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Excitatory drive onto dopaminergic neurons in the rostral linear nucleus is enhanced by norepinephrine in an α_1 adrenergic receptor-dependent manner

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Abstract

Dopaminergic innervation of the extended amygdala regulates anxiety-like behavior and stress responsivity. A portion of this dopamine input arises from dopamine neurons located in the ventral lateral periaqueductal gray (vPAG) and rostral (RLi) and caudal linear nuclei of the raphe (CLi). These neurons receive substantial norepinephrine input, which may prime them for involvement in stress responses. Using a mouse line that expresses eGFP under control of the tyrosine hydroxylase promoter, we explored the physiology and responsiveness to norepinephrine of these neurons. We find that RLi dopamine neurons differ from VTA dopamine neurons with respect to membrane resistance, capacitance and the hyperpolarization-activated current, I_h . Further, we found that norepinephrine increased the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) on RLi dopamine neurons. This effect was mediated through the α_1 adrenergic receptor

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(AR), as the actions of norepinephrine were mimicked by the α_1 -AR agonist methoxamine and blocked by the α_1 -AR antagonist prazosin. This action of norepinephrine on sEPSCs was transient, as it did not persist in the presence of prazosin. Methoxamine also increased the frequency of miniature EPSCs, indicating that the α_1 -AR action on glutamatergic transmission likely has a presynaptic mechanism. There was also a modest decrease in sEPSC frequency with the application of the α_2 -AR agonist UK-14,304. These studies illustrate a potential mechanism through which norepinephrine could recruit the activity of this population of dopaminergic neurons.

Keywords

rostral linear nucleus; dopamine; norepinephrine; excitatory transmission; extended amygdala

1. Introduction

Addiction exhibits significant comorbidity with mood and anxiety disorders, and stress is frequently stated as a cause for drug use relapse (Sareen et al 2006, Sinha et al 1999, Sinha & O'Malley 1999). The extended amygdala, which contains the bed nucleus of the stria terminalis (BNST) and the central nucleus of the amygdala (CeA), has been shown to play an important role in stress, anxiety, and addiction related behaviors (Davis et al 2010, Erb 2010, Koob 2009). Dopaminergic afferents in the extended amygdala are thought to regulate anxiety-like behavior and stress responsivity (Meloni et al 2006). While dopamine is classically regarded as a “reward” neurotransmitter, the firing of dopamine neurons increases following acute stress, and dopamine has also been proposed to encode aspects of aversive stimuli (Anstrom et al 2009, Anstrom & Woodward 2005, Brischoux et al 2009, Coco et al 1992, Deutch et al 1991, Lammel et al 2011, Matsumoto & Hikosaka 2009, Morrow et al 2000a). However, the impact of the midbrain dopamine system's sensitivity to stress is not well understood and requires further investigation.

Dopamine release in the extended amygdala has several actions. For example, in the CeA and BNST dopamine enhances spontaneous glutamatergic transmission (Kash et al 2008, Silberman & Winder 2013). In the BNST, dopamine decreases evoked glutamatergic and inhibitory transmission (Krawczyk et al 2011a, Krawczyk et al 2011b), mediates a form of long term potentiation at GABA synapses (Krawczyk et al 2013), decreases NMDA currents in cocaine self-administering rats (Krawczyk et al 2014), and regulates long-term intrinsic excitability of BNST neurons (Francesconi et al 2009). The BNST receives a substantial proportion of its total dopaminergic input from the ventral lateral periaqueductal grey (vIPAG) and the dorsal-caudal extension of the A10 dopamine cell group of the ventral tegmental area (VTA), termed the A10dc cell group, which is located in the dorsal, rostral linear (RLi), and caudal linear raphe nuclei (CLi) (Hasue & Shammah-Lagnado 2002, Meloni et al 2006). The dopamine neurons of the lateral VTA have been extensively studied while those of the A10dc group have often been overlooked. While overall very little is known about A10dc roles and properties, evidence suggests that the RLi is particularly sensitive to stressors (Deutch et al 1991).

There is an increasing appreciation regarding the functional heterogeneity of dopamine neurons. Electrophysiological properties of dopamine neurons, such as action potential width and the presence of a hyperpolarization-activated current (I_h), differ based on their projection targets and anatomical location (Lammel et al 2011, Li et al 2013, Margolis et al 2008). The modulation of excitatory synapses on dopamine neurons by aversive and rewarding stimuli also depend on their projection targets (Lammel et al 2011). For example, the dopamine neurons located in the medial posterior VTA, near the RLi, have no I_h and their glutamatergic synapses are modified by aversive, but not rewarding stimuli (Lammel et al 2011). Therefore, study of the basal electrophysiological properties of RLi dopamine neurons may give early insights into their actions.

The noradrenergic system is a key regulator of the stress response. Many stressors increase the firing of norepinephrine neurons and increase norepinephrine turnover in target regions (Abercrombie et al 1988, Cecchi et al 2002, Korf et al 1973). The locus coeruleus, A1, A2, and A5 norepinephrine groups send efferents to the RLi (Mejias-Aponte et al 2009). Activation of α_1 or α_2 adrenergic receptors (ARs) induce changes in burst firing of VTA dopamine neurons, (Grenhoff et al 1993, Grenhoff & Svensson 1989, Grenhoff & Svensson 1993, Guiard et al 2008) and glutamatergic transmission is crucial for burst firing of dopamine neurons (Overton & Clark 1992, Seutin et al 1993). Norepinephrine acts as a powerful modulator of excitatory neurotransmission in many limbic brain areas. Activation of β , α_1 , and α_2 -ARs have been shown to enhance or attenuate glutamatergic transmission in a variety of brain regions, including the hippocampus, CeA, BNST and VTA (Egli et al 2005, Flavin & Winder 2013, Gereau & Conn 1994, Jimenez-Rivera et al 2012, McElligott et al 2010, McElligott & Winder 2008, Nobis et al 2011, Shields et al 2009, Velasquez-Martinez et al 2012). Therefore, norepinephrine might modulate glutamatergic transmission on RLi dopamine neurons.

Given the interconnections of the RLi with circuitry mediating stress and anxiety and the stress sensitivity of RLi dopamine neurons, it is important to investigate the actions of norepinephrine on RLi dopamine neurons. Using a tyrosine hydroxylase (TH)-eGFP reporter mouse line, we found differences in basal electrophysiological properties, such as I_h , membrane resistance and capacitance, of VTA and RLi dopamine neurons. We also investigated the actions of norepinephrine on spontaneous glutamatergic transmission and found an increase in the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) on RLi dopamine neurons. Through the application of specific AR agonists and antagonists, we determined that this enhancement of excitatory transmission is due to the activation of α_1 -ARs. α_1 -ARs also increased the frequency of miniature EPSCs indicating a potential presynaptic locus for this modulation. In addition to this large α_1 -AR mediated enhancement of excitatory transmission, we also found a modest α_2 -AR mediated depression of excitatory transmission in RLi dopamine neurons.

2. Methods

2.1 Animals

Male mice ages 3–5 weeks were used in these experiments in accordance with animal use protocol approved by the Institutional Animal Care and Use Committee of Vanderbilt

University. All experiments were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023). Mice were group housed in the Vanderbilt vivarium under a 12 hour light/dark cycle with food and water *ad libitum*. All mice were obtained from a transgenic mouse line (Strain Name: STOCK Tg(Th-EGFP)DJ76Gsat/Mmnc). Mating pairs of mice were initially obtained from the Mutant Mouse Regional Resource Center in North Carolina. In this mouse line, the genome was modified to contain multiple copies of a modified BAC in which an eGFP reporter gene was inserted immediately upstream of the coding sequence of the gene for TH. Data presented here were obtained from two lineages of transgenic mice maintained in-house, one derived by cross-breeding with Swiss Webster mice (Taconic), and the other by cross-breeding with C57/B6 mice (Jackson Laboratories). The characterization of basal electrophysiology properties was performed in mice with the Swiss Webster background. The remainder of the electrophysiology studies and the immunohistochemistry were performed in TH-eGFP mice that were backcrossed with C57/B6 mice for many generations.

2.2 Immunohistochemistry & microscopy

Mice were transcardially perfused with ice-cold phosphate-buffered saline (PBS), followed by ice-cold 4% paraformaldehyde in PBS. Brains were removed from the skull and post-fixed in the same fixative overnight at 4°C, and were then transferred to 30% sucrose in PBS. Two to five days later, 40 µm thick coronal sections of brain were sliced on a cryostat (Leica CM3050S). Free-floating sections were washed in PBS (4 × 10 minutes), permeabilized with 0.5% Triton-X 100 in PBS, and then blocked with 10% normal donkey serum in PBS containing 0.1% Triton-X 100. Sections were then incubated with a mouse monoclonal anti-TH antibody (from ImmunoStar used at 1:2000 dilution) in blocking solution for 48 hours at 4°C, followed by PBS washes (4×10 minutes) and incubated with a Cy3-conjugated donkey anti-mouse secondary antibody (from Jackson ImmunoResearch used at 1:500 dilution) for 24 hours at 4°C in PBS with 0.1% Triton-X 100. Finally, sections were washed (4×10 minutes), mounted on slides, sealed with PolyAquamount, and left overnight to dry. Stained sections were examined with a Zeiss 510 scanning confocal microscope.

2.3 Electrophysiology recordings

2.3.1 Brain slice preparation—3–5 week old TH-eGFP mice were decapitated under Isoflurane anesthesia. The brains were quickly removed and placed in oxygenated ice-cold sucrose artificial cerebrospinal fluid (ACSF): 194 mM sucrose, 20 mM NaCl, 4.4 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 1.2 mM NaH₂PO₄, 10 mM glucose, and 26 mM NaHCO₃. Three-hundred µm coronal slices of the VTA or RLi were prepared using a vibrating microtome (Leica VT 1200). Two RLi slices per mouse were generated. Slices were incubated for 1 hour in oxygenated ACSF at 28°C: 124 mM NaCl, 2.5 mM KCl, 2.5 mM CaCl₂, 2 mM Mg₂SO₄, 1.25 mM NaH₂PO₄, 11 mM glucose, 26 mM NaHCO₃, and 0.4mM ascorbic acid. Slices then rested in a submerged superfusion chamber (Warner Instruments) for 30 minutes prior to recordings.

2.3.2 Whole-cell voltage clamp recordings—The TH-eGFP positive neurons were identified via fluorescent microscopy, and directly visualized with infrared video microscopy (Olympus) for patching. Recording electrodes were fabricated with a Flaming-Brown Micropipette Puller (Sutter Instruments) using thin-walled borosilicate glass capillaries (World Precision Instruments). Recording electrodes of resistance 2.5–5.5 M Ω were filled with an internal solution of 290–295 mOsmol, pH = 7.2–7.3, consisting of: 125 mM K⁺-gluconate, 5 mM NaCl, 4.4 mM KCl, 10 mM HEPES, 0.6 mM EGTA, 4 mM ATP, and 0.4 mM GTP for I_h current recordings and 117 mM Cs⁺-gluconate, 20 mM HEPES, 0.4 mM EGTA, 5mM TEA, 2mM MgCl₂, 4 mM ATP, and 0.3 mM GTP for the remainder of the electrophysiology recordings. All electrophysiology recordings were made using Clampex 9.2 and analyzed using Clampfit 10.2 (Molecular Devices). I_h currents were measured in the voltage-clamp recording configuration by stepping the holding potential from –60mV to –120mV for 1 second and then measuring the difference between the current immediately following the step to –120mV and the current at the end of the –120mV step. Whole-cell, voltage-clamp recordings were performed as described previously (Egli et al 2005, Kash et al 2008, Nobis et al 2011, Silberman & Winder 2013). Briefly, AMPA receptor-mediated sEPSCs were made at –70 mV and pharmacologically isolated by the addition of 25 μ M picrotoxin to the ACSF. Cells were allowed to equilibrate to whole-cell configuration for 5 min before recordings began. sEPSC recordings were acquired and analyzed in 2 min gap-free blocks, and access resistance was monitored between blocks of sEPSC recordings. Those experiments in which the access resistance changed by >20% were not included in the data analyses. For the experiment in which the effect of prazosin was determined, the antagonist was pre-applied for at least 15 minutes before application of norepinephrine and then remained onboard for the duration of the experiment. To isolate miniature EPSCs (mEPSCs) 1 μ M tetrodotoxin (TTX) was added in addition to sEPSC recording conditions.

2.4 Data analysis

2.4.1 Cell Counting—For counting single and multiply labeled neurons, z stacks of imaged brain sections were projected onto a single plane using Zeiss LSM software. Projected images were then opened in Metamorph and neurons counted with the help of manual logger functions. The signal intensity threshold for counting neurons as positive for a marker was set at twice that to the brightest neurons in no primary antibody control sections (TH), or for eGFP, neurons in brainstem areas that did not express eGFP. Quantitative data are expressed as mean $\pm$ S.E.M. unless otherwise noted.

2.4.2 Electrophysiology studies—Statistical analyses were performed using Microsoft Excel 2010 and GraphPad Prism 6. Specifically, when comparisons were made between RL_i and VTA basal excitability properties, an unpaired Student's *t*-test was used. When determining whether a compound had a significant effect, a Student's paired *t*-test was used, comparing the baseline value to the peak experimental value. Paired comparisons were made between baseline (the average of the last three recording blocks before drug is added) and the three recording blocks immediately following removal of drug application unless otherwise noted. When comparing antagonist effects on the norepinephrine-induced increase in sEPSCs, a repeated measures one-way ANOVA was used, followed by a Tukey's post-

test to determine the significance of specific comparisons. Degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity. For the electrophysiology studies, “n” refers to the number of cells. At least four animals were used in each electrophysiology experiment. All values given throughout the study are presented as mean $\pm$ S.E.M.

2.5 Pharmacology

Isoproterenol hydrochloride, tetrodotoxin citrate, and picrotoxin were purchased from Tocris Bioscience. Norepinephrine bitartrate salt monohydrate, methoxamine hydrochloride, and prazosin hydrochloride were purchased from Sigma-Aldrich. All experimental drugs were bath applied at their final concentrations as noted in the text. Dimethylsulfoxide (DMSO) was the solvent used for stock solution of picrotoxin where the maximum final concentration of DMSO in ACSF was 0.02% by volume. All other drugs, with the exception of prazosin, were easily dissolved in water. Prazosin was dissolved in water with vigorous heating and stirring.

3. Results

3.1 Electrophysiological characteristics of RLi dopamine neurons

We first wanted to confirm that the TH-eGFP mouse line would adequately report dopamine neurons in the RLi. To examine this, sections were processed for immunostaining for anti-TH and the numbers of eGFP+, TH+, and double labeled neurons were counted in images captured from each section with a 20 $\times$ lens. In the RLi, the percentage of eGFP+ cells that were co-localized with TH+ cells was $95 \pm 1\%$ ($n = 3$) (Fig. 1B–E). These data suggest that eGFP fluorescence in this mouse line is a good marker for dopamine neurons located in the RLi.

It has been reported that dopamine neurons have distinct electrophysiological profiles based on their projection targets and anatomical location (Lammel et al 2011, Margolis et al 2008). Therefore, we examined the basic electrophysiological properties of eGFP positive neurons located in the RLi and lateral VTA. Using whole-cell voltage clamp, we measured membrane resistance, membrane capacitance, and hyperpolarization-activated (I_h) current of TH-eGFP neurons in the RLi and VTA. RLi eGFP+ neurons exhibited a significantly higher membrane resistance ($745 \pm 115 \text{ M}\Omega$, $n = 16$) than eGFP+ neurons in the VTA ($195 \pm 53 \text{ M}\Omega$, $n = 10$, $p < 0.01$) (Fig. 1F). Further, the RLi neurons also displayed a smaller membrane capacitance ($25 \pm 2 \text{ pF}$, $n = 16$) than eGFP+ neurons in the VTA ($56 \pm 5 \text{ pF}$, $n = 10$, $p < 0.0001$) (Fig. 1G). Having established these differences in basic membrane properties, we next sought to determine if the eGFP+ neurons in the RLi exhibit an I_h current. The presence of I_h current has long been used as a marker for dopamine neurons in the VTA (Hopf et al 2007, Margolis et al 2006, Stuber et al 2008). Recently, it has been shown that a subset of dopamine neurons do not possess a robust I_h current (Lammel et al 2011, Margolis et al 2006, Margolis et al 2008). Consistent with this, we found that VTA eGFP+ neurons displayed an I_h current similar to those observed in previous studies ($-173 \pm 46 \text{ pA}$, $n = 10$) (Ungless et al 2003). However, the eGFP+ neurons in the RLi had a

significantly smaller I_h current (-14 ± 4 pA, $p < 0.001$, $n = 16$) when compared to the lateral VTA (Fig. 1H–J), with most neurons lacking any measurable current.

3.2 Norepinephrine enhances spontaneous excitatory transmission on TH-eGFP+ neurons in the RLi via activation of α_1 adrenergic receptors

The RLi projects to regions heavily involved in stress responding and receives a large noradrenergic projection (Mejias-Aponte et al 2009). Thus, we tested the hypothesis that norepinephrine modulates RLi dopamine neurons. We first examined the effects of norepinephrine on basal excitability and found no effect on holding current ($107 \pm 8\%$ of baseline, $p > 0.05$, $n = 8$) or membrane resistance ($106 \pm 6\%$ of baseline, $p > 0.05$, $n = 8$). Because norepinephrine has been shown to regulate excitatory drive in other brain regions, we measured sEPSCs on TH-eGFP neurons located in the RLi (average basal frequency, 2.4 ± 0.3 Hz; average basal amplitude, 20 ± 1 pA, $n = 28$) (Fig. 2A). We bath applied norepinephrine ($50 \mu\text{M}$) for 10 minutes while spontaneous glutamatergic transmission was monitored using whole cell patch-clamp recordings in acutely prepared brain slices. We found that this norepinephrine application resulted in an increase in the frequency of sEPSCs ($473 \pm 93\%$ of basal frequency, $p < 0.01$, $n = 8$) (Fig. 2B). Additionally, there was a modest, but significant effect on sEPSC amplitude ($128 \pm 11\%$ of basal amplitude, $p < 0.05$, $n = 8$) (Fig. 2C). This increase in amplitude was present during the last 5 minutes of norepinephrine application. There was no effect of norepinephrine on sEPSC rise time ($93 \pm 7\%$ of baseline, $p > 0.05$, $n = 8$) or decay time ($97 \pm 4\%$ of baseline, $p > 0.05$, $n = 8$).

Since norepinephrine can act on multiple adrenergic receptors (ARs), we next tested whether α_1 , α_2 , and/or β adrenergic receptors were responsible for the actions of norepinephrine on glutamatergic transmission in RLi TH-eGFP neurons. We found that application of the α_1 -AR agonist methoxamine ($100 \mu\text{M}$) increased sEPSC frequency ($581 \pm 169\%$ of basal frequency, $p < 0.05$, $n = 9$) (Fig. 3A) but not amplitude ($117 \pm 10\%$ of basal amplitude, $p = 0.10$, $n = 9$) (Fig. 3B) in TH-eGFP positive neurons in the RLi. Preapplication of the α_1 -AR antagonist prazosin ($10 \mu\text{M}$) prevented norepinephrine induced increases in sEPSC frequency and amplitude. In fact, application of norepinephrine in the presence of prazosin led to a decrease in sEPSC frequency ($60 \pm 6\%$ of basal frequency, $p < 0.01$, $n = 5$) and a modest, but significant, decrease in sEPSC amplitude ($91 \pm 2\%$ of basal amplitude, $p < 0.05$, $n = 5$) (Fig. 3C,D). To further investigate these decreases in sEPSC frequency and amplitude, we bath-applied the α_2 -AR agonist UK-14,304. A 10 minute application of UK-14,304 ($1 \mu\text{M}$) led to a significant decrease in sEPSC frequency ($60 \pm 9\%$ of basal frequency, $p < 0.05$, $n = 6$) with a trend toward a decrease in sEPSC amplitude ($89 \pm 5\%$ of basal amplitude, $p = 0.08$, $n = 6$) (Fig. 4A,B). Since activation of β -ARs can lead to increases in sEPSC frequency in other brain regions such as the BNST, we bath-applied the β -AR agonist isoproterenol ($3 \mu\text{M}$) for 10 minutes and found that this application of isoproterenol did not lead to any changes in sEPSC frequency ($104 \pm 16\%$ of basal frequency, $p > 0.05$, $n = 8$) or amplitude ($96 \pm 5\%$ of basal amplitude, $p > 0.05$, $n = 8$) (Fig. 4C,D). Taken together, these data indicate that norepinephrine enhances the spontaneous glutamatergic transmission in RLi TH-eGFP neurons via activation of α_1 -ARs.

3.3 Noradrenergic enhancement of excitatory transmission on TH-eGFP+ RLi neurons is transient

To further investigate the time course of norepinephrine's enhancement of excitatory transmission in RLi TH-eGFP neurons, we bath applied prazosin (10 μ M) to slices 10 minutes after a 10 minute application of norepinephrine was completed. The prazosin was able to reverse the increase in frequency of sEPSCs caused by norepinephrine (Fig. 5A,C) ($F(1.041, 5.206) = 8.935$, $p < 0.05$, $n = 6$). It also reduced the modest increase in amplitude of sEPSCs caused by norepinephrine (Fig. 5B,D) ($F(1.622, 8.111) = 13.48$, $p < 0.01$, $n = 6$). These results indicate that the increase in glutamatergic transmission in RLi TH-eGFP neurons is not due to long term potentiation of excitatory inputs but rather from transient effects of α_1 -AR activation.

3.4 α_1 -AR activation increases glutamatergic transmission in an activity-independent manner

To further investigate the mechanism of action of norepinephrine's effects on RLi TH-eGFP neurons, we examined the ability of methoxamine to modulate activity-independent miniature EPSCs (mEPSCs). mEPSCs were isolated by the addition of the sodium channel blocker TTX (1 μ M) to the bath solution (average basal frequency, 1.8 ± 0.8 Hz; average basal amplitude 15 ± 1 pA, $n = 7$). We found that methoxamine enhanced mEPSC frequency ($535 \pm 128\%$ of basal frequency, $p < 0.05$, $n = 7$) (Fig 6A–C), but had no effect on mEPSC amplitude ($112 \pm 6\%$ of basal amplitude, $n = 7$) (Fig. 6D) in RLi TH-eGFP neurons. These results indicate that the α_1 -AR induced enhancement of glutamatergic transmission in RLi TH-eGFP neurons occurs via an activity-independent mechanism and provide evidence that norepinephrine's site of action on RLi TH-eGFP neurons is presynaptic.

4. Discussion

Because of the small size of the RLi coupled with the lack of I_h in RLi dopamine neurons, a reporter method is necessary to identify these neurons for electrophysiological analysis. Thus we analyzed the properties of these neurons in a TH-eGFP line previously utilized for analysis of dopamine neurons (Li et al 2013). In an initial characterization of the basic membrane properties of eGFP positive neurons in the RLi of these transgenic mice, we found several significant differences as compared to eGFP positive lateral VTA neurons. The dopamine neurons in the RLi had a significantly greater membrane resistance when compared to the dopamine neurons in the VTA. Additionally, we found that the capacitance was significantly smaller in the RLi neurons. Finally, we found little to no I_h current in the RLi dopamine neurons. Other groups have also found a lack of I_h current in dopamine neurons with anatomical locations near the midline (Lammel et al 2011, Li et al 2013). Functionally, these properties are expected to affect differences in excitability and may influence plasticity of synaptic transmission. Our findings suggest fundamental differences in the basic membrane and excitability properties between the afferent dopaminergic projections to the BNST from the VTA and RLi. These findings raise the possibility that these neurons may exhibit different firing patterns *in vivo* and thus be regulated differently by exposure to stressors or drugs of abuse.

Norepinephrine acts as a powerful modulator of excitatory neurotransmission in many brain areas. We found that the most pronounced effect of norepinephrine on RLi dopamine neuron physiology was an increase in the frequency of sEPSCs. Since activation of β -ARs increases excitatory transmission in the hippocampus, CeA, and BNST, we attempted to mimic the effects of norepinephrine with the β -AR agonist isoproterenol (Egli et al 2005, Gereau & Conn 1994, Nobis et al 2011). However, isoproterenol had no effect on sEPSC frequency or amplitude.

In the BNST, activation of α_1 -ARs elicits long term depression of evoked glutamatergic transmission, but an increase in sEPSCs. Similarly, activation of α_1 -ARs leads to an enhancement of glutamatergic transmission onto VTA dopamine neurons (McElligott et al 2010, McElligott & Winder 2008, Velasquez-Martinez et al 2012). In the presence of the α_1 -AR antagonist prazosin, we found that norepinephrine did not increase excitatory transmission. In fact, when α_1 -ARs were blocked, norepinephrine decreased sEPSC frequency and amplitude. Since the α_2 -AR agonist UK-14,304 led to a depression of sEPSC frequency and a trend toward a decrease in sEPSC amplitude, this decrease in excitatory transmission is likely due to the activation of α_2 -ARs. Similarly, activation of α_2 -ARs decreases excitatory transmission in the BNST and onto VTA dopamine neurons (Egli et al 2005, Jimenez-Rivera et al 2012, Shields et al 2009). It is possible that this decrease in glutamatergic transmission is also due to the blockade of tonically active α_1 -ARs (Grenhoff & Svensson 1993). However, the timing of the effect coincides with norepinephrine application which supports α_2 -AR activation mediating this decrease in excitatory transmission. It is important to note that the overall effect of norepinephrine on RLi dopamine neurons is an increase in excitatory transmission and the small decrease in sEPSC frequency is surpassed by the large α_1 -AR mediated increase in sEPSC frequency.

Since the effect of norepinephrine on sEPSC frequency was blocked by the α_1 -AR antagonist, we attempted to mimic it with the α_1 -AR agonist methoxamine. Indeed, methoxamine mimicked the effects of norepinephrine on RLi sEPSC frequency. This finding is similar to that seen in VTA dopamine neurons (Velasquez-Martinez et al 2012). In the RLi, the frequency of mEPSCs increases with the activation of α_1 -ARs. The increase in mEPSC frequency indicates that α_1 -ARs may act presynaptically to increase excitatory transmission on RLi dopamine neurons and that their actions are independent of presynaptic action potentials. While activation of α_1 -ARs in VTA dopamine cells does not modulate mEPSCs, activation of α_1 -ARs in other brain regions, such as the hypothalamus, does lead to an increase in mEPSC frequency (Gordon & Bains 2003). The VTA dopamine neurons were identified via a large I_h current (> 200 pA) and are located more laterally, while the RLi dopamine neurons have very small, almost negligible, I_h current and are located along the midline. Therefore, these are entirely different populations of dopamine neurons which may differ in their response to inputs and outputs to projection targets. Both the lateral VTA and the RLi receive noradrenergic inputs from the locus coeruleus (LC) and the norepinephrine brainstem centers, A1, A2 and A5 (Mejias-Aponte et al 2009). However, the lateral VTA has a higher percentage of its norepinephrine input arising from the LC, while the RLi has a higher percentage of its norepinephrine input arising from the brainstem centers. This might lead to differences in how these two groups of dopamine neurons respond to stimuli, such as stress, that lead to the release of norepinephrine in target regions.

This study indicates a potential mechanism through which norepinephrine could recruit the activity of RLi dopamine neurons. Since activation of glutamatergic afferents mediates the firing of dopamine neurons, we would expect this α_1 -AR mediated enhancement in excitatory transmission to increase the firing of dopamine neurons and release of dopamine in target regions. Stressors have been shown to increase dopamine concentration in many target regions (Abercrombie et al 1989, Deutch et al 1991, Kalivas & Duffy 1995, Morrow et al 2000b, Sutoo & Akiyama 2002). Stress causes norepinephrine release, which our data suggests would lead to an increase in excitatory drive on RLi dopamine neurons via α_1 -AR activation. One possibility is that this drives dopamine release in target regions such as the BNST, where it may participate in reinforcement related behaviors as well as anxiety responses. Thus norepinephrine induced enhancement of excitatory drive on RLi dopamine neurons is a potential mechanism through which RLi dopamine neurons may be involved in the stress response.

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Highlights

- RLi dopamine neurons have distinct basal excitability profiles.
- Norepinephrine enhances spontaneous glutamate release onto RLi dopamine neurons.
- α_1 -AR activation enhances spontaneous glutamate release onto RLi dopamine neurons.
- α_1 -AR's effects on excitatory transmission are transient and activity independent.
- α_2 -AR activation decreases spontaneous glutamate release onto RLi dopamine neurons.

Figure 1.

RLi dopamine neurons have distinct basal excitability profiles. (A) Diagram of a coronal brain section image from Franklin & Paxinos mouse atlas showing region selected for VTA electrophysiology recordings. (B) Diagram of a coronal brain section image from Franklin & Paxinos mouse atlas showing region selected for RLi electrophysiology recordings. (C) Coronal section of the RLi corresponding to the black rectangle in B displaying eGFP fluorescence, (D) tyrosine hydroxylase immunohistochemistry, and (E) the merged image showing the co-localization of eGFP and tyrosine hydroxylase labeled neurons. The arrows

highlight examples of double labeled cells. (F) Bar graph showing the membrane resistance of eGFP positive neurons in the RLi and VTA. The eGFP positive neurons in the RLi exhibited a significantly higher membrane resistance compared to those in the VTA (** denotes $p < 0.01$). (G) Bar graph showing the capacitance of eGFP positive neurons in the RLi and VTA. The eGFP positive neurons in the RLi exhibited a significantly reduced capacitance compared to those in the VTA (**** denotes $p < 0.0001$). (H) Bar graph comparing the average peak I_h current for each group of dopamine neurons. There was a significant difference in the I_h current in the VTA dopamine neurons compared to dopamine neurons in the RLi (*** denotes $p < 0.001$). (I) A hyperpolarization voltage step in an eGFP positive neuron in the VTA leads to a robust inward current. (J) A hyperpolarization voltage step in an eGFP positive neuron in the RLi leads to a small but measureable inward current.

Figure 2.

Norepinephrine increases spontaneous glutamatergic transmission onto RLi dopamine neurons. (A) Representative sEPSC recordings in the RLi demonstrating the ability of norepinephrine (NE) to enhance glutamatergic transmission. (B) A 10 min application of 50 μ M norepinephrine increases sEPSC frequency onto RLi dopamine neurons. (C) A 10 min application of 50 μ M norepinephrine modestly increases sEPSC amplitude onto RLi dopamine neurons only during the last 5 minutes of drug application.

Figure 3.

Activation of α_1 -ARs increases sEPSC frequency onto RLi dopamine neurons. (A) A 20 min application of 100 μ M methoxamine (methox) increases sEPSC frequency onto RLi dopamine neurons. (B) A 20 min application of 100 μ M methoxamine has no effect on sEPSC amplitude onto RLi dopamine neurons. (C) In the presence of the α_1 -AR antagonist prazosin (10 μ M), a 10 min application of 50 μ M norepinephrine decreases the frequency of sEPSCs onto RLi dopamine neurons. (D) In the presence of the α_1 -AR antagonist prazosin (10 μ M), a 10 min application of 50 μ M norepinephrine modestly decreases the amplitude of sEPSCs onto RLi dopamine neurons.

Figure 4. α_2 -AR activation depresses glutamatergic transmission and β -AR activation has no effect on glutamatergic transmission onto RLi dopamine neurons. (A) A 10 min application of 1 μ M UK-14,304 decreases sEPSC frequency onto RLi dopamine neurons. (B) A 10 min application of 1 μ M UK-14,304 causes a trend toward a decrease in sEPSC amplitude onto RLi dopamine neurons. (C) A 10 min application of 3 μ M isoproterenol (Iso) has no effect on sEPSC frequency or (D) sEPSC amplitude onto RLi dopamine neurons.

Figure 5. Norepinephrine's actions on sEPSCs are transient. (A,C) The norepinephrine induced increase in sEPSC frequency does not persist when followed by a 15 min application of 10 μ M prazosin (* denotes $p < 0.05$). (B,D) The norepinephrine induced increase in sEPSC amplitude does not persist when followed by a 15 min application of 10 μ M prazosin (** denotes $p < 0.01$).

Figure 6.

Methoxamine increases mEPSC frequency but not amplitude. (A) Representative mEPSC recordings in the RLi demonstrating the ability of methoxamine to enhance glutamatergic transmission. (B) A representative experiment showing the effect of 100 μ M methoxamine on mEPSC frequency onto RLi dopamine neurons. (C) A 20 min application of 100 μ M methoxamine increases mEPSC frequency onto RLi dopamine neurons (* denotes $p < 0.05$). (D) A 20 min application of 100 μ M methoxamine has no effect on mEPSC amplitude onto RLi dopamine neurons.