Distinct forms of $G_q$-receptor-dependent plasticity of excitatory transmission in the BNST are differentially affected by stress

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Long-term depression (LTD) is an important synaptic mechanism for limiting excitatory influence over circuits subserving cognitive and emotional behavior. A major means of LTD induction is through the recruitment of signaling via $G_q$-linked receptors activated by norepinephrine (NE), acetylcholine, and glutamate. Receptors from these transmitter families have been proposed to converge on a common postsynaptic LTD maintenance mechanism, such that hetero- and homosynaptic induction produce similar alterations in glutamate synapse efficacy. We report that in the dorsolateral and ventrolateral bed nucleus of the stria terminalis (BNST), recruitment of $G_q$-linked receptors by glutamate or NE initiates mechanistically distinct forms of postsynaptically maintained LTD and these LTDs are differentially regulated by stress exposure. In particular, we show that although both mGluR5- and $\alpha_1$-adrenergic receptor (AR)-dependent LTDs involve postsynaptic endocytosis, the $\alpha_1$-AR-initiated LTD exclusively involves modulation of signaling through calcium-permeable AMPA receptors. Further, $\alpha_1$-AR but not $G_q$-dependent LTD is disrupted by restraint stress. $\alpha_1$-AR LTD is also impaired in mice chronically exposed to ethanol. These data thus suggest that in the BNST, NE- and glutamate-activated $G_q$-linked signaling pathways differentially tune glutamate synapse efficacy in response to stress.

adoption | norepinephrine | metabotropic glutamate receptor | calcium-permeable AMPA receptor | ethanol

A

iterations in key amygdalar and reward circuitries have been proposed as potential mechanisms underlying interrelated anxiety disorders and addiction. The bed nucleus of the stria terminalis (BNST) is a nucleus within a series of structures known as the “extended amygdala,” which receives a mix of glutamatergic inputs from cognitive and systemic brain centers and projects to key nuclei in both the reward and stress circuitries (1, 2). Consistent with this anatomy, a large literature indicates key roles of this region in anxiety-related behaviors, addiction, and other affective disorders (3).

The BNST receives intense noradrenergic innervation through the ventral noradrenergic bundle (VNB) (4). Disruption of the VNB or noradrenergic signaling in the BNST alters responses to stressors, preference for opiates, and stress-induced reinstatement to drug seeking (5, 6). $\alpha_1$-Adrenergic receptors (ARs) are $G_q$-linked G-protein-coupled receptors (GPCRs) that participate in shaping responses to stressors. Signaling through BNST $\alpha_1$-ARs within the BNST potently regulates the hypothalamic-pituitary-adrenal (HPA) stress axis and anxiety responses after stressors (7). The $\alpha_1$-AR antagonist prazosin has been shown to attenuate ethanol self-administration (SA) in ethanol-dependent rats (8) and reduces opiate SA (9). Furthermore, data from clinical trials have demonstrated that prazosin alleviates symptoms of post-traumatic stress disorder (PTSD) (10) and reduces alcohol drinking behavior in alcoholics (11).

Like $\alpha_1$-ARs, group I metabotropic glutamate receptors (mGluR1 and mGluR5) are also coupled to $G_q$. mGluR5 has been demonstrated to play a critical role in the reinforcing properties of abused drugs (12). Furthermore, multiple drugs of abuse hijack plasticity mechanisms at glutamatergic synapses in the BNST (13–15) and other reward nuclei (16). At hippocampal and cortical synapses, long-term depression (LTD) of excitatory transmission elicited by $G_q$-coupled GPCRs has been suggested to operate through a common pathway (17, 18). Previously, we described that mGluR5 activation induces LTD of excitatory transmission in the BNST (13). Additionally, norepinephrine (NE) can induce a time-dependent LTD via the $\alpha_1$-AR (19). A particularly interesting feature of $\alpha_1$-AR LTD is that it is heterosynaptic in nature in that it does not occur by augmenting NMDA receptor or mGluR5 signaling, although it can be elicited in the same neurons in BNST as mGluR5-LTD (19). Based on this interaction and the studies in hippocampus (17) and visual cortex (18), we hypothesized that $\alpha_1$-AR LTD and mGluR5 LTD share common mechanisms and are recruited by similar environmental stimuli. Here, we demonstrate that contrary to our hypothesis, $\alpha_1$-AR LTD but not mGluR5 LTD results in the loss of functional calcium-permeable AMPA receptors (CP-AMPARs) from the synapse, suggesting distinct mechanisms. Additionally, we demonstrate that $\alpha_1$-AR LTD and CP-AMPAR function are disrupted in chronic restraint-stress-exposed mice, although mGluR5 LTD remains intact. This result is opposite the profile of cocaine-induced modulation of these $G_q$-GPCR LTDs in BNST (13, 14, 19), suggesting that recruitment of these forms of plasticity is finely tuned to specific stimuli. Finally, potentially related to the ability of prazosin to reduce ethanol dependence-induced SA, $\alpha_1$-AR LTD is attenuated in mice undergoing withdrawal from chronic ethanol exposure.

Results $\alpha_1$-AR LTD Is Maintained via a Postsynaptic Mechanism. Previously, we demonstrated that mGluR5 LTD in the BNST is maintained postsynaptically (14); however, the synaptic locus of $\alpha_1$-AR modulation of excitatory transmission has not been thoroughly examined. $\alpha_1$-AR activation by either NE or methoxamine produces LTD of excitatory transmission in the dorsolateral (dl) BNST (18) (Fig. L4 and Fig. S1). This LTD is not associated with...
alterations in paired-pulse ratios (PPRs) of evoked excitatory responses after the induction of LTD, suggesting a postsynaptic mechanism (18) (Fig. S1 B and C). To explore this possibility further, we assayed the actions of methoxamine on excitatory transmission in the dBNST with low Ca\(^{2+}\) artificial cerebral spinal fluid (ACSF). Presynaptic modulation has been shown to be more robust in reduced extracellular Ca\(^{2+}\) (20). Previously, we have shown that altering the ACSF divalent cation concentrations to 1 mM Ca\(^{2+}\) and 2.8 mM Mg\(^{2+}\) (thus reducing the [Ca\(^{2+}\)]\text{free} from 2.5 mM) significantly increases basal PPR values in the BNST (14). This alteration, however, does not enhance the α\(_1\)-AR LTD in the BNST (LTD in normal Ca\(^{2+}\): \(n = 6\), 56.7 ± 3.6% of baseline; LTD in low Ca\(^{2+}\): \(n = 5\), 72.3 ± 5.6% of baseline; Fig. S1A), nor does it have any effect on the PPR following induction of the α\(_1\)-AR LTD (Fig. S1C).

To assess the maintenance mechanisms for α\(_1\)-AR LTD in the dBNST further, we next analyzed the effect of methoxamine on miniature excitatory postsynaptic currents (mEPSCs) in the presence of 1 μM tetrodotoxin (TTX). Classically, a reduction in mEPSC frequency is indicative of a decrease in glutamate release, whereas a decrease in amplitude is indicative of a decrease in postsynaptic sensitivity. We predicted that the observed LTD of evoked mEPSCs produced by methoxamine would be paralleled by a decrease in the amplitude and/or frequency of mEPSCs. Surprisingly, however, methoxamine failed to cause an alteration in either the frequency (\(n = 4\), baseline: 0.70 ± 0.3 Hz, following agonist: 0.68 ± 0.3 Hz, not significant (n.s.) or the amplitude (\(n = 4\), baseline: 16.3 ± 0.8 pA, following agonist: 15.8 ± 1.0 pA, n.s.) of the mEPSCs 36–40 min after agonist application (Fig. S2 A–C).

The absence of action of methoxamine on mEPSCs suggests that methoxamine-induced LTD is an activity-dependent process. Consistent with this idea, we previously reported that α\(_1\)-AR LTD requires L-type voltage-gated Ca\(^{2+}\) channel activity (19). We thus tested whether the lack of effect of methoxamine on mEPSCs was attributable to Na\(^{+}\) channel blockade by sampling spontaneous mEPSCs (sEPSCs) in the absence of TTX before and after methoxamine application (Fig. S3). Surprisingly, we found that α\(_1\)-AR activation robustly increased, rather than decreased, the frequency of sEPSCs in the BNST 40 min following methoxamine application (\(n = 10\), baseline: 1.48 ± 0.4 Hz, following agonist application: 4.30 ± 1.3 Hz; \(P < 0.05\); Fig. S3B), with a trend for a decreased sEPSC amplitude (baseline: 18.9 ± 1.9 pA; following agonist application: 17.4 ± 0.9 pA; Fig. S3A).

Recently showed that dopamine increases sEPSC frequency in the dBNST in an activity- and corticotropin-releasing factor 1 receptor (CRF\(_{1}\))-dependent process (21). α\(_1\)-AR signaling has been shown to increase spontaneous inhibitory postsynaptic currents (IPSCs) in the BNST (22). These IPSCs may originate either from intrinsic neurons or from extrinsic afferents from the central nucleus of the amygdala, both of which can contain corticotropin-releasing factor (CRF) (23). We thus hypothesized that activity-dependent release of CRF by methoxamine may mask a significant decrease of sEPSC amplitude by methoxamine (although we did observe a trend for amplitude decrease). The CRF\(_{1}\) antagonist 5-chloro-N-(cyclopentyImethyl)-2-methyl-N-propyl-N'-(2, 4, 6-trichlorophenyl)-4, 6-pyrirnodinediamine hydrochloride (NBI 27914; 1 μM) blocked the methoxamine-induced increase in sEPSC frequency (\(n = 5\), baseline: 3.23 ± 1.2, following agonist application: 2.48 ± 1.0 Hz, n.s.; Fig. S3D). Further, the presence of the CRF\(_{1}\) antagonist revealed that methoxamine produces a significant reduction in sEPSC amplitude (although we did observe a trend for amplitude decrease). The CRF\(_{1}\) antagonist 5-chloro-N-(cyclopentyImethyl)-2-methyl-N-propyl-N'-(2, 4, 6-trichlorophenyl)-4, 6-pyrirnodinediamine hydrochloride (NBI 27914; 1 μM) blocked the methoxamine-induced increase in sEPSC frequency (\(n = 5\), baseline: 3.23 ± 1.2, following agonist application: 2.48 ± 1.0 Hz, n.s.; Fig. S3D).

Fig. 1. α\(_1\)-AR LTD Requires Clathrin-Dependent Endocytosis. (A) Methoxamine (100 μM) produces LTD in control cells (\(n = 6\), ■) but not in cells infused with 2 μM (in patch solution) of the dynamin inhibitory peptide (○, \(n = 5\), P > 0.63). (B) Histogram comparing the EPSCs of the two conditions in A (min 50–55). The black bar is the control, and the white bar is the dynamin inhibitory peptide (\(P < 0.03\)). (C) Coapplication of 20 μM U0126 and 100 μM methoxamine induces LTD (\(n = 5\), P > 0.0001). methox, methoxamine.
AMPAR subunit protein–protein interactions in α1-AR LTD and mGluR5 LTD in the dIBNST. By infusing a peptide (via the patch pipette) corresponding to the C terminus of either the GluR1 protein (pep1-TGL; Tocris) or the GluR2 protein (pep2-SKVI; Tocris) into the cell for 30 min before recording, the subunit-specific interactions with intracellular proteins could be selectively disrupted (31). It was necessary to infuse the peptides for this duration because they can alter GluR subunit trafficking and alter basal EPSCs (32). After this initial incubation time, a baseline was collected and 100 μM methoxamine was applied to the cell for 15 min. The cells that were infused with the GluR1 peptide demonstrated significantly attenuated α1-AR LTD (n = 6, 70.5 ± 3.7% of baseline; Fig. 3A) as compared with the cells that were infused with the GluR2 peptide and naive no-peptide recordings (GluR2 peptide: n = 5, 54.6 ± 5.4% of baseline, GluR1 peptide vs. GluR2 peptide: P < 0.05 by one-way ANOVA; naive peptide: n = 7, 52.4 ± 6.4% of baseline, GluR1 vs. naive: P < 0.05 by one-way ANOVA; GluR2 vs. naive: n.s. by one-way ANOVA; Fig. 3A). Furthermore, when the same peptides were used to probe mGluR5 LTD in the dIBNST, neither peptide was able to attenuate LTD (n.s. by one-way ANOVA; GluR1 peptide: n = 7, 65.4 ± 6.8% of baseline; GluR2 peptide: n = 4, 71.1 ± 5.8% of baseline; naive: 70.3 ± 4.0% of baseline; Fig. 3B).

α1-AR LTD but Not mGluR5 LTD Is Disrupted by Stress-Inducing Manipulations. The BNST is a critical regulator of anxiety-like behaviors (33). Restraint stress is known to increase extracellular NE levels robustly in the BNST and to signal through BNST α1-ARs to increase anxiety-like behaviors and adrenocorticotropic hormone in blood plasma (7, 34). We hypothesized that 10 daily 2-h restraint stress sessions (see Methods) would modulate α1-AR LTD in vivo but not mGluR5 LTD. Recording from the ventrolateral BNST (vIBNST) [which receives the most robust NE projection (4)], we found that a 15-min 100-μM methoxamine application produced a large transient depression but failed to induce LTD in the restrained animals in the vIBNST (stress: n = 6, 95.7 ± 9.0% of baseline, n.s. change from baseline; naive: n = 4, 62.0 ± 9.7% of baseline; Fig. 4A). In the dIBNST, we found that α1-AR LTD was significantly attenuated in restrained mice as compared with naive controls (stress: n = 5, 84.6 ± 6.0% of baseline; naive: n = 5, 54.4% ± 8.1% of baseline; P < 0.05; Fig. 4B). We next examined the expression of mGluR5 LTD following the restraint stress protocol and found that mGluR5 LTD was not diminished in either the dIBNST or the vIBNST (dorsal: n = 5, 74.2 ± 5.4% of baseline; ventral: n = 7, 37.7 ± 6.8% of baseline; Fig. 4C and D) as compared with naive controls. Because of the disruption of α1-AR LTD in vIBNST in the stressed mice and the data suggesting that α1-AR LTD prevents signaling via CP-AMPARs (Fig. 2), we next probed the signaling through CP-AMPARs in the vIBNST in mice exposed to the same chronic restraint stress. Following application of 100 μM Naspm, naive mice exhibited a similar reduction in EPSCs in the
vIBNST as was observed in the dIBNST (n = 8, 71.1 ± 4.4% of baseline; Fig. 4E and F). Stressed mice, however, had a significantly attenuated Npasm-induced reduction in EPSCs as compared with their naive controls (n = 8, 88.3 ± 4.3% of baseline; P < 0.05; Fig. 4E and F).

α1-AR LTD Is Disrupted by Chronic Alcohol Exposure. Stress disorders and alcoholism are highly comorbid (35). Data from human studies suggest that NE is increased in the central nervous system (CNS) of alcoholics (36), where it may play a role in the pathogenesis of alcoholism (35). Additionally, the adrenergic system remains an attractive target for intervention in alcoholism (11). Recently, α1-AR signaling has been linked to drinking behavior in withdrawn-dependent animals (8). Because of these data and our chronic stress results, we examined the persistence of α1-AR LTD in the dIBNST and vIBNST in ethanol-exposed mice. Mice receiving chronic continuous ethanol (CCE) were exposed to 64 h of continuous ethanol vapor, whereas mice receiving chronic intermittent ethanol (CIE) were exposed to 4 days of 16 h of ethanol vapor with 8-h withdrawal periods interspersed. Both the ethanol-exposed and sham animals were administered i.p. pyrazole daily. Animals were killed 4–6 h into the final withdrawal under each condition. Although we examined a shorter time course following methoxamine application, both conditions resulted in significantly attenuated responses to α1-AR signaling from sham mice; however, the LTD was not fully occluded by these treatments (sham mice: n = 6, 55.5 ± 6.5% of baseline; CCE mice: n = 7, 81.1 ± 7.5% of baseline; CIE mice: n = 5, 79.1 ± 6.3% of baseline; one-way ANOVA, P < 0.05; Fig. 5). We did not observe a correlation in the degree of depression between the dIBNST or vIBNST in the ethanol-treated animals.

Discussion

We find that α1-AR activation produces LTD of excitatory transmission in the BNST that involves CP-AMPARs. Despite the fact that both α1-AR and mGluR5 are Gq11-linked and can elicit LTD on overlapping neuronal populations in the BNST, we find that these two forms of LTD have distinct maintenance mechanisms. These differences are also apparent when examining the persistence of these LTDs following environmental challenges. We previously found that cocaine disrupts mGluR5- but not α1-AR LTD (13, 14, 19). Here, we report that a chronic stressor disrupts α1-AR LTD and CP-AMPAR transmission but not mGluR5 LTD. Moreover, we report that α1-AR LTD is diminished by chronic ethanol exposure. Finally, our studies uncover an additional acute enhancement of glutamatergic transmission in the BNST by α1-ARs, which, as with dopamine actions in the region, occurs through a CRF1-dependent process.

α1-AR LTD Is Maintained by a Different Postsynaptic Mechanism Than mGluR5 LTD in the BNST. Previously, we found that α1-AR LTD induction occluded the further induction of mGluR5 LTD in the BNST (19), leading us to hypothesize that both LTDs involved similar mechanisms (18). Recently, we found that mGluR5 LTD in the BNST is maintained via postsynaptic mechanisms involving endocytosis and rearrangement of the actin cytoskeleton (14). We now show that α1-AR LTD also requires clathrin-dependent endocytosis. Unlike mGluR5 LTD, however, a difference in the required time course of agonist application and a lack of MEK1/2 involvement suggest that different mechanisms underlie both LTDs. Here, we present data suggesting that in contrast to mGluR5 LTD, the AMPARs targeted in α1-AR LTD are CP-AMPARs. Additionally, the mEPSC and sEPSC profiles are different between mGluR5 LTD and α1-AR LTD. In mGluR5 LTD, a decrease in the frequency of events is observed with sEPSCs but not with CP-AMPARs. The lack of an α1-AR agonist effect on the amplitude or frequency of mEPSCs suggests that α1-AR LTD is dependent on CP-AMPARs.
the activation of voltage-gated sodium channels. α1-AR signaling can actively desensitize mGluR5 signaling at inositol triphosphate (IP₃) receptors (37), providing an explanation for our observed occlusion of mGluR5 LTD following induction of α₁-AR LTD in our previous report (19) in the absence of a shared mechanism.

α₁-AR LTD but Not mGluR5 LTD Results in the Functional Loss of CP-AMPARs. CP-AMPARs are less abundant in the CNS than the more conventional Ca²⁺-impermeable AMPARs (30). Although our understanding of their roles is still developing (30, 38), it is apparent that CP-AMPARs are another means for Ca²⁺ signaling in cells (30). Another G₂q-linked LTD, mGluR1-mediated LTD in the VTA, can induce the removal of CP-AMPARs from the membrane (24). Additionally, it has been shown that group I mGluRs and CP-AMPAR activation facilitate a functional switch of AMPAR subunits in cerebellar stellate cells (39). We found that the polyamine Nasp₃, a selective external pharmacological blocker of CP-AMPARs, produced a reduction of ~30% of the EPSC in the BNST. This sensitivity was lost following induction of α₁-AR LTD, and a peptide inhibitor that mimics the C terminus of the GluR1 subunit attenuated α₁-AR LTD. These converging lines of evidence suggest that the loss of CP-AMPAR function comprises a portion of the mechanism underlying α₁-AR LTD. Although it is not known if signaling via CP-AMPARs alters plasticity within the BNST, a loss of CP-AMPARs following α₁-AR LTD may result in a metastable shift within the BNST that could alter circuit activity.

Additionally, we demonstrated that mGluR5 LTD in the BNST is neither maintained by the functional loss of CP-AMPARs from the postsynaptic density nor sensitive to the inclusion of the GluR1 C-terminal peptide in the intracellular solution. (We did not observe an affect of either C-terminal peptide on the GluR1 LTD; however, this LTD may alter the function of postsynaptic AMPARs via other means than C-terminal interactions.) These results, in conjunction with the differing mEPSC and sEPSC profiles, strongly suggest that although the same cell contains the required elements for the induction of either α₁-AR or mGluR5 LTD, they occur by distinctly different mechanisms. Furthermore, the induction of one form of G₂q-coupled LTD manipulates the plasticity of the other LTD (19), which differs from observations in other brain regions, where dual G₂q-coupled LTDs have been studied (17, 18). It is possible to envision that the occlusion of mGluR LTD in the BNST by α₁-AR LTD could influence subsequent behavior, and a lack of proper α₁-AR LTD induction/expression (see below) may result in pathological mGluR LTD expression.

Expression of α₁-AR LTD Is Manipulated by Chronic Stressors in Vivo. Previously, we demonstrated that α₁-AR LTD cannot be induced in two models of affective disorders: the α₂₅-AR KO mouse and the NE transporter KO mouse (19). We thus wanted to examine if stressful manipulations, which would increase adrenergic tone in vivo, could alter the expression of α₁-AR LTD ex vivo. Withdrawal from alcohol intoxication has been shown to increase anxiety (40), and patients experiencing withdrawal have elevated levels of NE and its metabolites (36, 41). Furthermore, CIE can increase anxiety-like behavior in animal models (44). We used a CCE protocol with a single withdrawal and a CIE protocol with four withdrawals. We originally expected to see differences only after the CIE repeated-withdrawal paradigm; however, both protocols significantly reduced the expression of LTD and were not significantly different from each other. This result, however, is perhaps not surprising, because animals are experiencing acute withdrawal in both conditions. Alternatively, other aspects of the exposure regimen, such as pharmacological effects of alcohol, may drive these alterations.

We also examined the expression of α₁-AR LTD following a prolonged chronic stressor. Restraint stress has been shown to increase NE levels within the BNST, and α₁-AR signaling therein increases anxiety-like behavior and activates the HPA axis (7). Mice that received chronic restraint stress failed to express α₁-AR LTD in the vBNST and demonstrated attenuated α₁-AR LTD in the dBNST. These stress experiments suggest that either the LTD had already been expressed in vivo or that the mechanisms for inducing α₁-AR LTD were not functioning following the stressor. Thus, we next probed CP-AMPAR function in the vBNST in animals exposed to the chronic stress paradigm. Alterations in the function of CP-AMPARs could serve as a physiological "marker" for the induction of LTD. Following chronic stress, the CP-AMPAR inhibitor Nasp₃ had a significantly reduced effect on evoked EPSCs as compared with naive controls (Fig. 4 E and F). These results suggest that the α₁-AR LTD is induced during the chronic restraint stress, which thus reduces transmission via CP-AMPARs. There is however, a slight but significant effect of Nasp₃ on EPSCs remaining in the stressed animals, suggesting complex poststressor effects on physiology. One possibility for the remaining CP-AMPAR function in the stressed animals is that although our bath application of α₁-AR agonist appears to target all synapses with CP-AMPARs, NE signaling in vivo may only target a subset of synapses.

Understanding the modulation of α₁-AR LTD is critical, because the BNST is thought to be part of the braking mechanism that inhibits activation of the paraventricular nucleus of the hypothalamus (PVN) (1). This LTD could thus serve to disengage the inhibitory influence of the BNST on the PVN under stressful conditions. Additionally, the HPA axis has altered function in both human disease states (43) and rodent models of affective disorders (44, 45), which may suggest a pathological role for this plasticity or the lack of α₁-AR LTD and CP-AMPAR function. In future studies, it will be important to delineate the pattern and duration of alcohol/stress exposures necessary to modulate α₁-AR LTD as well as the persistence of this modulation.

Although α₁-AR LTD was disrupted by alcohol exposure and chronic stress, mGluR5 LTD expression was not altered. Previously, we have observed that a single cocaine i.p. injection can prevent the expression of mGluR5 LTD but not α₁-AR LTD in the BNST (14, 19), which may suggest that different behavioral saliences can manipulate the induction of G₂q-coupled LTD on neurons within the BNST. Because we have demonstrated that prior induction of α₁-AR LTD can manipulate further induction of mGluR5 LTD (19), there may be cross-talk between the induction mechanisms that could contribute to stress/substance abuse pathological findings.

An intriguing result of this investigation is that α₁-AR activation leads to lasting increases in sEPSC frequency, presumably via the release of CRF acting on the CRF₁R₁, akin to dopamine signaling within the BNST (21). Patients with PTSD have been shown to have overengaged central CRF systems (43). In addition, CRF administration within the BNST can potentiate anxiety (44). Moreover, animals experiencing protracted withdrawal from alcohol have altered CRF tone in a component of the dBNST, which leads to decreased plasticity of the intrinsic excitability of these neurons (46). Heightened adrenergic tone may be one mechanism by which the CRF system is dysregulated in these dependent animals.

In total, the current data, together with our previous results in models of affective disorders, strongly correlate the loss of α₁-AR LTD with a number of psychiatric ailments and provide a potential mechanism for the therapeutic effects observed in clinical trials that have demonstrated α₁-AR antagonists as potential therapeutic agents for the alleviation of PTSD and alcoholism (14–17).

**Methods**

**Animal Care.** All mice used in experiments were male C57BL/6j mice 5–8 weeks old (The Jackson Laboratories). All animals were provided with food and water ad libitum, with the exception of the 2-h stress (see below) experiments, and were housed in groups within the Vanderbilt Animal Care Facilities. Approved guidelines from the Vanderbilt University and University
of North Carolina at Chapel Hill Animal Care and Use Committees were used for all experiments.

**Brain Slices and Whole-Cell Recordings.** Brain slices were prepared, and whole-cell recordings were made as previously described (21, 29) (see **SI Methods**). dBNST recordings focused heavily on the undifferentiated anterolateral regions and ventral aspects of the oval nucleus.

**Stress Procedures.** Mice were stressed for 2 h for 10 consecutive days and recorded from on the 11th day. Restraint devices were 50-mL conical tubes with several (≥15) holes in the front and rear (cap) to maintain airflow. While in restraint devices, animals were placed inside separate sound- and light-attenuating boxes, and they were returned to their home cage immediately following restraint.

**Ethanol Procedures.** Ethanol chamber experiments were performed in accordance with the Integrative Neurosciences Initiative on Alcoholism (INIA-Stress) standard operating procedure and as previously reported by Healey et al. (47). For a full description, please refer to **SI Methods**.

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