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Noradrenergic modulation of activity in a vocal control nucleus *in vitro*

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Abstract

Norepinephrine (NE) can profoundly modulate sensory processing, but its effect on motor function is less well understood. Birdsong is a learned behavior involving sensory and motor processes that are influenced by NE. A potential site of NE action is the robust nucleus of the arcopallium (RA): RA receives noradrenergic inputs and has adrenergic receptors, and it is a sensorimotor area instrumental to song production. We hypothesized that NE modulates RA neurons, and as a first test, we examined the effect of NE on RA activity *in vitro*.

We recorded spontaneous activity extracellularly from isolated RA neurons in brain slices made from adult male zebra finches. These neurons exhibited regular tonic activity with firing rates averaging 5.5 Hz. Bath application of NE rapidly and reversibly decreased firing for the majority of neurons, to the extent that spontaneous activity was often abolished. This was likely a direct effect on the cell recorded, since it occurred with blockade of fast excitatory and inhibitory synaptic transmission, or of all synaptic transmission. The NE-induced suppression involved the $\alpha 2$ -adrenergic receptor: yohimbine, an antagonist, completely reversed the suppression, and clonidine, an agonist, partially mimicked it. Perforated patch recordings revealed that NE induced a conductance increase in RA neurons; however, this did not prevent cells from firing when stimulated by afferents in HVC. For some neurons, NE application resulted in an increase in signal-to-noise ratio for spikes evoked by HVC stimulation. Thus, NE could strongly modulate the spontaneous activity of RA cells, potentially enhancing signals relayed through RA.

INTRODUCTION

Noradrenergic cells project expansively throughout the brain, giving norepinephrine (NE) the potential to modulate diverse brain functions. NE can strongly modify sensory processing, increasing the salience of sensory responses (Berridge and Waterhouse 2003). In contrast, the effect of NE on motor and sensorimotor systems is less well understood. NE has been linked to increased motor neuron excitability (Fung et al. 1991), sensorimotor gating (Oranje et al. 2004; Sallinen et al. 1998), facilitation of behavioral responses (Clayton et al. 2004), and motor learning (Plewania et al. 2004). Thus, in addition to its better-known sensory role, NE can influence motor function. A recent theory proposes that NE functions to optimize task-related performance, either by facilitating responses within a task or by enabling a switch to a task with greater utility to the organism (Aston-Jones and Cohen 2005).

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To further explore the role of NE on motor outputs, it is useful to consider the song control system in oscine birds. The song system is the neural circuit controlling song behavior (Figure 1A) (Nottebohm et al. 1982; Nottebohm et al. 1976), which is a learned motor output composed of stereotyped sequences of vocalizations. The song system is widely innervated by a number of neuromodulatory inputs, including catecholaminergic and cholinergic afferents (Bottjer 1993; Lewis et al. 1981; Mello et al. 1998; Ryan and Arnold 1981; Soha et al. 1995). These neuromodulators potentially mediate the sensitivity of song behavior to social (Collins 2004; Nowicki and Searcy 2004) and seasonal (Brenowitz 2004) factors. In particular, norepinephrine (NE) modulates auditory responses in the song system (Cardin and Schmidt 2004; Dave et al. 1998). Moreover, NE has been associated with song production: decreases in NE levels decrease singing frequency levels (Barclay et al. 1996) and singing-related gene expression (Castelino and Ball 2005). The combination of a stereotyped behavior with a discrete neural circuit provides a model system for exploring the diverse roles of NE in the brain, including motor systems.

The robust nucleus of the arcopallium (RA) is a song system nucleus that is a potential site for neuromodulator-induced effects on song behavior. RA is part of the motor pathway, which connects the forebrain to the syrinx, the avian vocal organ, and to premotor areas controlling respiration (Figure 1A). RA neurons display premotor activity during singing that is notable for its precision (Chi and Margoliash 2001; Leonardo and Fee 2005; McCasland 1987; Yu and Margoliash 1996). Although RA has both auditory and premotor responses, its function is essential for vocal production: lesions effectively mute the bird (Nottebohm et al. 1976; Simpson and Vicario 1990). Activity in RA can be influenced by the anterior forebrain pathway (AFP) (Kao et al. 2005; Ölveczky et al. 2005), a pathway that is important for song learning but not for production (Bottjer et al. 1984). RA activity is also potentially modified by neuromodulators, including NE. RA receives moderately dense NE terminals (Mello et al. 1998; Sakaguchi and Saito 1989) that originate from the locus coeruleus (LC) (Appeltants et al. 2002), and RA cells have adrenergic receptors (Revilla et al. 1999; Ritters and Ball 2002). To begin to understand the influence of NE on RA activity, we studied the effect of NE on spontaneously active RA neurons *in vitro*. We found that NE markedly reduced spontaneous activity through α_2 -adrenergic receptors, and activated an increase in conductance which did not prevent spikes evoked by afferent activity.

MATERIALS AND METHODS

Slice preparation

Data were obtained from 38 adult male zebra finches (*Taeniopygia guttata*) purchased from suppliers. All procedures were in compliance with a protocol approved by the Institutional Animal Care and Use Committee of the University of Washington. Birds were anesthetized with isoflurane and decapitated. The brain was quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 119 NaCl, 2.5 KCl, 1.3 MgSO₄, 2.5 CaCl₂, 1 NaH₂PO₄, 16.2 NaHCO₃, 11-D glucose, and 10 HEPES (osmolarity 275–290 mOsm). Parasagittal or coronal slices 350–400 μ m thick were cut with a vibrating microtome in ice-cold ACSF, then transferred to a storage chamber containing ACSF heated to 35°C. Once slicing was completed, the storage chamber was allowed to cool to room temperature. The storage and recording ACSF was the same as the slicing ACSF, except that the HEPES was replaced with equiosmolar NaHCO₃; this solution is also referred to as “control Ringer” in the Results. All solutions were bubbled with a 95% O₂ and 5% CO₂ mixture.

Electrophysiology

Recording began >1 hour after slices were made. For recording, a slice was submerged in a small chamber perfused with the HEPES-free ACSF at 2 mL/min. ACSF temperature was

maintained at 30°C. RA, HVC, and the axon tract between them are visible in brain slices when trans-illuminated and viewed with a microscope.

Extracellular and perforated patch techniques were used to record RA cells. Both types of electrodes were pulled with a Sutter Instruments (Novato, CA) P-97 micropipette puller. Extracellular recordings were made using glass pipettes pulled to tip widths of 5–10 μm . The pipettes were filled with a 0.9% NaCl solution, and the resulting resistances ranged from 3 to 10 M Ω . Single units were isolated, and their waveforms had a signal-to-noise ratio of at least 3. Voltage signals were initially amplified with an Axoclamp 2B (Axon Instruments, Foster City, CA), then low-pass filtered (5 kHz) and further amplified with a Brownlee Model 410 amplifier (San Jose, CA). Total signal amplification for extracellular recordings was 1000x. The filtered signals were digitized at 10 kHz with a National Instruments (Austin, TX) digitizing board and stored on a PC using customized data acquisition programs written in LabView (National Instruments) by M. Farries (University of Washington) and D. Perkel. Data were collected during 5 s long trials with 5 s between each trial (except for experiments in which NE was pressure-applied locally; see below). Similar extracellular recording methods in RA slices have been used by Park et al. (2005).

Current clamp recordings were made with the gramicidin-perforated patch method (Rhee et al. 1994). Glass electrodes were pulled to a tip width <2 μm , and the tip of the pipette was filled with internal solution that consisted of (in mM) 120 Kmethysulfate, 10 HEPES, 2 EGTA, 8 NaCl, 2 ATP, 0.3 GTP and 1 MgCl₂; pH was 7.3 and osmolarity was 0–5% less than the ACSF osmolarity. The rest of the pipette was filled with the same internal solution supplemented with a gramicidin solution: gramicidin (Sigma; St. Louis, MO) stock solution was made fresh in dimethyl sulfoxide (DMSO) (Fisher Scientific; Fair Lawn, NJ) at a concentration of 0.1–0.3 mg/mL; this solution was then added to the internal solution to make a final concentration of 0.1–0.3 $\mu\text{g/mL}$. Final electrode resistances were 5–8 M Ω . Once a gigaohm seal was achieved using the blind patch technique (Blanton et al. 1989), the recorded potential stabilized within 10 minutes, and the series resistance stabilized at ~200 M Ω within 20 minutes. Input resistance and resting potential were monitored throughout the experiment, and a cell was not included in the data set if either varied by >20%. In some cases, 10 mM biocytin (Vector Laboratories, Burlingame, CA) was included in the internal solution for histological identification of the cells recorded. To allow access of the biocytin to the cell, at the end of the experiment the patch was ruptured with gentle negative pressure. Current clamp voltage signals were amplified 100x, low-pass filtered at 3 kHz, and digitized at 6 kHz.

For stimulation experiments, a stimulating electrode was placed within HVC or immediately ventral to HVC within the HVC-RA fiber tract. The stimulating electrode was either a stainless steel bipolar electrode or a platinum/iridium concentric bipolar electrode (FHC, Bowdoinham, ME). Single stimulus pulses to HVC or the HVC-RA fiber tract were delivered until a spike was consistently evoked from an RA cell (recorded extracellularly) within 5 ms; across cells, the average latency between stimulus pulse onset and the spike (measured at the maximum negative-going deflection) was 3.3 ms (SD=0.8). The minimum stimulus intensity required to obtain consistent spiking was used. This averaged 33.8 V (SD=19.0). Once evoked spikes were obtained, short, high-frequency stimulus trains were delivered consisting of 3 trains of 100 μs long monophasic pulses delivered at 100 Hz for 20–30 ms (i.e., 3 or 4 pulses per train, respectively); the interval between each train was 1 sec. An example of this stimulus is illustrated in Figure 10B. Spikes evoked by this stimulation were counted as those spikes occurring within 5 ms of each stimulation pulse. The total number of spikes (evoked or not) was counted during a period circumscribed by the first pulse of the first train and the last pulse of the third train (+ 5 ms).

Drugs used in these experiments included 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Tocris Cookson, Ellisville, MO), 2-amino-5-phosphonovaleric acid (APV) (Tocris), kynurenic acid (KA) (Sigma, St. Louis, MO), picrotoxin (PTX) (Sigma), CdCl₂ (Sigma), norepinephrine (NE) (Sigma), yohimbine (Sigma), and clonidine (Sigma). Unless otherwise noted, drugs were bath applied. We marked the start of drug application as the first trial with any drug present. The duration of bath application of the drug varied between cells (range: 1.4–11.4 minutes).

Local application of NE was made to four cells using a glass pipette with a 10 µm tip diameter that was filled with 10 mM NE and placed within 100 µm of the recording electrode. The NE solution was then pressure applied for brief durations (10–40 ms) at 10–14 PSI using a Pressure System IIe (Toohey Company; Fairfield, NJ). This resulted in local application of approximately 100–200 nL of the NE solution near the cell. \ For these experiments, trial duration was extended up to 20 s.

Histology

In cases in which cells were filled with biocytin, slices were immersion-fixed in paraformaldehyde (4% in 0.1 M phosphate buffer) and kept at 4°C at least overnight. Slices were subsequently cryoprotected in a sucrose solution (30% in 0.1 M phosphate buffer) and stored at 4°C overnight. Slices were then resectioned to 40 µm thickness with a freezing microtome, and processed for visualization with an avidin-biotin horseradish peroxidase complex kit, (Vector ABC Elite Kit; Vector Laboratories, Burlingame, CA) using diaminobenzidine as the peroxidase substrate. Sections were counterstained with cresyl violet.

Data analysis

Spikes were detected using procedures written in IGOR 4.0 (Wavemetrics, Lake Oswego, OR) by M. Solis. Spike detection depended on two parameters set by the user: 1) a minimum height of the differentiated waveform (expressed in SDs of the entire waveform), which constrained the initial slope of an acceptable spike waveform, and 2) a minimum amplitude of the spike. If a spike met both criteria, the user was prompted to accept or reject the spike. The user could compare the spike's height and width to previously accepted spikes in making this decision. These parameters were maintained for detecting post-suppression spiking. Thus, this offline spike discrimination could further confirm that the recording came from a well-isolated cell. All cells used in this study had stable spike waveforms during a baseline period of 5 minutes: their spike amplitudes had a coefficient of variation (CV) of 0.10 or less (mean=0.07, SD=0.02, n=108).

When spiking resumed after a period of NE-induced suppression, we verified that we were recording from the same cell. For this subset of cells (n=53), the same parameters for spike detection were used both prior to and following NE-induced suppression. In comparing pre-suppression spike amplitude to post-suppression spike amplitude, the average percent change in amplitude was 8.5% (SD=8.1, n=53). 89% of these cells had a percent change in amplitude less than 20%; 74% had a percent change in amplitude less than 10%. For cells with percent changes in amplitude greater than 20%, we verified that they were the same cell based on spike shape, and the lack of other units in the recording. If this subset of cells was eliminated from this study, our findings would not change.

Once spikes were detected, spike frequency and the coefficient of variation (CV) of frequency were measured for each trial. Spike frequency was calculated as the mean of the reciprocals of the individual intervals between spikes (i.e., average instantaneous frequency). The CV was the SD of each frequency measurement divided by the mean. To assay the effect of drugs on these parameters, values were averaged from trials collected during the last minute prior to

drug application and compared to the averaged values from the trials collected during the last minute of drug application. Unpaired t-tests (IGOR) determined whether these average values were significantly different from each other for a single cell. Effects of drug were described as increases or decreases in firing only when this test determined a significant difference from control. The change induced by a drug was calculated as a percent change relative to predrug values: $(\text{drug value} - \text{predrug value}) / \text{predrug value}$. Positive values indicated increases, negative values indicated decreases, and values near 0 reflected no change relative to control values.

For perforated patch recordings, we measured input resistance by delivering hyperpolarizing current pulses (1 s long). The steady-state voltage was measured during the last 200 ms of the current pulse, and compared to 200 ms of baseline just prior to current pulse delivery. These values were used to construct IV plots.

Statistical analyses of cell populations were done with Prism (GraphPad Software, San Diego, CA); tests were two-tailed. Parametric tests were used for distributions that passed the Kolmogorov-Smirnov goodness of fit test for normality; otherwise, non-parametric methods were used.

A cluster analysis was used to determine whether the effects of NE on RA cells obtained from the same bird were more similar than expected by chance. To do this, the variance of the NE-induced percent change in frequency values obtained from one bird was compared to a simulated distribution of variances that resulted from random draws from the pool of all percent change in frequency values obtained from all birds. The distribution was constructed from 100 Monte Carlo simulations, which randomly selected n percent change in frequency values from the entire pool of values, where n equals the number of cells recorded in each bird. The median of the simulated variance distribution was compared to the variance of the values obtained from the bird, using a one-sample test (one-tailed). If the experimental variance was significantly less than the median variance of the simulated distribution, then the values for that bird were considered “clustered.” This procedure was repeated for each bird.

RESULTS

Extracellular recordings of the spontaneous activity of 108 isolated RA neurons (median peak-to-peak spike height was 0.52 mV) were made in the brain slices. As previously reported *in vivo* (Adret and Margoliash 2002; Yu and Margoliash 1996) and *in vitro* (Mooney 1992), RA neurons exhibited spontaneous activity that was characterized by regular inter-spike intervals (Figure 1B). The mean firing rate in control Ringer was 5.5 Hz (SD=2.8, range: 1–16 Hz, $n=88$) and mean CV of firing rate was 0.15 (SD=0.12, $n=88$). Figure 1C plots the mean firing rate and CV for each cell, revealing an indirect relation between the two; however, no distinct clusters emerged in this plot, suggesting that these recordings were made from a fairly homogeneous population of RA cells with respect to spontaneous activity.

Effects of NE on spontaneous activity in RA

The predominant effect of NE on RA neurons was to suppress spontaneous activity: 75% of cells significantly decreased their firing. Figure 2A illustrates a case in which 10 μM NE applied to the bath abolished the cell's firing quickly and reversibly. Comparisons of pre- and post-suppression spike waveforms verified that the same cell was recorded upon washout of NE (Figure 2B and C). In all, 47.9% (23/48) of cells tested exhibited complete suppression of their firing in NE. The effect of NE on all cells is plotted in Figure 2D: in addition to complete suppression, NE decreased (but did not abolish) firing, increased firing, or did not change firing rates (Table 1). This range of effects was quantified by calculating the percent change in firing rate in NE relative to control (see Methods). The resulting percentage is plotted for each cell in the summary graph in Figure 8 (“NE” column). For the population of cells tested, NE caused

a significant reduction in firing rate, from an average of 4.7 Hz in control conditions to an average of 2.0 Hz in the presence of NE (Figure 2E; paired t-test, $p < 0.0001$; $n=48$). We investigated whether the NE-induced suppression resulted from a direct effect of NE on the cell recorded, or from an indirect effect produced by other cells. Thus, we examined the effect of NE under two conditions: when fast excitatory and inhibitory transmission was blocked with 1 mM kynurenic acid (KA) and 150 μ M picrotoxin (PTX), or when synaptic transmission altogether was blocked with 100 μ M CdCl₂. Under both conditions, the suppressive effect of NE was maintained. Figure 3A shows an example in which NE abolished the spontaneous firing of an RA cell in the presence of 100 μ M CdCl₂. The scatter plot in Figure 3B shows the effect of all applications of NE to cells under these two conditions. Half of the cells tested under these conditions showed complete suppression of their firing rates in the presence of NE (30/60 cells), and for the population there was a significant decrease in firing in the presence of NE (Figure 3C; paired t-test, $p < 0.0001$; $n=60$). As for experiments conducted with control Ringer, neurons exhibited a range of responses, including significant decreases, increases, and no changes in their firing rates in response to NE (Figure 3B and Table 1). This range of effects is also shown in Figure 8 (“NE in KA+PTX” and “NE in CdCl₂” columns) where the percent change in firing rate in NE for each cell is plotted. The average percent change in frequency was -63% for NE, -50% for NE in KA+PTX, and -96% for NE in CdCl₂. The effect of NE did not differ across all three conditions (i.e., first 3 columns in Figure 8; Kruskal-Wallis test, $p < 0.1080$). For the subset of neurons with significant reductions in firing, the average percent change in frequency was -82% ($n=23$) for NE in control Ringer, -85% ($n=35$) for NE in KA+PTX, and -96% for NE in CdCl₂ ($n=8$). The measurements of spike frequency in the presence of NE were taken during the last minute of drug application, and thus reflect a stabilized change in spike frequency. The duration of drug application averaged 4.3 min ($SD=1.8$, $n=108$), but varied from cell to cell. To check for transient changes in spike frequency to NE application, and to compare responses across cells at the same time point, we also measured the frequency of RA spiking during the second minute of NE application. These “early” spike rates were compared to the later spike rates previously measured for each cell in Figure 3D. Whereas some cells showed slightly higher or lower spike rates at this earlier time point than the later measurement, overall there were no consistent differences between these two time points ($p < 0.8893$, paired t-test; $n=98$); some cells were not included in this analysis because NE application in these cases was not longer than 2 minutes. Similarly, the average percent change in frequency at this early point of NE application was -42% relative to control (“early NE” column in Figure 8).

For neurons that decreased but maintained spontaneous activity in the presence of NE, there was a decrease in spiking regularity. For these cells, there was a significant two-fold increase in CV during NE application: the mean CV was 0.13 ($SD=0.18$) prior to NE application (includes all Ringer types) and 0.30 ($SD=0.20$) in NE ($p < 0.0058$, paired t-test; $n=28$). For those cells with significant changes (increases or decreases) in firing rate to NE, the relationship between frequency and CV was similar to that in control Ringer (Figure 3E); thus, spontaneous firing rate and regularity covaried, and may be similarly regulated.

Next we examined whether those cells that did not decrease their firing rates in NE had a suppressive response to higher doses. When exposed to higher concentrations of NE (ranging from 20 to 100 μ M), these cells maintained their spontaneous activity levels (Figure 4A). The average spontaneous rate of these cells slightly increased in response to high doses of NE relative to that measured for 10 μ M NE (Figure 4B; paired t-test, $p < 0.0235$; $n=12$). There was no effect of the maximum dosage on firing CV (Figure 4C; paired t-test, $p < 0.3649$; $n=12$). Thus, a subpopulation of RA cells could not be induced to exhibit a suppressive response to NE.

The response of a cell to NE was not predictable from its baseline firing rate, its firing regularity (as measured by CV), or the individual bird from which it came. No correlation was found between the percent change in firing rate in NE and the baseline firing rate for each cell ($r^2=0.027$, $p<0.1016$). Similarly, there was no clear relation between the percent change in NE and CV for each cell ($r^2=0.003$, $p<0.5970$). Finally, neural responses to NE did not vary according to bird. Figure 4D plots the percent change in NE for each cell as a function of bird; in several birds, cells with a suppressive response coexisted with cells that did not (e.g., bird 2). A cluster analysis (see Methods) did not find significant clustering of the percent change in NE values obtained from each bird. Thus, within the population of spontaneously active neurons in RA, there were distinct differences in responsiveness to NE.

NE-induced suppression was mediated by $\alpha 2$ -adrenergic receptors

Given the extensive evidence for $\alpha 2$ -adrenergic receptors in RA (Ball 1994; Casto and Ball 1996; Ritters and Ball 2002; Ritters et al. 2002), we investigated their role in the NE-induced suppression using yohimbine, a selective $\alpha 2$ -adrenergic receptor antagonist. Yohimbine readily reversed the suppressive effect of NE (Figure 5A). This was true for all 11 cells tested (Figure 5B). The firing rate in the presence of NE+yohimbine (1 or 5 μM) was significantly greater than the firing rate in the presence of NE alone (Figure 5B; paired t-test, $p<0.0001$; $n=11$). Thus, the suppression of spontaneous activity by NE was mediated by $\alpha 2$ -adrenergic receptors.

We also noticed that the firing rate in the presence of NE+yohimbine exceeded that of baseline (measured prior to NE application): there was a statistically significant difference between the rates measured under baseline conditions and those measured with both NE and yohimbine present (Figure 5B; paired t-test, $p<0.0006$; $n=11$). This supra-recovery was also apparent in the percent change in frequency in NE+yohimbine for all cells ("NE+yoh" column in Figure 8): the average was 140%. This suggested that, in addition to antagonizing the exogenously applied NE, yohimbine may have blocked the effects of endogenously released NE.

To test this, we monitored the effect of yohimbine alone on the firing rates of RA cells that were previously determined to suppress their firing in NE. Following NE washout, application of yohimbine alone did not increase firing rates relative to baseline (pre-yohimbine) levels ($n=14$). Figure 5C compares the baseline firing frequency for each cell to the frequency in the presence of yohimbine alone; most points lie along the diagonal line, which indicates similar firing rates under both conditions. Overall, there was no significant difference between baseline firing rates and those measured in the presence of 1 or 5 μM yohimbine alone ($p<0.6044$, paired t-test; $n=14$). This lack of change in yohimbine alone was also reflected in the percent change in frequency in the presence of yohimbine alone ("yoh alone" column in Figure 8), which averaged 12%. Moreover, significant effects were not apparent when the two concentrations of yohimbine were considered separately (paired t-tests between control and yohimbine firing rates; for 1 μM $p<0.9162$, $n=8$ and for 5 μM $p<0.3396$, $n=6$). Thus, we found no evidence for endogenous NE-induced suppression of firing rates.

Another possible explanation is that the supra-recovery observed with NE+yohimbine treatment reflected suppression-induced plasticity in spontaneous firing rate (Nelson et al. 2003). To examine this, we compared each cell's firing rate prior to NE application to its recovered firing rate upon washout of NE (as defined as the rate at five minutes following the return of spiking); both the pre-NE and recovery time points are illustrated in Figure 6A. Only cells with NE-induced suppression were included in this analysis. Figure 6A shows an example of a neuron that had a slightly (and significantly) elevated firing rate after recovery from NE-induced suppression. Its pre-NE firing rate was 5.4 Hz, whereas upon recovery its rate was 6.8 Hz; this amounted to an increase of 25.9% relative to baseline. Many cells had similar elevations during recovery, as illustrated in Figure 6B, which plots pre-NE baseline rates against recovery rates. Significant increases were observed for 48.8% of cells (20/41), and for

the population there was a significant increase in firing rate upon recovery from NE effects (Figure 6C) ($p < 0.0242$, paired t-test; $n = 41$). This constituted a 39.8% average increase relative to baseline (“post NE” column in Figure 8). Whether a cell showed an elevated firing rate during recovery was not apparent from the amount of time NE was applied (percent change in frequency during recovery vs. minutes NE applied; $r^2 = 0.046$, $p < 0.1791$) nor from the degree of NE-induced suppression (percent change in frequency during recovery vs. percent change in frequency in NE; $r^2 = 0.0596$, $p < 0.1242$). There was an inverse relationship between the baseline firing rate and the percent change in firing during recovery from NE: cells with lower firing rates were more susceptible to supra-recovery than cells with higher firing rates. This trend was weak, however ($r^2 = 0.1356$, $p < 0.0179$). Regardless, washout from the suppressive effects of NE was associated with increases in firing rates.

Because the NE-induced suppression was blocked by yohimbine, we tried to mimic the effect of NE with a selective α_2 -adrenergic receptor agonist, clonidine. Clonidine reliably decreased the spontaneous firing rates of RA cells, as illustrated by the cell in Figure 7A. All cells showed a decrease in the presence of 10 or 50 μM clonidine (Figure 7B); for 18/19 cells (94.7%) this decrease was significant. Across the population, clonidine caused a significant decrease in firing rate relative to baseline levels ($p < 0.0006$, paired t-test; $n = 19$), and the average percent change in frequency was -47.2% (“clonidine” column in Figure 8). For the subset of cells with a significant reduction in the presence of clonidine, the average percent change in frequency was -48.3% . Thus, consistent with a role for α_2 -adrenergic receptors, clonidine mimicked the suppressive effect of NE. Unlike NE, however, clonidine rarely abolished spontaneous activity of RA cells (Figure 7B and Figure 8). Clonidine eliminated firing in 5.3% (1/19) of cells tested, whereas NE eliminated firing in 49.1% (53/108) of cells tested; this difference in incidence was significant ($p < 0.0003$, Fisher’s exact test). Finally, clonidine did not significantly alter spiking regularity, as measured by the CV of firing frequency ($p < 0.1054$, Wilcoxon; $n = 19$).

Given the range of responses to NE, we directly compared the response of a cell to NE with its response to clonidine (Figure 7C). In these experiments, NE application preceded clonidine application, since effects of clonidine were not readily reversible. After NE was applied, a period of washout allowed spiking to resume its pre-drug frequency and regularity (on average 8 minutes post NE application); clonidine application followed. We found no significant difference in the average percent change in frequency elicited by NE and by clonidine ($p < 0.1280$, paired t-test; $n = 14$); however, clonidine and NE did not have identical effects in the same cell. For example, cells that lost all spontaneous activity in the presence of NE maintained firing in the presence of clonidine, albeit at a decreased level relative to control. Moreover, three cells that slightly increased their firing in response to NE had a suppressive response revealed in the presence of clonidine. This raised the possibility of roles for other adrenergic receptor types in regulating spontaneous activity in RA. Overall, clonidine mimicked the suppressive effect of NE, but not to the same degree.

The NE-induced suppression involved a conductance increase

To determine whether the NE-induced suppression involved a change in conductance, we made perforated patch recordings from spontaneously active RA cells. Perforated patch recordings maintained the spontaneous activity of RA neurons more reliably than whole-cell recordings, which allowed us to assay the effect of NE on spontaneous activity as well as on conductance. The top panel of Figure 9A shows the average voltage responses of one cell to a -20 pA current pulse under control conditions and in the presence of 10 μM NE. The bottom panel compares the neuron’s responses to multiple hyperpolarizing current pulses in control conditions and in NE in an IV plot. NE induced a decrease in input resistance for this cell, as indicated by the decrease in slope of the IV relationship in NE. Similarly, increases in conductance were measured for all cells that significantly decreased their firing in the presence of NE; the average

increase in conductance was 1.59 nS (SD=1.08, n=10). This was equivalent to a 81.4% increase relative to control conductance; the individual values for all 10 cells are shown on the ordinate of Figure 9B. The degree of conductance change tended to be larger for cells with higher baseline firing rates (Figure 9B); however, this was not statistically significant ($r^2=0.2827$, $p<0.1138$). Furthermore, there was no significant correlation between the percent change in conductance and percent change in firing rates induced by NE.

NE also decreased excitability for three cells that were tested with depolarizing current pulses. This is illustrated by the voltage responses in the top part Figure 9C, where a +40 pA current pulse evoked spiking under control conditions, but not in the presence of NE. Spiking was nearly eliminated in the presence of NE for all cells tested (Figure 9C). For each cell, the decreases in excitability were significant: when matched for current pulse amplitude and membrane potential, the number of spikes elicited under control conditions was significantly less than the number evoked in the presence of NE (paired t-tests, $p<0.01$). Thus, NE also decreased excitability in RA cells.

The NE-induced conductance had a reversal potential consistent with a K^+ conductance. The reversal potential for the NE-induced conductance in the cell shown in Figure 9A was -81 mV; the average across all cells was -82.8 mV (SD=21.01, n=10). Also consistent with a K^+ conductance, NE induced a slight hyperpolarization in some cells, as indicated by the positive holding current required in NE to match V_m to control levels (mean=22.5 pA, SD=12.6; n=4 cells that hyperpolarized). Still, some cells had less negative reversal potentials that might also reveal a role for Cl^- conductances (range -65.4 to -135.5 mV). Five cells were filled with biocytin after rupturing the patch at the end of the experiment; each cell had thick spinous dendrites, consistent with RA projection neuron morphology (Spiro et al. 1999).

Finally, we examined whether hyperpolarization of RA cells could induce long-lasting increases in firing rate (Nelson et al. 2003). Four cells were hyperpolarized with negative holding current to a level that eliminated spontaneous activity for ~two minutes. Upon release of hyperpolarization, there was an average increase of 4.1% in firing rates relative to control, but this did not reflect a significant difference between pre- and post-hyperpolarization firing rates (paired t-test, $p<0.2385$; n=4). Thus, at least short periods of hyperpolarization did not enhance firing rates.

Spikes could be synaptically evoked from RA neurons silenced by NE

NE could have rapid effects on spontaneous activity in RA. Figure 10A shows the effect of a 25 ms puff of 10 mM NE from a pipette positioned near a neuron recorded extracellularly. The cell lost its spontaneous activity within two seconds of the puff, and resumed firing in less than 10 seconds. This effect was blocked by yohimbine. NE was similarly fast-acting and reversible for three other cells, indicating that it is capable of operating on a timescale of seconds.

Despite elimination of spontaneous activity by NE, RA neurons retained the capacity to fire action potentials. Stimulation of HVC, a source of afferent input to RA, in the same slice could induce NE-silenced cells to fire (see ticks enclosed by box in Figure 10A). Figure 10B shows these evoked spikes in more detail. When 3 trains of 100 Hz stimulus pulses were delivered to HVC, the RA neuron followed with nearly one spike per stimulus pulse. Thus, NE did not block the ability of HVC inputs to drive action potential firing in RA neurons. This was true in 3 other cells tested.

These results raised the possibility that NE could function to raise the signal-to-noise ratio of evoked RA activity through elimination of spontaneous activity. For example, in cell 1 of Figure 10C the ratio of evoked spikes (those occurring during HVC stimulus trains; see Methods) to the total number of spikes (counted between the first and last stimulus pulse)

under control conditions was 0.55, whereas the ratio in the presence of NE was 0.93. This change represents a 60.5% increase in the signal-to-noise ratio. In four cells tested, NE induced a mean increase in signal to noise ratio of 62.1% (SD=47.4). One of these cells, however, showed a negligible increase in signal-to-noise of 1.5% in NE ("cell 2" in Figure 10D). The lack of a strong increase in this case was due to stimulation-induced silencing of spontaneous activity under control conditions. This suggests that when RA neurons are driven strongly, intrinsic (e.g., after hyperpolarizations) or polysynaptic mechanisms may also suppress spontaneous firing.

The increase in signal-to-noise ratio was accompanied by a decrease in signal: the number of evoked spikes in NE was significantly less than the number evoked under control conditions in 3 out of 4 cells ($p < 0.05$, unpaired t-tests). This probably reflects the increased conductance and decreased excitability that RA cells experienced in the presence of NE.

DISCUSSION

We found that NE modulated the neural activity of vocal premotor cells *in vitro*. NE suppressed spontaneous activity in RA, either decreasing the firing rate or abolishing firing altogether. This suppression likely resulted from a direct effect of NE on the cell recorded, and was mediated by α_2 -adrenergic receptors. The NE-induced suppression involved an increase in conductance, which did not prevent RA neurons from firing when activated by HVC afferents. This could result in increased signal-to-noise ratio for evoked spiking in RA.

Mechanisms of NE action

The predominant effect of NE was to suppress spontaneous activity in RA. This was likely mediated by α_2 -adrenergic receptors because the suppression was blocked by the antagonist yohimbine and partially mimicked by the agonist clonidine. This pharmacology agrees with previous studies that have localized α_2 -adrenergic receptors to RA (Riters and Ball 2002). Given the moderate noradrenergic projections to RA (Appeltants et al. 2002; Mello et al. 1998), NE is the likely ligand for these receptors. However, a role for dopamine cannot be ruled out since it can also activate adrenergic receptors (Cornil et al. 2002; Malenka and Nicoll 1986), and is prevalent in RA as well (Harding et al. 1998; Sakaguchi and Saito 1989).

The inability of clonidine to consistently abolish firing, even in RA cells that were silenced by NE, is puzzling. One possibility is that a complete loss of spontaneous activity requires coordinated activation of multiple adrenergic receptor subtypes; however, this seems unlikely because yohimbine completely reversed such a loss of activity in NE. Alternatively, the disparate actions of yohimbine and clonidine may reflect small differences in their affinities for α_2 -adrenergic receptor subtypes: in mammals, yohimbine has highest affinity for the α_2C subtype, whereas clonidine has highest affinity for the α_2A subtype (MacDonald et al. 1997). This difference could be more pronounced in avian adrenergic receptors. Nevertheless, studies in other species have found a similar inability of clonidine to mimic fully α_2 -adrenergic receptor mediated effects (Blanton and Kriegstein 1992; Liu and Alreja 1998; Williams and Reiner 1993), which may indicate the existence of a novel α_2 -adrenergic receptor subtype. A third possibility is that the action of clonidine on imidazoline receptors could interfere with the suppressive effect of α_2 -adrenergic receptors activation, although previous studies have found only a synergistic effect between these two receptor types (Georges and Aston-Jones 2003; Georges et al. 2005).

There was some heterogeneity in RA cellular responses to NE. While the majority of neurons decreased their firing in response to NE, other neurons showed no change or even slight increases in their firing rates. Similar to this latter type, RA neurons recorded *in vivo* in anesthetized birds did not suppress their firing in response to NE infusion (Dave et al. 1998);

however, it is difficult to compare these results with ours given the difficulty in knowing NE concentration near cells recorded *in vivo*. The heterogeneous responses to NE in our study could be due to RA neurons that had different complements of adrenergic receptor types. For example, those cells lacking a suppressive response to NE may have lacked the α_2 -adrenergic receptor, or had in addition other receptor types that counteracted the effect mediated by α_2 -adrenergic receptors. The latter possibility was raised by three cells that did not exhibit NE-induced suppression, yet decreased their firing when tested with clonidine (Figure 7C). Although not tested for in this study, there is some evidence for β -adrenergic receptors in RA (Revilla et al. 1999). The supra-recovery observed under NE+yohimbine conditions could also be interpreted as reflecting the actions of other receptor types: these would increase firing rate, and the effect would be more evident in the absence of α_2 -mediated suppression.

Another potential source of response heterogeneity is androgen level: α_2 -adrenergic receptor binding is seasonally regulated in starlings, and is sensitive to androgen levels (Riters et al. 2002). Although zebra finches are not seasonal birds, differences in their androgen levels could change the density of α_2 -adrenergic receptors in RA between individual birds, resulting in heterogeneity of responses to NE either within or between birds. Although the effect of NE on RA cells did not appear to vary according to bird in our dataset, this remains a possibility given the restricted sampling of neurons in each bird.

The NE-induced suppression of spontaneous activity in RA was mediated by a conductance increase. The average reversal potential for the NE-induced conductance was consistent with a K^+ conductance, although reversal potentials for some cells also suggested a role for a Cl^- conductance. Similar α_2 -adrenergic receptor-mediated increases in K^+ conductance are found in several brain areas, including locus coeruleus (Aghajanian and VanderMaelen 1982; Egan et al. 1983), brainstem cholinergic neurons (Williams and Reiner 1993), hypothalamus (Li and van den Pol 2005), septum (Liu and Alreja 1998), and cerebral cortex (Blanton and Kriegstein 1992). Thus, the action of NE described here on avian forebrain neurons is shared across diverse brain areas and taxa.

Consequences of NE action in RA

The RA neurons modulated by NE *in vitro* were similar to those with auditory and premotor activity *in vivo* in that they displayed regular spontaneous activity. Although the firing rates of RA neurons in our slice preparation had lower frequencies than reported *in vivo* (Adret and Margoliash 2002; Dave et al. 1998; Leonardo and Fee 2005; Vicario and Raksin 2000; Vicario and Yohay 1992; Yu and Margoliash 1996), the activity *in vitro* was still marked by regularity. The lower firing rates in this study likely reflect differences between *in vitro* and *in vivo* preparations, including synaptic drive, temperature, and oxygenation. Regarding the cell types modulated by NE in this study, spontaneous activity is a property shared by both projection neurons and interneurons (Spiro et al. 1999; see Figure 2 legend). Although we cannot rule out the possibility that some of the neurons in this study were interneurons, we think the majority were projection neurons given the regularity of their spontaneous activity and the morphology of the subset of filled cells (Mooney 1992; Spiro et al. 1999). Thus, NE-induced suppression could profoundly influence the auditory and premotor output of the nucleus.

NE-mediated increases in signal-to-noise ratio of sensory responses have been found in the auditory (Foote et al. 1975), visual (Kasamatsu and Heggelund 1982), and somatosensory (Waterhouse and Woodward 1980) systems. These resulted from decreases in spontaneous activity and/or increases in evoked activity (Madison and Nicoll 1982). In the song system, a similar role has been found for NE in modulating the salience of auditory signals. NE application to the forebrain nucleus interface of the nidopallium (NIf) increases or decreases auditory responses in a dose-dependent manner (Cardin and Schmidt 2004). Similarly, NE could increase the salience of signals within RA, either through decreasing spontaneous

activity, increasing evoked activity, or both. The increases in signal-to-noise ratio that we observed for RA cells resulted from the loss of spontaneous activity alone. Although NE also decreased evoked activity and excitability, this reduction in signal was outweighed by the decrease in noise, resulting in an increased signal-to-noise ratio overall. One cell showed a loss of spontaneous activity between bouts of HVC stimulation under control conditions (in the absence of NE), suggesting the activation of an afterhyperpolarization (Spiro et al. 1999) or inhibitory circuitry when RA neurons are strongly driven by HVC. Thus, a combination of NE, intrinsic, and synaptic properties could contribute to increasing the gain of auditory or premotor signals relayed through RA.

Upon release from the effect of NE (either during washout of NE or during yohimbine+NE application), enhanced firing rates relative to pre-NE levels were observed. This raised the possibility that a prolonged loss of spontaneous activity could cause a compensatory increase in firing rate, as in the vestibular nucleus, where prolonged hyperpolarization leads to firing rate plasticity (Nelson et al. 2003). Although we found no lasting effect of short hyperpolarizations on the firing rates of a subset of RA neurons, future experiments may reveal a similar type of plasticity.

Implications for behavior

LC activation is related to the state of arousal of an animal (Berridge and Waterhouse 2003), and has been associated with awake states (Aston-Jones and Bloom 1981), processing of salient sensory cues (Aston-Jones et al. 1994), and execution of motor responses after simple decisions (Aston-Jones and Cohen 2005; Clayton et al. 2004). In songbirds, NE gates state-dependent auditory responses in the song system (Cardin and Schmidt 2004; Dave et al. 1998). Because RA is a sensorimotor nucleus, NE release in RA could modulate both auditory and premotor activity during singing. Consistent with this, wide-scale NE depletion decreases singing frequency (Barclay et al. 1996) and modulates singing-related gene expression (Castelino and Ball 2005).

The NE-induced suppression we found *in vitro* is reminiscent of the diminished spontaneous activity that occurs in RA *in vivo* during the transition from tonic to burst firing. During singing, RA cells reduce their tonic, spontaneous firing and produce premotor bursts that are remarkably precise (Chi and Margoliash 2001; Dave et al. 1998; Leonardo and Fee 2005; Yu and Margoliash 1996). Similar changes also occur during auditory responses in anesthetized birds, but they are less pronounced (Dave et al. 1998; Shea and Margoliash 2003). NE could contribute to this transition to burst firing, thus promoting enhanced signaling through RA. To explore whether NE contributes to this transition, and to better understand its effect on premotor activity, it will be important to block NE action in RA during singing.

Neuromodulators endow complex behaviors with flexibility, fine-tuning their execution to be appropriate for particular contexts. For example, NE may contribute to the sensitivity of song behavior to social cues. When birds sing to other birds, they produce “directed” song, which is a type of highly aroused and stereotyped song. In contrast, “undirected” song is produced when a bird is by itself, and is characterized by variability in certain song features (Sossinka and Böhner 1980). This variability is driven by AFP activity, in particular the lateral magnocellular nucleus of the anterior nidopallium (LMAN) (Kao et al. 2005). Thus, directed song could represent a configuration of the song system circuit in which the AFP does not influence RA activity. NE could induce such a configuration: NE can abolish LMAN synaptic inputs onto RA cells *in vitro* (Perkel 1995), thus diminishing AFP influence on the motor pathway. This effect, combined with the increased signal-to-noise ratio for HVC input on RA activity reported here, could selectively constrain and enhance signaling through the motor pathway during singing, and thus contribute to the behavioral stereotypy characteristic of directed song.

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Figure 1.

Song system schematic and RA properties. A) The song system consists of two main pathways: the motor pathway shown in black and the anterior forebrain pathway (AFP) in white. RA lies within the motor pathway, which connects to motor neurons controlling the syrinx (nXIIts) and premotor neurons involved in respiration (RAm and PAm). B) Voltage trace of spontaneous activity recorded extracellularly from a cell in a slice preparation of RA. The CV of the spike amplitude during a 5 minute baseline was 0.06, indicating a stable recording. C) There was an inverse relationship between spike frequency and the CV of spike frequency measured for each cell (open circles).

Figure 2.

NE suppressed spontaneous activity of RA cells. A) A raster plot shows that application of 10 μM NE abolished the spontaneous activity of a single cell recorded in RA. Each tick mark indicates a spike that occurred during a 5 second sweep. The thick line on the right indicates the time of NE application, which lasted 1.42 minutes. Regular spontaneous activity recovered quickly during washout. B) On the left, recovery (post-NE, black) spike waveforms overlay baseline (pre-NE, red) waveforms. On the right, the area in red denotes the mean waveform $\pm$ SD of the baseline spikes. The black line plots the mean waveform for the recovery spikes, and the error bars are SD. For both panels, the last 10 spikes prior to silencing are compared to the first 10 spikes recorded during recovery. C) A scatter plot of spike height and width

compares baseline spikes (red circles, $n=371$) to recovery spikes (black dots, $n=613$). D) A scatter plot compares the firing rate in control Ringer ("control") to that measured during the last minute of NE application. Each open circle represents a neuron. The diagonal line is where points would lie if there was no change in firing rate in the presence of NE. A majority of cells showed a decrease in firing (i.e., lie below the diagonal line). E) On average, there was a significant decrease in firing rate in NE for the population of RA cells shown in D (asterisk marks significant difference). Error bars represent SEMs.

Figure 3.

NE-induced suppression was likely a direct effect. A) NE abolished spontaneous activity of a neuron in RA recorded in the presence of 100 μM CdCl_2 . Each point represents the frequency of the cell measured for each trial. B) A scatter plot compares the spike rate under baseline conditions to the spike rate during NE application. Each point represents a cell; those recorded with fast excitatory and inhibitory transmission blocked ("KA+PTX") are indicated with open circles, and those recorded with all synaptic transmission blocked (" CdCl_2 ") are marked with solid circles. The diagonal line indicates where points would lie if firing rates were the same for baseline and NE conditions. C) On average, NE decreased the firing rate from baseline frequencies, which were measured during either type of blockade of synaptic transmission. Error bars indicate SEMs, and the asterisk marks the significant decrease. D) The effect of NE was similar at early and later time points of NE application. The scatter plot compares the degree of NE effect at the end of NE application ("later") and during the second minute of NE application ("early"). Each point represents a cell. The diagonal indicates where points would lie if the effect was the same for these two time points. E) Spike frequency is plotted against CV for each neuron that showed significant changes in firing rate in response to NE (solid

circles). These data lie within the range of values obtained from RA cells recorded under control conditions (open circles); the control data are the same points shown in Figure 1C. Each point represents a neuron.

Figure 4.

A subset of RA neurons did not exhibit a suppressive response to NE. A) Firing rate is plotted for baseline and for increasing concentrations of NE for each neuron. Lines connect responses from the same neurons. Each cell did not exhibit a significant decrease in firing in response to 10 μ M NE. B) The mean percent change in firing rate relative to baseline is shown for 10 μ M NE and for the maximum concentration of NE applied to each cell. 0% change indicates no change from baseline. The bars are averages taken from the population of cells shown in A; error bars are SEMs. The asterisk marks a statistically significant increase in firing rate. C) The mean percent change in CV relative to baseline is shown for 10 μ M NE and for the maximum concentration of NE applied to each cell; graph conventions are as described in B. D) The percent change in frequency for each neuron is plotted as a function of bird. Along the abscissa, each number refers to an experimental bird, and the points show the percent change values in NE obtained from each cell recorded from slices made from that bird.

Figure 5.

Yohimbine reversed the suppressive effect of NE. **A)** The firing rate of a RA neuron is plotted against time during an experiment in which 1 mM KA+150 μ M PTX, 10 μ M NE, and 1 μ M yohimbine were applied sequentially. Spontaneous activity returned in the presence of yohimbine+NE. **B)** The firing rates of all neurons involved in the same experiment described in **A** are shown for baseline conditions, in the presence of 10 μ M NE, and in the presence of 10 μ M NE+1 or 5 μ M yohimbine (“yoh”). Lines connect responses recorded from the same neuron. Brackets and asterisks indicate those comparisons that yielded statistically significant differences. **C)** A scatter plot compares the baseline firing rate to that obtained in the presence of yohimbine alone. Each point represents a neuron, and the diagonal line indicates where points would lie if these values were equal. Cells tested with 1 μ M or 5 μ M yohimbine are indicated by circles or squares, respectively.

Figure 6.

Enhanced firing rates could follow NE-induced suppression. A) The firing rate of a RA neuron is plotted against time during an experiment in which 10 μ M NE abolished firing. Once firing resumed, the rate during the recovery time period was slightly and significantly higher than that during the pre-NE baseline. B) A scatter plot compares the firing rate during the pre-NE period to that during the recovery period for each cell. The line indicates where points would lie when values were equal. C) A bar graph plots the average firing rates for the population of cells shown in B; firing rates during the pre-NE baseline and recovery period are compared. Error bars indicate SEMs, and the asterisk denotes a significant difference.

Figure 7.

Clonidine partially mimicked the suppressive effect of NE. A) The firing rate of a neuron in RA is plotted against time during an experiment in which 50 μ M clonidine was applied. B) The scatter plot compares the baseline firing rates to those measured during 10 or 50 μ M clonidine application (circles and squares, respectively). The diagonal line indicates where points would lie if firing rates in baseline and yohimbine conditions were equal; each point represents a neuron. C) Responses to NE and clonidine are compared for individual RA cells. The percent change in frequency to NE for a cell is plotted alongside that in clonidine; lines connect values obtained from the same cell. The dotted line at 0% indicates no change relative to baseline (i.e., pre-drug values); positive values indicate increases relative to baseline, and negative values indicate decreases relative to baseline.

Figure 8.

Summary graph of percent change in firing rate relative to baseline for all experiments. Each point represents an individual neuron. Thick horizontal lines in each column indicate the mean percent change obtained from the population of cells for a particular experiment. The gray line across the first 3 columns marks the mean for all NE experiments, regardless of baseline conditions. The dotted line at 0% indicates no change relative to baseline, positive values indicate increases in firing rates, and negative values indicate decreases in firing rates. In the 5th and 6th columns, solid and open circles mark cases of 1 and 5 μ M yohimbine, respectively. In the 8th column, solid and open circles indicate cases of 10 μ M and 50 μ M clonidine, respectively.

Figure 9.

NE induced a conductance increase in RA neurons. A) Top panel, The voltage responses of an RA neuron to -20 pA current pulses are shown for control conditions and in the presence of NE. Each voltage response is the average of three traces. The membrane potential was held at -80 mV. Scale bars are 500 ms and 4 mV. Bottom panel, An IV plot for an individual RA neuron shows its voltage response to current injection in control (solid circles) and NE (open circles) conditions. The lines show the best linear fits through the points. B) The percent increase in conductance in response to application of NE (relative to control) is plotted against baseline firing rate for each neuron. The solid circle represents the neuron shown in A. Four cells required current injection to maintain their baseline V_m . C) Top panel, A neuron's voltage

responses to a +40 pA current pulse are shown under control conditions and in the presence of NE. The resting potential in both cases was -65 mV. Scale bars are 500 ms and 20 mV. Bottom panel, A scatter plot shows the silencing effect of NE on spiking elicited by suprathreshold current injection. The mean number of spikes evoked by depolarizing current pulses (1 s long) under control conditions is compared to that evoked in the presence of NE at the same membrane potential. Symbol types indicate different cells ($n=3$ neurons total; data from two different current pulses plotted for each cell). The line indicates where points would lie if there was no change in spiking between conditions. The inset shows voltage responses from a neuron to a +40 pA current pulse under control conditions and in the presence of NE. The resting potential in both cases was -65 mV.

Figure 10.

NE-induced suppression of spontaneous activity did not prevent RA cells from spiking in response to afferent input. A) A raster plot shows how the spontaneous activity of a single RA neuron was briefly interrupted by local application of NE. The dotted vertical line indicates the time of a 25 ms puff of 10 mM NE. The effect of the NE puff was blocked by 1 μ M yohimbine (indicated by thick vertical line on right-hand side of raster plot). During trials 5–7, HVC was stimulated with three pulse trains (indicated by arrowheads), which evoked spiking from the RA cell. The boxed area is enlarged in B. B) Enlarged view of boxed area in the raster plot in A shows how stimulation of HVC can evoke spikes in a RA neuron whose spontaneous activity has been silenced by local application of NE. Each stimulus consisted of three trains, made up of 3 pulses occurring at 100 Hz each. One spike was evoked for most stimulus pulses. C) Bath applied NE caused an increase in the ratio of evoked to spontaneous spikes for cell 1. The raster plot shows that, under control conditions evoked RA spikes (arrowheads indicate stimulus trains delivered to HVC) occurred with a continuing background of spontaneous activity, whereas in NE the spikes were evoked during a silenced background. This resulted in an increase in signal-to-noise ratio (see text). D) A raster plot for a different cell shows how, under control conditions, HVC-driven activity in RA also exhibited brief suppressions of spontaneous activity. Thus, the ratio of evoked to spontaneous spikes for this cell was comparable between control and NE conditions.

TABLE 1
Counts of NE effects on spontaneous firing rates in RA

condition ^a	decreased (abolished) ^{bc}	increased	no change
control, n=48	38 (23)	4	6
KA+PTX, n=52	35 (24)	12	5
CdCl ₂ , n=8	8 (6)	0	0
TOTAL, n=108	81 (53)	16	11
	75% (49.1%)	14.8%	10.2%

^a Condition refers to the Ringer solution prior to NE application; n refers to number of cells tested.

^b Changes in firing (decrease, increase, or no change) were determined for each cell by unpaired t-tests between pre-NE and NE trials (see Methods).

^c Numbers in parentheses indicate the subset of cells that were silenced by NE application.