity (26–32). Another line of investigation indicates that, in emotionally arousing conditions, postlearning BLA activity facilitates striatal-dependent memories. Indeed, injection of amphetamines in the BLA just after training increases striatal-dependent storage of response information (17). Yet, intraamygdala injections of lidocaine just before testing retention have no effect on performance (17). Together, these findings suggest that the BLA enhances the formation of striatal-dependent memories by facilitating NMDA-induced corticostriatal plasticity.

Our results support this view. First, BLA inputs were found to express a higher NMDA-to-AMPA ratio than cortical synapses. Second, pairing the activation of cortical and BLA inputs with somatic APs enhanced the incidence and amplitude of corticostriatal LTP. This facilitation depended on NMDA receptors located at BLA synapses and was input-specific, occurring only at cortical synapses that were activated within 500 ms of BLA inputs. Considered together, these findings suggest that the facilitating effects of amygdala activity on corticostriatal LTP result from the ability of BLA inputs to raise, via NMDA receptors, the postsynaptic Ca^{2+} concentration beyond levels critical for LTP induction.

Lax Timing Requirements for the BLA-Mediated Facilitation of Corticostriatal LTP. Despite the NMDA dependence of the BLA effect, the LTP facilitation did not require precise coactivation of BLA and cortical inputs, provided that both inputs were paired to postsynaptic APs. In fact, the LTP facilitation was seen even when BLA and cortical stimuli were separated by as much as 0.5 s during induction, which contrasts with earlier reports on spike-timing-dependent plasticity where simultaneous pre- and postsynaptic activation, within <40 ms, was required for LTP induction (45). Thus, our findings imply that strong BLA inputs trigger an intracellular signal that can affect the fate of cortical synapses activated within 0.5 s of the BLA input. Given the NMDA dependence of the BLA effect, it is possible that the Ca^{2+} influx caused by BLA stimuli decays slowly enough for cortical inputs occurring 0.5 s later to be significantly affected. Another possibility is that this Ca^{2+} influx triggers one or more downstream signaling cascade(s).

On the surface, the loose temporal coordination required for BLA inputs to facilitate corticostriatal LTP might seem to represent an obstacle for the selective enhancement of particular sets of corticostriatal synapses during learning. However, this problem might be circumvented if the BLA effect required that multiple pairings with cortical inputs occurred over time before a significant potentiation is achieved. Although 60 such pairings were sufficient to induce LTP in our conditions, the minimum number of coincident cortical–BLA firings might be different *in vivo* where corticostriatal neurons exhibit sustained spontaneous activity (46, 47). In these conditions, BLA inputs might only facilitate cortical synapses that consistently and repeatedly exhibit phasic increases in activity around BLA spikes.

Relation to Previous Work on the BLA-Mediated Facilitation of Synaptic Plasticity. Although many studies have examined how the BLA facilitates synaptic plasticity in its targets, their relevance to corticostriatal plasticity is unclear. For instance, one model centers on the ability of the BLA to recruit corticopetal basal forebrain cholinergic neurons (48). This BLA-driven release of acetylcholine would facilitate plasticity in cortical networks. Indeed, it was found that muscarinic receptor blockade interferes with the stabilizing and facilitating effects of BLA stimulation on LTP of thalamocortical (24) and perforant path (23) synapses. However, because the cholinergic innervation of the striatum mostly has an intrinsic origin (49) and BLA axons do not target striatal cholinergic interneurons (34), such a mechanism is unlikely to explain our results. Together, these data suggest that BLA axons may influence synaptic plasticity through several parallel mechanisms.

These data are further supported by differences in the connectivity of these various networks. Indeed, although BLA directly projects to the striatum, it has no direct projections to the dentate gyrus or visual cortex (50). Yet, BLA stimulation after LTP induction can enhance and stabilize LTP of perforant path and geniculocortical synapses. In further contrast with our findings, the BLA enhancement of the perforant path LTP is independent of NMDA receptors (23). Thus, it seems likely that in this case, the BLA-mediated facilitation of synaptic plasticity was entirely indirect, via the cholinergic basal forebrain. A challenge for future studies will be to examine the effect of BLA inputs on activity-dependent plasticity in the rhinal cortices where the BLA has direct as well as indirect (via the cholinergic basal forebrain) projections.

Relevance to BLA-Related Modulation of Striatal-Dependent Learning. Much evidence indicates that different parts of the striatum participate in different forms of memory (51, 52). The dorsal striatum is required for habit formation, where fixed stimulus-response associations are acquired gradually. In contrast, the ventral striatum, the region investigated here, is believed to be involved in flexible place learning. Although BLA projections to the striatum are densest in its ventral part, substantial BLA projections reach more dorsal striatal sectors (53). In addition, pharmaco-behavioral studies indicate that the BLA can facilitate the formation of stimulus–response associations (17). In light of these data, the mechanisms evidenced here may well apply to both dorsal and ventral striatal memory functions. However, this contention awaits behavioral testing.

Materials and Methods

Slice Preparation. Experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with the approval of the Institutional Animal Care and Use Committee of Rutgers University. Coronal brain slices were obtained from Hartley guinea pigs (200–250 g). Animals were deeply anesthetized i.p. with 80 mg/kg ketamine, 12 mg/kg xylazine, and 60 mg/kg pentobarbital. The brain was extracted and cut in 400-μm-thick slices in ice-cold oxygenated aCSF with a vibrating microtome. The aCSF contained 126 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose (pH 7.3, 300 mOsm). Before recordings, slices were kept in an oxygenated chamber for at least 1 h at room temperature and then transferred one at a time to a recording chamber perfused with oxygenated aCSF at a rate of 4 ml/min. The temperature of the chamber was gradually increased to 32°C before the recordings began.

Electrophysiology. Stimulating electrodes were placed in the BLA and at two cortical sites (Fig. 1*C1*). Whole-cell patch recordings were performed with pipettes (4–6 MΩ) pulled from borosilicate glass capillaries and filled with a solution containing 130 mM K-gluconate, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 10 mM KCl, 2 mM MgCl2, 2 mM ATP-Mg, and 0.2 mM GTP-Tris(hydroxy-methyl)aminomethane (pH 7.2, 280 mOsm). In some experiments, neurobiotin (0.2%) was added to the pipette solution for post hoc morphological identification of recorded cells. Patch recordings of MSNs were obtained under visual control in the ventral striatum. All recordings were performed in current-clamp mode with the exception of the occlusion tests (carried out in voltage-clamp mode).

LTP Induction and Monitoring. During LTP experiments, MSNs were maintained in current-clamp mode, at a membrane potential of −90 mV by intracellular current injection (±0.01 nA).

Input resistance was monitored throughout the experiment, and recordings with fluctuations >10% were excluded from the analysis. Unless otherwise noted, LTP was induced by pairing stimuli delivered at two sites (cortex–cortex or BLA–cortex) with postsynaptic APs that were elicited by brief intracellular current pulses (2 ms, 1.5–3 nA). The stimuli were timed such that the AP peak coincided with the rising phase of the summated synaptic responses. This protocol was repeated 60 times at 2 Hz, after which responses were monitored for >30 min. Recordings were analyzed offline. Only monosynaptic responses were included in the analysis, and the initial half of the rising phase was analyzed for changes in slope. Because BLA and cortical projections are believe to arise from glutamatergic neurons (34, 35), it is highly unlikely that the early part of the responses evoked by BLA and cortical stimuli were reversed inhibitory postsynaptic potentials (IPSPs). Moreover, the striatum does not project back to the cortex or amygdala. As a result, electrical stimulation of the BLA or cortex cannot backfire principal striatal neurons, greatly simplifying interpretation of the results. Results are expressed as averages ± SE.

Statistical Analyses. To determine whether the different induction protocols induced a significant amount of LTP, we used Student's *t* test analysis. The average response recorded 20–30 min after LTP induction was calculated for each cell within a group, and the distribution of these values was compared with baseline. The changes were considered significant when P values <0.05 were obtained. The incidence of LTP induction in different groups was compared with a χ^2 test by using a significance threshold of $P < 0.05$.

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