NIH Public Access
Author Manuscript
Eur J Neurosci. Author manuscript; available in PMC 2010 June 1.

Published in final edited form as:
Eur J Neurosci. 2008 May ; 27(9): 2433–2443.

Chronic cold exposure increases RGS7 expression and decreases α_2-autoreceptor-mediated inhibition of noradrenergic locus coeruleus neurons

Hank P. Jedema[1,2], **Stephen J. Gold**[3], **Guillermo Gonzalez-Burgos**[2,4], **Alan F. Sved**[1,2,4], **Ben J. Tobe**[5], **Theodore G. Wensel**[5], and **Anthony A. Grace**[1,2,4,6]

[1]Dept. of Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA, USA

[2]Center for Neuroscience at the University of Pittsburgh (CNUP), Univ. of Pittsburgh, Pittsburgh, PA, USA

[3]Dept of Psychiatry, UTSW, Dallas, TX, USA

[4]Dept. of Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA, USA

[5]Dept. of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX, USA

[6]Dept. of Psychology, Univ. of Pittsburgh, Pittsburgh, PA, USA

Abstract

Chronic stress exposure alters the central noradrenergic neurons originating from the locus coeruleus (LC). Previously, we demonstrated that evoked increases in the firing rate of LC neurons and their release of norepinephrine are enhanced following chronic cold exposure. In the present studies, we tested the hypothesis that reduced feedback inhibition of LC neurons might underlie these alterations in LC activity by examining the effect of α_2-autoreceptor stimulation on LC activity in chronically stressed rats using *in vivo* and *in vitro* single unit recordings. Given that Regulators of G-protein Signaling (RGS) proteins can impact the coupling of α_2-autoreceptors to downstream signaling cascades, we also explored the expression of several RGS-proteins following chronic stress exposure. We observed that the α_2-autoreceptor-evoked inhibition of LC neurons was reduced and that the expression of RGS7 was increased following chronic stress exposure. Finally, we demonstrated that intracellular administration of RGS7 via patch clamp electrodes mimicked the stress-induced decrease in clonidine-evoked autoreceptor-mediated inhibition. These novel data provide a mechanism to explain how chronic stress-induced alterations in receptor coupling can result in changes in α_2-autoreceptor control of noradrenergic function throughout the central nervous system potentially leading to alterations in anxiety-related behaviors and may suggest novel therapeutic targets for the treatment of mood and anxiety disorders.

Keywords

coeruleus; norepinephrine; chronic stress; α_2-adrenergic receptor; clonidine; RGS protein

Locus coeruleus (LC) neurons are the main source of norepinephrine (NE) in the central nervous system {Foote, 1983 #602; Aston-Jones, 1995 #1972} and play an important role in the behavioral response to stress. Thus, LC neuron firing rate and NE release from axon

Corresponding author/current address: Hank P. Jedema Dept. of Psychiatry, Univ. of Pittsburgh 4071 BST3 3501 Fifth Avenue Pittsburgh, PA 15261 USA Jedema@bns.pitt.edu .

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

terminals are increased by acute stress exposure {Grant, 1984 #2110; Abercrombie, 1987 #18; Abercrombie, 1992 #933}. Furthermore, chronic stress exposure can alter LC neuron responses to subsequent stressors {Stanford, 1995 #1541; Zigmond, 1995 #921; Morilak, 2005 #3176}. Consequently, the evoked increase in LC firing rate and stress-evoked NE turnover or release is enhanced following chronic cold, repeated restraint, or repeated tailshock exposure {Mana, 1997 #450; Simson, 1988 #1443; Adell, 1988 #649; Anisman, 1990 #693; Thierry, 1968 #587; Nisenbaum, 1991 #455}. We focused on chronic cold exposure because it reliably reproduces the persistent sensitization in central noradrenergic function and plasma corticosterone levels observed in humans afflicted with stress-related mood and anxiety disorders {Vernikos, 1982 #604; Bremner, 1997 #945; Wong, 2000 #1305}. These experiments extend our previous behavioral, neurochemical, electrophysiological, and ultrastructural investigations using this specific stress paradigm to the molecular level, thus permitting assessment of how these changes act in concert to alter central noradrenergic function.

The effects of chronic cold exposure on the NE system are most apparent during evoked activity {Finlay, 1997 #893; Nisenbaum, 1991 #455; Mana, 1997 #450; Jedema, 2001 #1472; Jedema, 2003 #1562}. Based on the preferential increase in evoked activity, the increased slope of the dose-response relationship to corticotrophin-releasing hormone {Jedema, 2001 #1472}, and the steeper current-response curve in response to intracellular current injection {Jedema, 2003 #1562}, we hypothesized that inhibitory feedback of LC neurons was reduced following chronic cold exposure. Altered feedback inhibition might be mediated by changes in α_2-autoreceptor function, local GABAergic interneurons, and/or conductances intrinsic to LC neurons{Acosta, 1993 #917; Andrade, 1984 #678; Simson, 1988 #1443}. Therefore, we directly assessed the effect of α_2-autoreceptor stimulation on LC firing rate following chronic stress exposure using *in vivo* and *in vitro* recordings.

The response to stimulation of G-protein coupled receptors (GPCR), such as α_2-receptors, is modulated by changes in proteins of the regulators of G-protein signaling (RGS) family. RGS proteins decrease the efficacy of GPCR stimulation by increasing the GTPase activity of the G_α-subunit, leading to a more rapid termination of the response to receptor stimulation {Xie, 2007 #3778; Hollinger, 2002 #3165; Ross, 2000 #3160}. Over 20 RGS proteins have been described in vertebrates, and at least 6 of them are expressed in LC neurons {Gold, 1997 #2191; Taymans, 2002 #3500}. RGS proteins are involved in altering opiate receptor function in LC neurons following opiate tolerance and withdrawal {Gold, 2003 #2556; Xie, 2005 #3135} and in other cell types, RGS proteins modulate α_2-autoreceptor coupling to their intracellular targets {Cavalli, 2000 #2185; Bahia, 2003 #3137}. Furthermore, RGS mRNA expression can be regulated by stress or corticosterone exposure {Ni, 1999 #1430}, suggesting a potential involvement in the effects of chronic cold exposure on LC activity. Thus, we examined the involvement of 3 RGS proteins, highly expressed in LC and implicated in stress and anxiety-related disorders {Ni, 1999 #1430; Oliveira-dos-Santos, 2000 #2183; Yalcin, 2004 #3132}, on the response to chronic cold.

METHODS

Animals

Upon arrival, adult male Sprague Dawley rats (Hilltop, Scottdale, PA) were housed in hanging stainless steel cages in a colony room maintained at an ambient temperature of 23°C. Throughout the experiments, lights were maintained on a 12 hr light/dark cycle (lights on at 08.00 a.m.), with food (Laboratory rodent diet 5001, PMI Feeds, St. Louis, MO) and water available *ad libitum*. All rats were housed in pairs in the colony room for 5-10 days prior to any treatment and all protocols were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh and were in accordance with the USPHS *Guide for the Care and Use of Laboratory Animals*.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Cold exposure

Rats were randomly assigned to a cold-exposed or control group. Control rats were housed singly in hanging stainless steel cages in a colony room maintained at an ambient temperature of 23°C for 14-17 days. Given that it increases the effectiveness of chronic cold exposure on catecholamine systems without interfering with normal adaptive thermoregulatory behaviors {Fluharty, 1983 #958; Moore, 2001 #1542}, the body fur of rats in the cold exposure group was partially shaved from the rump to the forelimbs immediately prior to cold exposure. Cold-exposed rats were housed singly in hanging stainless steel cages in a cold room maintained at an ambient temperature of 5°C, where they remained undisturbed for 14-17 days. They were removed from the cold room the afternoon prior to the experiment and housed overnight in a colony room maintained at an ambient temperature of 23°C, in order to maintain a protocol similar to that used previously for studying the stress-induced sensitization of NE neurons {Gresch, 1994 #751; Miner, 2006 #3198; Finlay, 1997 #893; Mana, 1997 #450; Nisenbaum, 1991 #455; Jedema, 2001 #1472; Jedema, 2003 #1562}. Cold-exposed rats readily adapted to the cold environment {Folk Jr., 1974 #943} and appeared healthy and continued to eat and increase their body weight. The present stress paradigm of 14 days of cold exposure is likely at the threshold for producing sensitization in rats, in that it leads to consistent sensitization of the noradrenergic system, whereas shorter duration or intermittent exposure to cold does not lead to noradrenergic sensitization in Sprague-Dawley rats {Jedema, 1999 #1279; Pardon, 2003 #3181; Finlay, 1997 #893; Mana, 1997 #450}. Furthermore, during the initial days in the cold, rats typically do not gain as much weight as the rats housed at room temperature but their bodyweight gain normalizes with continued exposure {Folk Jr., 1974 #943}. Finally, there is evidence that chronic cold may be a comparatively less extreme stressor compared to other paradigms (e.g., chronic multiple stressors, repeated footshock, etc) based on the limited level of c-fos induction observed in response to cold exposure even in a more stress-sensitive rat strain {Baffi, 2000 #2079}.

In the present electrophysiology experiments, the bodyweight of cold-exposed rats did not differ from the control group (*in vivo*: cold 309±8g (N=6) vs control 319±13g (N=4); *in vitro*: cold 266±9g (N=8) versus control 260±19g (N=12)). However, for the Western blotting groups, the body weight was significantly lower in the cold-exposed rats: cold 271±6 (N=10) versus control 302±4g (N=10); $t(18)= -4.116$; $p<0.001$). In some of our previous experiments we observed small but significant changes in body weight between groups {Gresch, 1994 #751;Jedema, 1999 #1279; Jedema, 2001 #1472}, whereas in other studies we found no significant differences {Finlay, 1997 #893; Jedema, 2003 #1562; Mana, 1997 #450}. The decreased variability of body weight (as a result of all animals being sacrificed on the same day) may have resulted in the observation that the comparison of body weight reached statistical significance for the Western-blot groups. Cold-exposed rats and their control group were tested during the same time period to avoid confounding the results with potential changes of the responses over time.

In Vivo Electrophysiology

Single unit activity was recorded *in vivo* as previously described {Jedema, 2001 #1472}. Under halothane anesthesia (1.5-3.0% in O_2; Halocarbon Laboratories, River Edge, NJ), a femoral venous catheter (PE-10) was inserted before the rat was positioned in a stereotaxic frame (David Kopf, Tujunga CA) with the nose down at an approximate 15° angle (difference in DV coordinates of bregma and lambda was 3.0 mm). Core temperature was maintained at 37°C using a heating pad (Fintronics VL-20F, New Haven, CT) and rectal probe thermometer. Following exposure of the skull, a hole was drilled in the area overlying the LC. Glass electrodes (Omegadot, 2mm; WPI, New York, NY) were pulled using a vertical puller (Narishige PE-2, Tokyo, Japan) and filled with 2M NaCl/2% Pontamine Sky Blue (PSB; impedance 6-12 MΩ). Electrodes were positioned in the LC (3.5mm caudal to lambda, 1.1mm

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

lateral from the midline, and 5.0-6.0 mm ventral from the dorsal brain surface) using a hydraulic microdrive (Kopf model 640). LC neurons were tentatively identified based on well-established criteria including spike waveform, firing pattern, and response to paw compression {Foote, 1983 #602; Jedema, 2001 #1472; Mana, 1997 #450}. Signals from the electrodes were amplified using a high-impedance headstage amplifier connected to an amplifier/window discriminator (Fintronics WDR 420; Fintronics, Orange, CT). Electrophysiological activity was monitored using an audio monitor (Grass AM-8, West Warwick, RI) and a storage oscilloscope (Hitachi V134, Brisbane, CA). In addition, data were monitored and analyzed using a data acquisition board (Microstar Labs™, Bellevue, WA) interfaced with a Windows based PC and custom made software (Neuroscope®, Brian Lowry). Following the recording of stable baseline activity of an individual LC neuron for at least 5 min, increasing doses of the α_2-receptor agonist, clonidine HCl (0.5-8.0 μg/kg; 10μg/ml), were infused via the femoral vein at 12 min intervals until cessation of spontaneous LC activity occurred. At the end of the experiment, the location of the recording site was marked by iontophoresis of PSB and verified to be within the LC in 60μm thick, Nissl-stained, coronal sections. Only one neuron was recorded per rat.

In Vitro Electrophysiology

Horizontal brainstem slices containing the LC were prepared as previously described {Jedema, 2003 #1562}. Briefly, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and perfused through the ascending aorta with an ice-cold, oxygenated (low Na/high sucrose) perfusion solution (1.9 mM KCl, 1.2 mM Na_2HPO_4, 6 mM $MgCl_2$, 33 mM $NaHCO_3$, 20 mM glucose, 229 mM sucrose saturated with 95% O_2/5% CO_2). Following decapitation, the brain was rapidly removed, placed in cold perfusion solution and 300 μm thick horizontal slices containing the LC were prepared using a Microslicer (DSK). Tissue was transferred to cold, oxygenated aCSF (124 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 2.4 mM $CaCl_2$, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, 10 mM glucose saturated with 95% O_2/5% CO_2). After a minimal recovery period of 60-90 min, sections were transferred to the recording chamber where they were superfused with oxygenated aCSF at a flow rate of 0.8-1.5 ml/min at 35°C.

Extracellular single unit recordings were obtained with the same equipment in a similar manner as described above. LC neurons were tentatively identified by their location within the trans-illuminated slice, their regular and spontaneous activity and their action potential waveform. Following the recording of stable baseline activity of each individual LC neuron for at least 5 min, increasing doses of the α_2-receptor agonist, clonidine HCl (0.3-100 nM), were bath applied at 5 min intervals until cessation of spontaneous activity occurred.

Whole cell recordings were obtained from visually-identified LC neurons using an infrared differential interference contrast video microscope (Olympus BX51WI or BX61WI) and a Multiclamp 700A amplifier (Axon Instruments, MDC, Sunnyvale, CA) in current-clamp mode, using bridge balance and pipette capacitance compensation. Data were low-pass filtered at 3kHz and digitized at 10kHz using a data acquisition board (CED 1401; Cambridge Electronic Design Ltd, Cambridge, UK) interfaced with a Windows based PC and Signal 3.02 software (Cambridge Electronic Design). Borosilicate pipettes (4-6 MΩ) were filled with a freshly prepared pipette solution containing (in mM): K-gluconate 115, KCl 20, HEPES 10, EGTA 0.5, ATP 4.5, GTP 0.3, and phosphocreatine 14, with pH adjusted to 7.2–7.3. The series resistance was monitored periodically throughout the experiment and any changes in series resistance were less than 20%. The membrane potential was not corrected for liquid junction potentials.

For intracellular administration of RGS7, a stock solution of RGS7 with its obligatory binding protein Gβ5 was prepared using methods previously described {He, 2000 #3449}. Briefly, recombinant baculoviruses of N-terminal GST-tagged RGS7 and untagged $G\beta_{5S}$ were

generated according to the manufacturer's instructions (BD PharMingen, San Jose, CA). Sf9 cells, grown in Sf-900 II SFM media (Invitrogen, Carlsbad, CA) in spinner flasks (cell density 10^6 cells/ml), were co-infected with baculovirus and harvested after 48 hours. Cells were suspended in lysis buffer (50 mM Tris, 500 mM NaCl, 5 mM dithiothreitol, 1% Nonidet P-40, pH 8) and protease inhibitors (0.03 mg/ml leupeptin, 17 μg/ml pepstatin A, 5 μg/ml aprotinin, 30 μg/ml lima bean trypsin inhibitor, and solid phenylmethylsulfonyl fluoride) and lysed by multiple passes through a microfluidizer (Microfluidics,Newton, MA). The lysate was clarified by centrifugation at 20,000*x g* for 30 minutes and applied to a column of Glutathione Sepharose 4 Fast Flow resin (GE Healthcare, Piscataway, NJ) at 4°C. The resin was washed with lysis buffer and wash buffer (10 mM Tris, 100 mM NaCl, 2 mM $MgCl_2$, 2 mM dithiothreitol, pH 7.4) and finally, eluted with 100 mM glutathione in wash buffer. Protein was exchanged under N_2 gas into whole cell pipette solution (without ATP and GTP; as described above) at 4°C in a 50 ml stirred cell with a YM100 ultrafiltration membrane (NMWL:100 kDa; Millipore, Billerica, MA). The concentration of RGS7 was quantified by gel densitometry using a bovine serum albumin (BSA) standard and prior to use the RGS7 stock solution was further diluted in fresh pipette solution to a final concentration of 100nM RGS7 (and containing 4.5mM ATP and 0.3mM GTP). The concentration of RGS7 was based on dose response curves reported for RGS4 {Xu, 1999 #3775} and concentrations of RGS proteins used previously in electrophysiological experiments carried out in native tissues {Cabrera-Vera, 2004 #3779; Gold, 2003 #2556}. Control experiments were performed using fresh pipette solution without the addition of the RGS7 stock solution.

Western Blots

Western blot analysis was performed using standard procedures as previously described {Harlow, 1988 #3170}. Following decapitation using a guillotine, the brain was rapidly removed from the skull and after removal of the cerebellum, frozen in isopentane on dry ice and stored at −80°C. Subsequently, under microscopic visualization three 400 μm thick coronal sections separated by a 50μm thick section (for histological verification) were taken from the frozen brainstem using a cryostat at −16°C (Microm HM505E, Richard Allan Scientific, Kalamazoo, MI). Using a 1mm tissue punch (FST, Foster City, CA) a semicircular segment containing the LC was punched bilaterally from each thick section and all punches from each brain were pooled.

Frozen samples were solubilized by sonication in buffer containing 1.0% sodium dodecyl sulphate, 10μg/mL lima bean trypsin inhibitor, 10μg/mL leupeptin, 15μg/mL phenylmethylsulphonyl fluoride, 15μg/mL L^{-1}–*p*-tosylamino-2-phenylethyl chloromethyl ketone (TPCK), 15μg/mL (3S)-7-amino-1-chloro-3-tosylamino-2-heptanone hydrochloride (TLCK), and 10μM MG-132 (Calbiochem), and protein concentrations were determined by the method of Lowry with BSA as standard. Aliquots of LC extracts containing 35μg of total protein were subjected to SDS-PAGE. The proteins in resulting gels were transferred to nitrocellulose and comparable lane loading and transfer was confirmed by Ponceau staining. Nitrocellulose filters were blocked for 1 hour in 3% nonfat milk in phosphate-buffered saline containing Tween-20 and incubated overnight at 4°C with primary antibodies diluted into Tris-buffered saline BSA. RGS2, RGS4, and RGS7 were selected for analysis based on high expression levels in the LC, the availability of specific primary antibodies, and because they have been implicated in stress and anxiety-related disorders {Ni, 1999 #1430; Oliveira-dos-Santos, 2000 #2183; Yalcin, 2004 #3132}. Rabbit antiserum against the C-terminus of RGS2 (CKKPQITTEPHAT; 1:1000; a generous gift from Dr. D. Siderovski); rabbit antisera against rat RGS4 {Krumins, 2004 #3155}; 1:2000; a generous gift from Dr. S. Mumby), affinity purified anti-RGS7 antibodies (Upstate Biotechnologies;1:10,000), and SGS rabbit antisera against Gβ5 {Zhang, 1996 #3202}; 1:5000; a generous gift from Dr. W. Simonds, NIDDK). The specificity of these RGS and Gβ5 antibodies was demonstrated previously {Oliveira-dos-

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

Santos, 2000 #2183; Krumins, 2004 #3155; Zhang, 1996 #3202}. The next day membranes were washed, incubated in appropriate horseradish peroxidase-conjugated secondary antibodies (1:10,000; Vector labs) for 30 min at 23°C, processed for enhanced chemiluminescence, and exposed to autoradiographic film. Multiple exposure times were conducted to insure relative optical densities that were within the linear range of the films response curve. Images were captured with a CCD camera (Sony ExwaveHAD) and quantified densitometrically with NIH image (version 1.61). As an additional control for loading, blots were re-processed for total Gβ immunoreactivity using rabbit antisera against pan-Gβ (antibody B600 {Linder, 1993 #3199}; a generous gift from S. Mumby). There was no difference in total Gβ abundance between the 2 groups. All chemicals were obtained from Sigma (St. Louis, MO) except where specified otherwise.

Data analysis

For electrophysiological experiments, sliding averages over 10secs were calculated from the 1sec bins of the firing rate histogram. Baseline firing rate was defined as the average firing rate over the 2 min prior to clonidine administration. For *in vivo* experiments, the effect of a bolus administration of clonidine was defined as the minimum (sliding average) firing rate following drug injection. For *in vitro* experiments, the effect of continuous clonidine administration was defined as the average firing rate recorded over the final 2 min at each given concentration. The magnitude of the drug effect was expressed as percent inhibition from baseline and the ED_{50} for each neuron was calculated based on the 3-parameter sigmoidal curve fit of the data (Sigmaplot).

Previously, we used criteria that were established for dopamine neurons to define bursts in LC neurons in chloral-hydrate anesthetized rats {Mana, 1997 #450; Jedema, 2001 #1472}. In the analysis of more recent data we observed that the use of these dopaminergic criteria resulted in a failure to identify all bursts correctly because several bursts just exceeded the 80msec interspike interval (ISI) threshold established for dopamine neurons of the substantia nigra {Grace, 1984 #1267}. As the number of events classified as bursts would be expected to increase by raising the threshold criterion, we determined if and at which burst-threshold criterion the number of bursts determined by such criterion would reach a “plateau” before continuing to increase as a result of incorrectly characterizing rapid spike discharge as bursts. When the relative number of bursts of 10 LC neurons during 3 minutes of basal discharge activity from a separate group of control rats showing clear burst activity was plotted versus the ISI interval used as a threshold to define bursting, a relationship was evident that could be well fitted by a cubic equation (Fig 1). The point of inflexion of this curve was calculated to be at 114msec, which was consistent with our visual examination of burst activity during spontaneous discharge of action potentials and our observation that the 80msec ISI threshold for burst was too low for LC neurons. We therefore adjusted the threshold criterion for bursting of LC neurons to 110msec, and set this as our standard criterion for the burst analysis of LC neurons.

For Western blots, background-subtracted optical densities of the blots for each sample were compared between cold and control groups using 2-tailed *t*-tests for independent samples.

For whole cell recordings, the membrane potential was averaged over a 2 min period immediately prior to the administration of clonidine. The effect of clonidine was quantified as the most hyperpolarized deflection of the membrane potential following agonist administration and the average effects for the control and RGS7 groups were compared using a 1-tailed *t*-test for independent samples based on the directional null hypothesis.

All statistical comparisons were performed using Sigmastat 3.11 (Systat Software Inc., San Jose, CA). Average data was compared between groups using t-tests where appropriate or

NIH-PA Author Manuscript

Mann-Whitney rank sum tests when the normality assumption was not met. The α-level was set to 0.05.

RESULTS

In vivo single unit recordings

The basal firing rate (BL FR) of LC neurons in the control rats was 0.9±0.1 Hz (Table 1) which is similar to numerous previous reports on LC FR in anesthetized rats {Jedema, 2001 #1472; Mana, 1997 #450; Curtis, 1997 #1008; Foote, 1983 #602}. LC neurons of cold-exposed rats had a slightly higher basal discharge rate (1.7±0.3Hz; *t*(8)=2.38; p=0.045) than in control rats and exhibited occasional discharge of spikes in bursts which was not observed in the same duration of baseline activity of control rats (Table 1). As the level of anesthesia can influence the basal discharge rate of LC neurons {Valentino, 1988 #1354}, the level of anesthesia during recording was carefully monitored using testing of corneal and plantar reflexes and there was no significant difference in the concentration of halothane used between groups.

In response to IV administration of the α_2-receptor agonist clonidine (0.5-6.0μg/kg), the firing rate of LC neurons was transiently inhibited in a dose-dependent manner (Fig. 2), consistent with previous studies {Aghajanian, 1982 #677; Lacroix, 1991 #1183; Pineda, 1997 #1235}. In response to similar doses of clonidine, LC neurons of cold-exposed rats exhibited a smaller inhibition compared to control rats. The ED_{50} calculated for individual neurons was significantly higher in cold-exposed rats (control 1.64±0.20 μg/kg versus cold 2.40±0.22 μg/kg; *t*(8)= 2.64, p=0.030). No correlation was observed between basal firing rate and the ED_{50} for clonidine in our *in vivo* experiments.

In vitro single unit recordings

The BL FR of LC neurons of control rats was 1.1±0.1 Hz (Table 2), similar to previous reports on LC FR in brainstem slices {Jedema, 2003 #1562; Williams, 1984 #1107}. Similar to the *in vivo* experiments, the firing rate of LC neurons in slices from cold-exposed rats was significantly higher (1.6±0.1 Hz; Mann-Whitney rank sum test *T*(29)=296.50, p=0.004) and there was a trend for more spikes discharged in bursts. The reduced effect of cold exposure on bursting of LC neurons *in vitro* compared to the *in vivo* experiments described above is likely related to the greatly reduced extrinsic input in the slice, which contributes to transient burst activation of LC neurons.

In response to bath application of clonidine (0.3-100nM), the firing rate of LC neurons was decreased in a dose-dependent manner (Fig 3). Unlike the transient response *in vivo*, the clonidine-evoked decrease in firing rate did not readily reverse. In several slices in which LC FR was monitored following the return to aCSF superfusion, the discharge rate did not return to basal levels within 20-30min. However, bath application of the α_2-receptor antagonist, RX821,002 readily reversed the clonidine-evoked inhibition and resulted in the resumption of spontaneous discharge of action potentials (data not shown). We hypothesize that the transient effect of clonidine *in vivo* is a reflection of the highly lipophilic nature of this drug.

Although the maximal effect of clonidine (i.e. complete inhibition) was not altered, the firing rates of LC neurons in slices from cold-exposed rats were less inhibited by comparable doses of clonidine, with the ED_{50} calculated for individual neurons being significantly higher in slices from cold-exposed rats (control 8.6±1.7 nM versus cold 14.5±2.3 nM; Mann-Whitney rank sum test *T*(28)=267.00, p=0.04). The ED_{50} determined here for inhibition of spontaneous action potential discharge of LC neurons in control slices is consistent with previous reports of clonidine-evoked hyperpolarization of LC neurons {Williams, 1985 #1104}. In addition,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

there was a significant correlation between basal firing rate and the ED_{50} for clonidine (Pearson's correlation coefficient across groups 0.607; p=0.0004).

Quantitative Western Blots

Quantification of the Western blots (Fig 4) demonstrated that the expression of RGS2 and RGS4 were similar in LC tissue from control and cold-exposed animals ($t(18)= -0.52$ and -0.35, respectively). However, the expression of RGS7 was significantly increased in LC tissue from cold-exposed animals ($t(17) = 2.22$, p=0.04). In contrast to the significant increase in RGS7 levels there was no change in levels of the obligatory binding partner of RGS7, Gbeta5, that was processed on the same membranes. RGS7 expression in tissue punches from the ventromedial pons of the same sections was not altered following chronic cold.

Whole cell recordings

Because the effect of RGS7 on α_2-receptors or in LC neurons has not been demonstrated, whole cell recordings were performed with purified RGS7 and its obligatory binding partner, Gβ5, administered intracellularly (100nM) via the patch pipette (Fig 5). Intracellular administration of RGS7 in LC neurons reduced the magnitude of the hyperpolarization evoked by bath administration of clonidine at a concentration close to its ED_{50} (20nM) [$t(5) = 2.051$, p=0.0475]. Administration of RGS7 did not affect basic spike properties of the neurons. In addition to the reduced hyperpolarization, it appeared that the response to clonidine desensitized (in 3 out of 4 neurons) with continuous clonidine administration in the presence of exogenous RGS7, but we were unable to quantify this accurately in the limited number of neurons. Independent of its time course, the peak effect of autoreceptor stimulation was reduced by intracellular RGS7 administration supporting a functional role of RGS7 in coupling of NE autoreceptor to its effectors.

DISCUSSION

This study demonstrates that LC neurons of rats exposed to chronic cold exhibit a reduced response to α_2-autoreceptor stimulation both *in vivo* and *in vitro*. Furthermore, RGS7 expression was selectively increased in LC following cold exposure, and intracellular RGS7 administration reduced the response to noradrenergic autoreceptor stimulation. This reduced autoreceptor function was accompanied by a small but significant increase in spontaneous baseline LC activity, which could be due to decreased autoreceptor feedback inhibition mediated by NE release from LC neurons {Huang, 2007 #3495}. A significant or a trend toward an increase in firing rate and burst firing has consistently been observed in LC neurons following chronic cold {Jedema, 2001 #1472; Jedema, 2003 #1562; Mana, 1997 #450}; an observation further supported by a meta-analysis of all basal firing rates of cold-exposed and control animals across all of our electrophysiological studies ($N_{control}$=115, N_{cold}=83; $p<0.001$). Small increases in basal firing rate and bursting of LC neurons were also observed following repeated tail shock and repeated immobilization stress {Pavcovich, 1990 #476; Simson, 1988 #1443}. Furthermore, both basal and evoked LC activity would be expected to change if chronic stress alters electrophysiological properties of LC neurons resulting in increased input resistance without changing spike-threshold {Jedema, 2003 #1562}. If the affected conductances were selectively activated during action potential discharge, the spike waveform characteristics would likely be altered, and we have previously demonstrated that this does not occur.

The observed rightward shift of the dose-response curve for clonidine-evoked inhibition of LC neurons in both *in vivo* and *in vitro* preparations localize the alterations in autoreceptor function to the LC and provide a potential mechanism to explain the enhanced activation of LC neurons. A reduction of α_2-autoreceptor-evoked opening of potassium conductances following cold

NIH-PA Author Manuscript

exposure increases input resistance of LC neurons, likely enhancing the LC response to all excitatory input {Jedema, 2003 #1562}. This reduction would likely exert a larger effect on evoked compared to basal LC activity {Simson, 1987 #1445}. It is unknown whether the autoreceptor-coupled potassium conductances have specific effects on the bursting patterns of LC neurons, but similar to chronic stress, increased basal firing and bursting activity of LC neurons have been reported previously following irreversible inactivation of α_2-autoreceptors {Pineda, 1997 #1235}.

Various changes of α_2-receptor binding or mRNA were reported in LC neurons following chronic stress exposure {Flugge, 1996 #2208; Featherby, 2004 #3060; Meyer, 2000 #2203}, but few studies have examined the impact on receptor function of the combined effect of changes in receptor affinity, number, and coupling to intracellular targets. Following repeated tail shock, the effect of noradrenergic autoreceptor antagonists is decreased in rats exhibiting noradrenergic sensitization and behavioral depression {Simson, 1988 #1443}. Chronic cold and repeated tailshock are two paradigms known to cause noradrenergic sensitization whereas some other stress paradigms do not {Jedema, 1999 #1279; Zigmond, 1995 #921}, which is important to consider when evaluating chronic stress paradigms and their potential clinical implications. We predict that the chronic cold or repeated tail shock paradigm leading to sensitization of noradrenergic function in rodents would be a more applicable model of the sensitization of the noradrenergic system observed clinically. Whether repeated restraint leads to enhanced NE activity is unknown, but this different stress paradigm or the much higher doses of clonidine {Pavcovich, 1990 #476; Aghajanian, 1982 #677; Lacroix, 1991 #1183; Pineda, 1997 #1235} may explain the contrasting leftward shift of the dose response curve for clonidine-evoked inhibition of LC neurons reported following repeated restraint {Pavcovich, 1990 #476}. The present demonstration of decreased autoreceptor function is consistent with the reduction of autoinhibitory control over LC neuronal activity as suggested by decreased autoreceptor antagonism following repeated tail shock exposure {Simson, 1988 #1443} and in a prenatal cocaine model leading to enhanced noradrenergic activation {Elsworth, 2007 #3518}. Furthermore, it complements previous neurochemical and electrophysiological changes in the noradrenergic system following cold exposure {Finlay, 1997 #893; Gresch, 1994 #751; Jedema, 2001 #1472; Jedema, 2003 #1562; Jedema, 1999 #1279; Mana, 1997 #450; Nisenbaum, 1992 #463; Nisenbaum, 1991 #455}. In a previous study, chronic cold exposure increased α_2-mRNA but α_2-binding in LC was not changed {Featherby, 2004 #3060}. Those data complement our observations and suggest that the decreased α_2-receptor response may be a consequence of selective modulation of intracellular signaling cascades without alterations in receptor binding, while the increased α_2-mRNA levels may reflect a compensatory attempt to increase autoreceptor control over LC activity.

Aside from down-regulation of autoreceptor function, chronic cold exposure selectively increased RGS7 expression in the LC. Increased RGS7 expression in whole brain has been reported following a systemic high dose of LPS or TNF-α {Benzing, 1999 #3459}, but to our knowledge, this is the first report of a physiological manipulation resulting in altered expression of RGS7 combined with functional impact in a specific cell group *in vivo*. An increase in RGS7 expression in LC neurons likely enhances the GTPase activity of the $G_{\alpha i}$ subunits activated by autoreceptor stimulation {Hooks, 2003 #3157}, thereby leading to a reduced efficacy of autoreceptor-mediated inhibition and providing a mechanism for the sensitization of noradrenergic function induced by chronic stress (Fig 6). A twofold increase in RGS4 expression in LC neurons after chronic morphine has been reported previously, but no functional assessment of opioid receptor function was reported {Gold, 2003 #2556}. Although the magnitude of the change in RGS7 expression following chronic stress may be smaller than can be elicited using pharmacological and genetic manipulations in expression systems, the coincident change in autoreceptor function is constent with the notion that that the present increase in RGS7 expression has a functional impact on autoreceptor function and

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

noradrenergic activity. Increased RGS7 may also diminish signaling via $G_{\alpha q}$ coupled receptors as has been described in heterologous systems {Ghavami, 2004 #3287; Witherow, 2003 #3774}. In addition to the acceleration of termination of the effect of autoreceptor- (and perhaps other Gi/o-coupled receptor) stimulation, RGS7 may alter gene expression via direct actions in the nucleus {Hepler, 2005 #3168; Drenan, 2005 #3150}, perhaps contributing to the increased TH mRNA levels observed following chronic cold {Seiple, 1997 #1438}. Increased RGS7 abundance could result from elevated concentrations of its obligatory binding partner, Gβ5. However, our Western blot analysis demonstrated that Gβ5 levels were not altered in cold-exposed rats (data not shown). Reduced RGS7 degradation or increased synthesis might also mediate the observed increase in RGS7 expression. The p38-mediated reduction of RGS7 degradation in response to TNF-α and the discrepancy between RGS protein and mRNA expression in LC suggest that stabilization of RGS proteins is also an important contributor to the increased RGS7 expression after cold exposure {Benzing, 1999 #3459}.

Given the increased RGS4-mRNA expression in LC following chronic unpredictable stress (CUS) {Ni, 1999 #1430}, it is surprising that the RGS4 expression was not altered by chronic cold exposure. These data may further underscore the importance of post-translational control over RGS protein expression or they may suggest that different stressors differentially regulate different RGS proteins, each affecting specific downstream pathways. It is unknown whether the CUS paradigm causes sensitization of the noradrenergic system, but it does not affect autoreceptor sensitivity in hippocampal synaptosomes {Prieto, 2003 #3506} suggesting that alterations in RGS4 do not affect autoreceptor function in LC neurons. Although RGS2 expression has been related to anxiety {Oliveira-dos-Santos, 2000 #2183; Yalcin, 2004 #3132}, the lack of change in its expression following treatment that alters α_2-autoreceptor function was not surprising. Noradrenergic α_2-receptors signal predominantly via pertussis toxin-sensitive Gαi G-proteins to open GIRK channels for their electrophysiological effect {Aghajanian, 1986 #1437; Bunemann, 2001 #3175} and RGS2 selectively modulates Gαq function {Heximer, 1999 #3173}. These data further support the specificity of the regulation of GPCR signaling.

Importantly, we demonstrated that increased intracellular RGS7 levels in individual neurons is associated with a reduction in autoreceptor responsivity. It is difficult to estimate relevant intracellular RGS7 concentrations given the non-homogenous concentrations throughout the neuron and RGS7 binding-proteins greatly affecting the activity of RGS7 {Drenan, 2006 #3428}. Regardless, the observed RGS7-evoked decrease of α_2-receptor signaling suggests that the chronic stress-evoked increase in RGS7 and decrease in α_2-receptor function are related.

If present throughout LC neurons, the decreased efficacy of α_2-autoreceptor stimulation and potentially other Gαi-coupled receptors could further contribute to the enhanced release of NE at the terminal. However, reverse dialysis-induced inhibition of NE efflux in hippocampus of cold-exposed rats did not suggest a rightward shift of the dose-response curve for clonidine {Nisenbaum, 1993 #461}. These data may point to differential control of α_2-receptors at terminal versus soma level, which has been hypothesized previously {Featherby, 2004 #3060}.

The present data combined with previous observations demonstrate how the increase in a specific RGS protein may result in decreased efficacy of α_2-autoreceptor control of noradrenergic neurons without changes in number or affinity of α_2-receptors, leading to altered noradrenergic tone in multiple terminal regions throughout the central nervous system. Such a condition could potentially contribute to the alteration we have observed in anxiety-related behavior of these rats in the elevated plus-maze {Seiple, 1997 #1438}. Given the abnormalities in central noradrenergic function in mood and anxiety disorders {Aston-Jones, 2000 #1481; Wong, 2000 #1305; Southwick, 1997 #950} and recent implications of abnormalities in RGS

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

expression in other psychiatric disorders such as schizophrenia {Mirnics, 2001 #2180}, it will be important to examine RGS expression in humans afflicted with mood and anxiety disorders in future studies.

Acknowledgments

The authors thank Niki Macmurdo, Bryan Potts, and Christy Smolak for technical assistance, and Brian Lowry for Neuroscope data analysis software. This work was supported by USPHS DA15408 (AAG) and a 2006 NARSAD Young Investigator Award (HPJ).

REFERENCES

Abercrombie ED, Jacobs BL. Single-unit response of noradrenergic neurons in the locus coeruleus of freely moving cats. I. Acutely presented stressful and nonstressful stimuli. Journal of Neuroscience 1987;7:2837–2843. [PubMed: 3625275]

Abercrombie, ED.; Nisenbaum, LK.; Zigmond, MJ. Impact of acute and chronic stress on the release and synthesis of norepinephrine in brain: microdialysis studies in behaving animals. In: Kvetnansky, R.; McCarty, R.; Axelrod, J., editors. Stress: Neuroendocrine and molecular approaches. Gordon and Breach Science Publishers; New York: 1992. p. 29-42.

Acosta GB, Losada ME Otero, Rubio MC. Area-dependent changes in GABAergic function after acute and chronic cold stress. Neuroscience Letters 1993;154:175–178. [PubMed: 8361637]

Adell A, Garcia-Marquez C, Armario A, Gelpi E. Chronic stress increases serotonin and noradrenaline in rat brain and sensitizes their responses to a further acute stress. Journal of Neurochemistry 1988;50:1678–1681. [PubMed: 2453609]

Aghajanian GK, VanderMaelen CP. alpha 2-adrenoceptor-mediated hyperpolarization of locus coeruleus neurons: intracellular studies in vivo. Science 1982;215:1394–1396. [PubMed: 6278591]

Aghajanian GK, Wang YY. Pertussis toxin blocks the outward currents evoked by opiate and alpha 2-agonists in locus coeruleus neurons. Brain Research 1986;371:390–394. [PubMed: 3697768]

Andrade R, Aghajanian GK. Intrinsic regulation of locus coeruleus neurons: electrophysiological evidence indicating a predominant role for autoinhibition. Brain Research 1984;310:401–406. [PubMed: 6488034]

Anisman H, Zacharko RM. Multiple neurochemical and behavioral consequences of stressors: implications for depression. Pharmacology & Therapeutics 1990;46:119–136. [PubMed: 2181488]

Aston-Jones, G.; Shipley, MT.; Grzanna, R. The locus coeruleus, A5 and A7 noradrenergic cell groups. In: Paxinos, G., editor. The rat nervous system. Second Edition. Academic Press; New York: 1995. p. 183-213.

Aston-Jones G, Rajkowski J, Cohen J. Locus coeruleus and regulation of behavioral flexibility and attention. Progress in Brain Research 2000;126:165–182. [PubMed: 11105646]

Baffi JS, Palkovits M. Fine topography of brain areas activated by cold stress. A fos immunohistochemical study in rats. Neuroendocrinology 2000;72:102–113.

Bahia DS, Sartania N, Ward RJ, Cavalli A, Jones TLZ, Druey KM, Milligan G. Concerted stimulation and deactivation of pertussis toxin-sensitive G proteins by chimeric G protein-coupled receptor-regulator of G protein signaling 4 fusion proteins: analysis of the contribution of palmitoylated cysteine residues to the GAP activity of RGS4. Journal of Neurochemistry 2003;85:1289–1298. [PubMed: 12753087]

Benzing T, Brandes R, Sellin L, Schermer B, Lecker S, Walz G, Kim E. Upregulation of RGS7 may contribute to tumor necrosis factor-induced changes in central nervous function. Nat Med 1999;5:913–918. [PubMed: 10426315]

Bremner JD, Innis RB, Ng CK, Staib LH, Salomon RM, Bronen RA, Duncan J, Southwick SM, Krystal JH, Rich D, Zubal G, Dey H, Soufer R, Charney DS. Positron emission tomography measurement of cerebral metabolic correlates of yohimbine administration in combat-related posttraumatic stress disorder. Archives of General Psychiatry 1997;54:246–254. [PubMed: 9075465]

Bunemann M, Bucheler MM, Philipp M, Lohse MJ, Hein L. Activation and deactivation kinetics of alpha 2A- and alpha 2C-adrenergic receptor-activated G protein-activated inwardly rectifying K+ channel currents. Journal of Biological Chemistry 2001;276:47512–47517. [PubMed: 11591725]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Cavalli A, Druey KM, Milligan G. The regulator of G protein signaling RGS4 selectively enhances alpha 2A-adreoreceptor stimulation of the GTPase activity of Go1alpha and Gi2alpha. Journal of Biological Chemistry 2000;275:23693–23699. [PubMed: 10807934]

Curtis AL, Lechner SM, Pavcovich LA, Valentino RJ. Activation of the locus coeruleus noradrenergic system by intracoerulear microinfusion of corticotropin-releasing factor: effects on discharge rate, cortical norepinephrine levels and cortical electroencephalographic activity. Journal of Pharmacology & Experimental Therapeutics 1997;281:163–172. [PubMed: 9103494]

Drenan RM, Doupnik CA, Boyle MP, Muglia LJ, Huettner JE, Linder ME, Blumer KJ. Palmitoylation regulates plasma membrane-nuclear shuttling of R7BP, a novel membrane anchor for the RGS7 family. Journal of Cell Biology 2005;169:623–633. [PubMed: 15897264]

Drenan RM, Doupnik CA, Jayaraman M, Buchwalter AL, Kaltenbronn KM, Huettner JE, Linder ME, Blumer KJ. R7BP augments the function of RGS7*Gbeta5 complexes by a plasma membrane-targeting mechanism. J Biol Chem 2006;281:28222–28231. [PubMed: 16867977]

Elsworth JD, Morrow BA, Nguyen VT, Mitra J, Picciotto MR, Roth RH. Prenatal cocaine exposure enhances responsivity of locus coeruleus norepinephrine neurons: Role of autoreceptors. Neuroscience 2007;147:419–427. [PubMed: 17543464]

Featherby T, Lawrence AJ. Chronic cold stress regulates ascending noradrenergic pathways. Neuroscience 2004;127:949–960. [PubMed: 15312907]

Finlay JM, Jedema HP, Rabinovic AD, Mana MJ, Zigmond MJ, Sved AF. Impact of corticotropin-releasing hormone on extracellular norepinephrine in prefrontal cortex after chronic cold stress. Journal of Neurochemistry 1997;69:144–150. [PubMed: 9202305]

Flugge G. Alterations in the central nervous alpha 2-adrenoceptor system under chronic psychosocial stress. Neuroscience 1996;75:187–196. [PubMed: 8923533]

Fluharty SJ, Snyder GL, Stricker EM, Zigmond MJ. Short- and long-term changes in adrenal tyrosine hydroxylase activity during insulin-induced hypoglycemia and cold stress. Brain Research 1983;267:384–387. [PubMed: 6135493]

Folk, GE, Jr.. Textbook of environmental physiology. 2nd Edition. Lea and Febiger; Philadelphia: 1974.

Foote SL, Bloom FE, Aston-Jones G. Nucleus locus ceruleus: new evidence of anatomical and physiological specificity. Physiological Reviews 1983;63:844–914. [PubMed: 6308694]

Gold SJ, Ni YG, Dohlman HG, Nestler EJ. Regulators of G-protein signaling (RGS) proteins: region-specific expression of nine subtypes in rat brain. Journal of Neuroscience 1997;17:8024–8037. [PubMed: 9315921]

Gold SJ, Han MH, Herman AE, Ni YG, Pudiak CM, Aghajanian GK, Liu RJ, Potts BW, Mumby SM, Nestler EJ. Regulation of RGS proteins by chronic morphine in rat locus coeruleus. European Journal of Neuroscience 2003;17:971–980. [PubMed: 12653973]

Grace AA, Bunney BS. The control of firing pattern in nigral dopamine neurons: burst firing. Journal of Neuroscience 1984;4:2877–2890. [PubMed: 6150071]

Grant SJ, Redmond DE Jr. Neuronal activity of the locus ceruleus in awake Macaca arctoides. Experimental Neurology 1984;84:701–708. [PubMed: 6723890]

Gresch PJ, Sved AF, Zigmond MJ, Finlay JM. Stress-induced sensitization of dopamine and norepinephrine efflux in medial prefrontal cortex of the rat. Journal of Neurochemistry 1994;63:575–583. [PubMed: 8035182]

Harlow, E.; Lane, D. Immunoblotting. In: Harlow, E.; Lane, D., editors. Antibodies: A laboratory manual. Cold Spring Harbor Laboratory Press; Cold Spring Harbor: 1988. p. 471-501.

He W, Lu L, Zhang X, El-Hodiri HM, Chen CK, Slep KC, Simon MI, Jamrich M, Wensel TG. Modules in the photoreceptor RGS9-1.Gbeta 5L GTPase-accelerating protein complex control effector coupling, GTPase acceleration, protein folding, and stability. J Biol Chem 2000;275:37093–37100. [PubMed: 10978345]

Hepler JR. R7BP: A surprising new link between G proteins, RGS proteins, and nuclear signaling in the brain. Sci STKE 2005;294:pe38. 2005. [PubMed: 16046666]

Heximer SP, Srinivasa SP, Bernstein LS, Bernard JL, Linder ME, Hepler JR, Blumer KJ. G protein selectivity is a determinant of RGS2 function. Journal of Biological Chemistry 1999;274:34253–34259. [PubMed: 10567399]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Hollinger S, Hepler JR. Cellular regulation of RGS proteins: modulators and integrators of G protein signaling. Pharmacological Reviews 2002;54:527–559. [PubMed: 12223533]

Huang HP, Wang SR, Yao W, Zhang C, Zhou Y, Chen XW, Zhang B, Xiong W, Wang LY, Zheng LH, Landry M, Hökfelt T, Xu ZQD, Zhou Z. Long latency of evoked quantal transmitter release from somata of locus coeruleus neurons in rat pontine slices. Proc Natl Acad Sci U S A 2007;104:1401–1406. [PubMed: 17227848]

Jedema HP, Grace AA. Chronic exposure to cold stress alters electrophysiological properties of locus coeruleus neurons recorded *in vitro*. Neuropsychopharmacology 2003;28:63–72. [PubMed: 12496941]

Jedema HP, Sved AF, Zigmond MJ, Finlay JM. Sensitization of norepinephrine release in medial prefrontal cortex: effect of different chronic stress protocols. Brain Research 1999;830:211–217. [PubMed: 10366677]

Jedema HP, Finlay JM, Sved AF, Grace AA. Chronic cold exposure potentiates CRH-evoked increases in electrophysiologic activity of locus coeruleus neurons. Biological Psychiatry 2001;49:351–359. [PubMed: 11239906]

Krumins AM, Barker SA, Huang C, Sunahara RK, Yu K, Wilkie TM, Gold SJ, Mumby SM. Differentially regulated expression of endogenous RGS4 and RGS7. Journal of Biological Chemistry 2004;279:2593–2599. [PubMed: 14604980]

Lacroix D, Blier P, Curet O, de Montigny C. Effects of long-term desipramine administration on noradrenergic neurotransmission: electrophysiological studies in the rat brain. Journal of Pharmacology & Experimental Therapeutics 1991;257:1081–1090. [PubMed: 1646320]

Mana MJ, Grace AA. Chronic cold stress alters the basal and evoked electrophysiological activity of rat locus coeruleus neurons. Neuroscience 1997;81:1055–1064. [PubMed: 9330367]

Meyer H, Palchaudhuri M, Scheinin M, Flugge G. Regulation of alpha(2A)-adrenoceptor expression by chronic stress in neurons of the brain stem. Brain Research 2000;880:147–158. [PubMed: 11032999]

Miner LAH, Jedema HP, Moore FW, Blakely RD, Grace AA, sesack SR. Chronic Stress Increases the Plasmalemmal Distribution of the Norepinephrine Transporter and the Coexpression of Tyrosine Hydroxylase in Norepinephrine Axons in the Prefrontal Cortex. Journal of Neuroscience (Online) 2006;26:1571–1578. [PubMed: 16452680]

Mirnics K, Middleton FA, Stanwood GD, Lewis DA, Levitt P. Disease-specific changes in regulator of G-protein signaling 4 (RGS4) expression in schizophrenia. Molecular Psychiatry 2001;6:293–301. [PubMed: 11326297]

Moore H, Rose HJ, Grace AA. Chronic cold stress reduces the spontaneous activity of ventral tegmental dopamine neurons. Neuropsychopharmacology 2001;24:410–419. [PubMed: 11182536]

Morilak DA, Barrera G, Echevarria DJ, Garcia AS, Hernandez A, Ma S, Petre CO. Role of brain norepinephrine in the behavioral response to stress. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2005;29:1214–1224. [PubMed: 16226365]

Ni YG, Gold SJ, Iredale PA, Terwilliger RZ, Duman RS, Nestler EJ. Region-specific regulation of RGS4 (Regulator of G-protein-signaling protein type 4) in brain by stress and glucocorticoids: in vivo and in vitro studies. Journal of Neuroscience 1999;19:3674–3680. [PubMed: 10233999]

Nisenbaum LK, Abercrombie ED. Enhanced tyrosine hydroxylation in hippocampus of chronically stressed rats upon exposure to a novel stressor. Journal of Neurochemistry 1992;58:276–281. [PubMed: 1345767]

Nisenbaum LK, Abercrombie ED. Presynaptic alterations associated with enhancement of evoked release and synthesis of norepinephrine in hippocampus of chronically cold-stressed rats. Brain Research 1993;608:280–287. [PubMed: 8098649]

Nisenbaum LK, Zigmond MJ, Sved AF, Abercrombie ED. Prior exposure to chronic stress results in enhanced synthesis and release of hippocampal norepinephrine in response to a novel stressor. Journal of Neuroscience 1991;11:1478–1484. [PubMed: 1674004]

Oliveira-dos-Santos AJ, Matsumoto G, Snow BE, Bai D, Houston FP, Whishaw IQ, Mariathasan S, Sasaki T, Wakeham A, Ohashi PS, Roder JC, Barnes CA, Siderovski DP, Penninger JM. Regulation of T cell activation, anxiety, and male aggression by RGS2. Proceedings of the National Academy of Sciences of the United States of America 2000;97:12272–12277. [PubMed: 11027316]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Pardon M-C, Ma S, Morilak DA. Chronic cold stress sensitizes brain noradrenergic reactivity and noradrenergic facilitation of the HPA stress response in Wistar Kyoto rats. Brain Research 2003;971:55–65. [PubMed: 12691837]

Pavcovich LA, Cancela LM, Volosin M, Molina VA, Ramirez OA. Chronic stress-induced changes in locus coeruleus neuronal activity. Brain Research Bulletin 1990;24:293–296. [PubMed: 2157529]

Pineda J, Ruiz-Ortega JA, Ugedo L. Receptor reserve and turnover of alpha-2 adrenoceptors that mediate the clonidine-induced inhibition of rat locus coeruleus neurons in vivo. Journal of Pharmacology & Experimental Therapeutics 1997;281:690–698. [PubMed: 9152374]

Prieto, Mn; GÃ3mez, FM.; Giralt, M Teresa. Effects of acute, repeated and chronic variable stress on in vivo tyrosine hydroxylase activity and on alpha(2)-adrenoceptor sensitivity in the rat brain. Stress 2003;6:281–287. [PubMed: 14660060]

Ross EM, Wilkie TM. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. Annual Review of Biochemistry 2000;69:795–827.

Seiple SD, Jedema HP, Sved AF, Zigmond MJ, Austin MC, Finlay JM. Chronic stress: effects on behavior and tyrosine hydroxylase mRNA in the locus coeruleus. Society for Neuroscience abstract 1997;23:1078.

Simson PE, Weiss JM. Alpha-2 receptor blockade increases responsiveness of locus coeruleus neurons to excitatory stimulation. Journal of Neuroscience 1987;7:1732–1740. [PubMed: 2885401]

Simson PE, Weiss JM. Altered activity of the locus coeruleus in an animal model of depression. Neuropsychopharmacology 1988;1:287–295. [PubMed: 2908021]

Southwick SM, Bremner AD, Grillon CG, Krystal JH, Nagy LM, Charney DS. Noradrenergic alterations in posttraumatic stress disorder. Annals of the New York Academy of Sciences 1997;821:125–141. 3rd CAM. [PubMed: 9238199]

Stanford SC. Central noradrenergic neurones and stress. Pharmacology & Therapeutics 1995;68:297–242. [PubMed: 8719972]

Taymans JM, Wintmolders C, Te Riele P, Jurzak M, Groenewegen HJ, Leysen JE, Langlois X. Detailed localization of regulator of G protein signaling 2 messenger ribonucleic acid and protein in the rat brain. Neuroscience 2002;114:39–53. [PubMed: 12207953]

Thierry AM, Javoy F, Glowinski J, Kety SS. Effects of stress on the metabolism of norepinephrine, dopamine and serotonin in the central nervous system of the rat. I. Modifications of norepinephrine turnover. Journal of Pharmacology & Experimental Therapeutics 1968;163:163–171. [PubMed: 5673703]

Valentino RJ, Foote SL. Corticotropin-releasing hormone increases tonic but not sensory-evoked activity of noradrenergic locus coeruleus neurons in unanesthetized rats. Journal of Neuroscience 1988;8:1016–1025. [PubMed: 3258021]

Vernikos J, Dallman MF, Bonner C, Katzen A, Shinsako J. Pituitary-adrenal function in rats chronically exposed to cold. Endocrinology 1982;110:413–420. [PubMed: 6276134]

Williams JT, Henderson G, North RA. Characterization of alpha 2-adrenoceptors which increase potassium conductance in rat locus coeruleus neurones. Neuroscience 1985;14:95–101. [PubMed: 2579354]

Williams JT, North RA, Shefner SA, Nishi S, Egan TM. Membrane properties of rat locus coeruleus neurones. Neuroscience 1984;13:137–156. [PubMed: 6493483]

Wong ML, Kling MA, Munson PJ, Listwak S, Licinio J, Prolo P, Karp B, McCutcheon IE, Geracioti TD Jr. DeBellis MD, Rice KC, Goldstein DS, Veldhuis JD, Chrousos GP, Oldfield EH, McCann SM, Gold PW. Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: relation to hypercortisolism and corticotropin-releasing hormone. Proceedings of the National Academy of Sciences of the United States of America 2000;97:325–330. [PubMed: 10618417]

Xie, G-x; Palmer, PP. RGS proteins: new players in the field of opioid signaling and tolerance mechanisms. Anesthesia & Analgesia 2005;100:1034–1042. [PubMed: 15781518]

Yalcin B, Willis-Owen SAG, Fullerton J, Meesaq A, Deacon RM, Rawlins JNP, Copley RR, Morris AP, Flint J, Mott R. Genetic dissection of a behavioral quantitative trait locus shows that Rgs2 modulates anxiety in mice. Nature Genetics 2004;36:1197–1202. [see comment]. [PubMed: 15489855]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Zhang S, Coso OA, Lee C, Gutkind JS, Simonds WF. Selective activation of effector pathways by brain-specific G protein beta5. Journal of Biological Chemistry 1996;271:33575–33579. [PubMed: 8969224]

Zigmond, MJ.; Finlay, JM.; Sved, AF. Neurochemical studies of central noradrenergic responses to acute and chronic stress. In: Friedman, MJ.; Charney, DS.; Deutsch, AY., editors. Neurobiological and clinical consequences of stress: from normal adaptation to PTSD. Lippincott-Raven Publishers; Philadelpia: 1995. p. 45-60.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

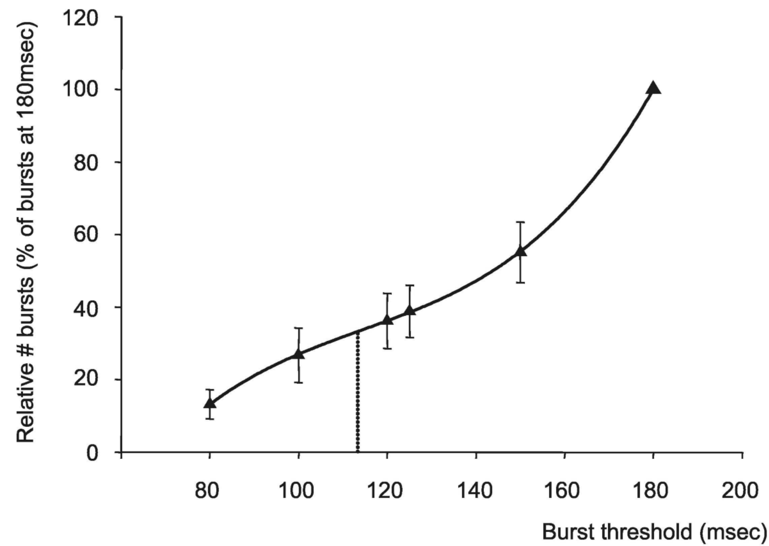

Figure 1. The number of spikes in bursts as a function of burst threshold

Based on our empirical observation that 80msec was too low of a threshold to define burst firing in LC neurons, we examined the influence of varying the threshold criterion on bursting in a separate group of control rats showing clear burst activity (N=10 rats). Plotting the relative number of bursts as a function of threshold revealed an inflexion point in the relationship at 114msec; this definition was then visually confirmed to accurately identify burst events. Therefore, we used 110msec as the burst threshold criterion for LC neurons.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

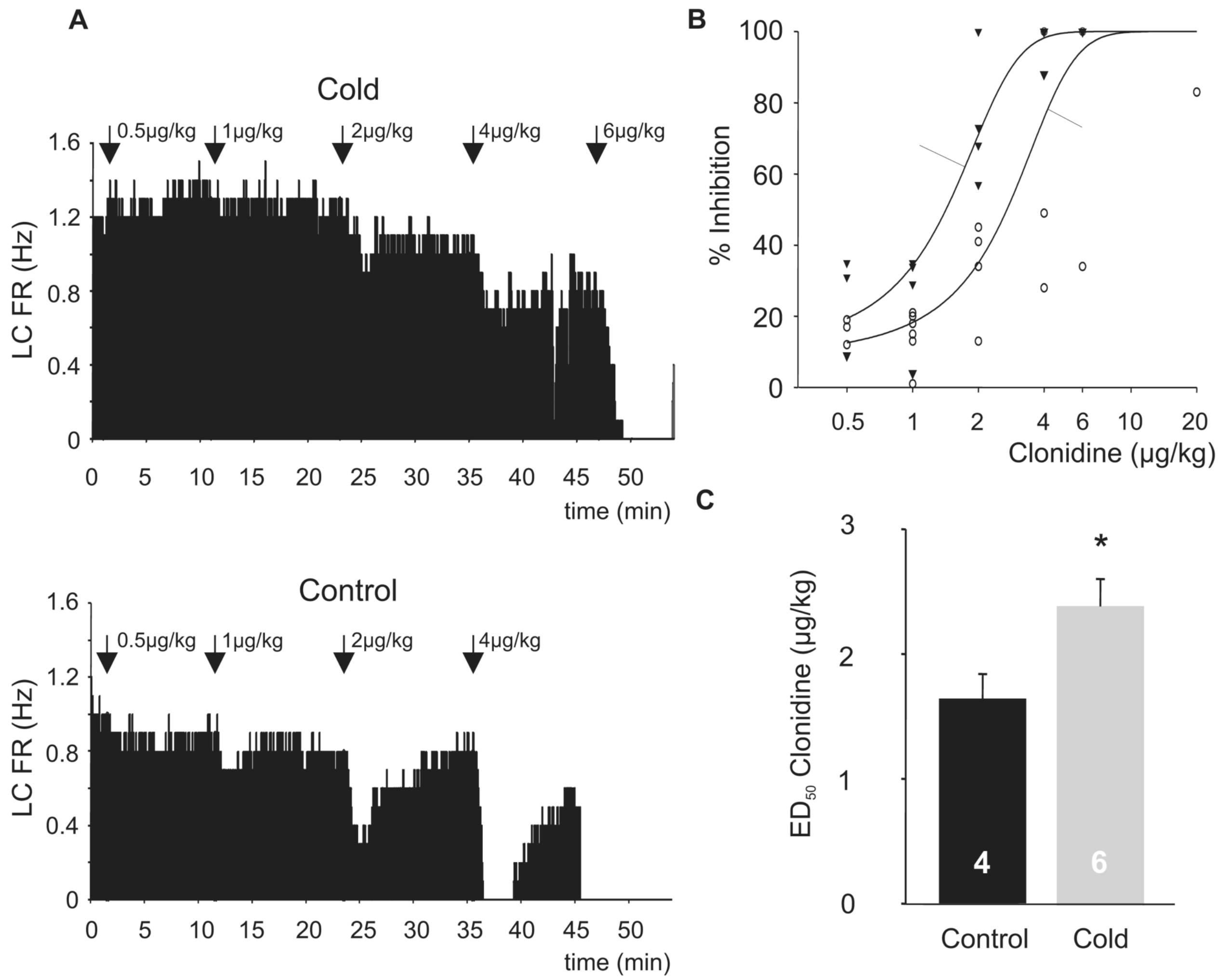

Figure 2. Clonidine-evoked inhibition of LC neuron activity *in vivo*

(A) A firing rate histogram of an LC neuron in a halothane-anesthetized, cold-exposed (top) and control (bottom) rat demonstrates the inhibitory effect of escalating doses of IV administration of clonidine. The brief period of LC activity at the far right of the top histogram reflects the response to a foot pinch which was consistently used following cessation of activity to verify the quality of the neuronal recording. (**B**) The maximal inhibitory effect of clonidine occurred at higher doses in rats previously exposed to chronic cold. Open circles represent individual data points of cold-exposed rats (N=6) and triangles represent data points of cold exposed rats (N=4). The regression line for all data points in each group demonstrates a right-ward shift in cold-exposed animals. (**C**) The ED_{50} calculated for individual neurons was higher in previously cold-exposed rats.

* p=0.03

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

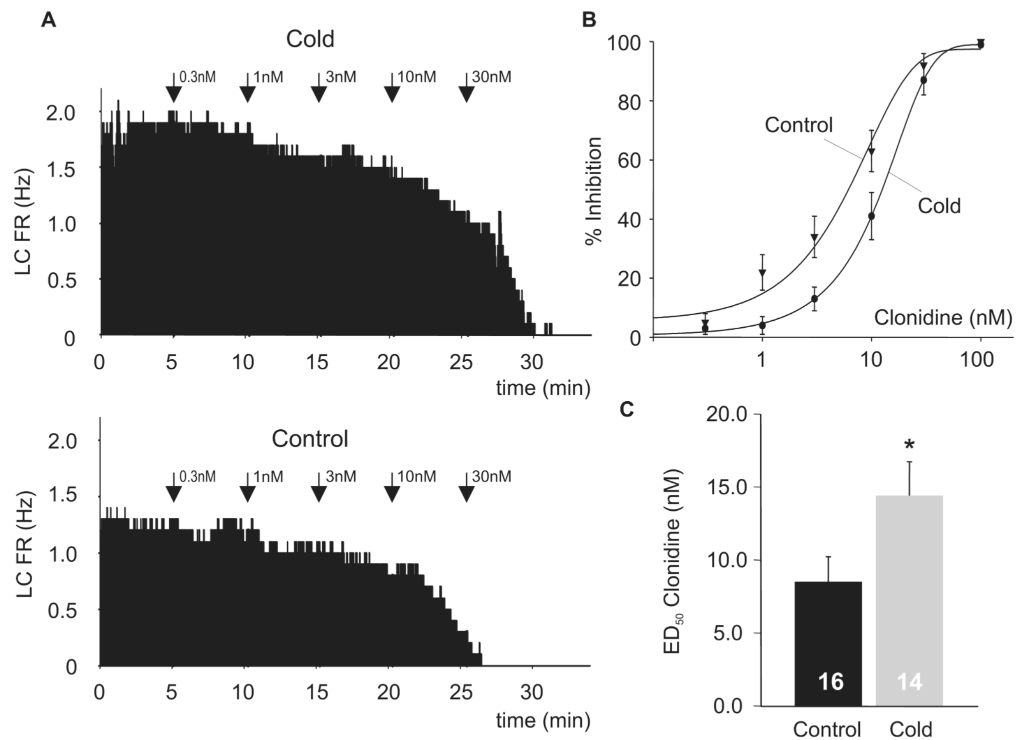

Figure 3. Clonidine-evoked inhibition of LC neuron activity *in vitro*

(A) A firing rate histogram of an LC neuron in a horizontal brain stem slice demonstrates the inhibitory effect of escalating doses of bath application of clonidine. (**B**) When the inhibitory effect of clonidine (average firing rate over 2 min period starting 3min after clonidine entered the bath) was plotted as a function of dose, it was apparent that LC neurons in slices from cold-exposed rats (circles; N=16) were less responsive to clonidine compared to controls (triangles; N= 14). (**C**) The ED_{50} calculated for individual neurons was higher in slices from previous cold-exposed rats.

* p=0.04

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

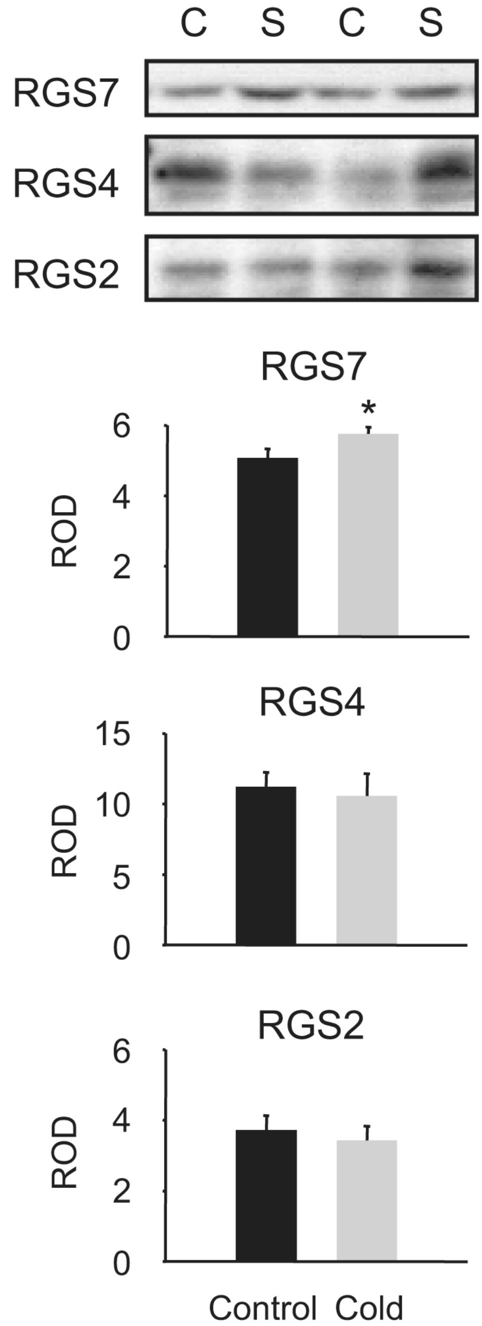

Figure 4. Expression of Regulators of G-protein Signaling proteins in LC

Representative autoradiograms from quantitative Western blots demonstrate the expression of RGS2, RGS4, and RGS7 in tissue punches of LC tissue from control (C) and cold-stressed (S) rats. There was no difference in the expression of RGS2 and RGS4 immunoreactivity between previously cold-exposed and control rats, but the levels of RGS7 immunoreactivity were significantly increased. (N=9-10 rats/group). ROD Relative Optical Density

* p=0.04

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

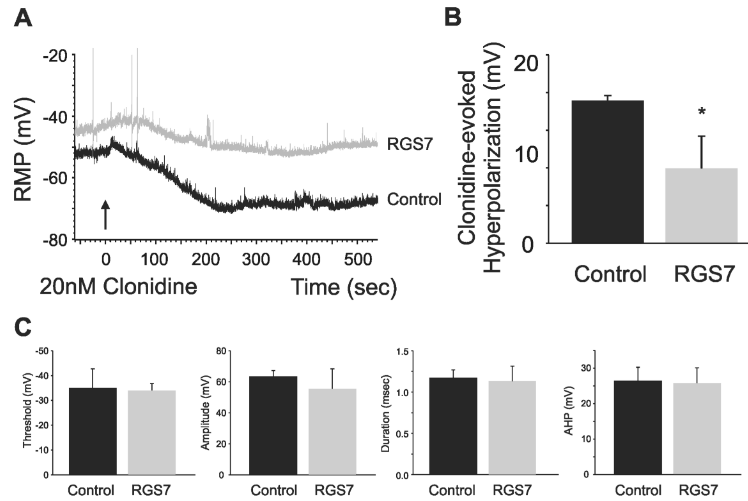

Figure 5. Exogenous intracellular RGS7 decreases the clonidine-induced hyperpolarization of LC neurons

(**A**) Administration of clonidine (20nM) hyperpolarized control neurons (black; N=3) to a greater extent than neurons in which RGS7 was infused through the patch pipette (grey; N=4). (**B**) The peak effect of clonidine was significantly reduced in neurons in which RGS7 was infused, while (**C**) the basic spike properties of these neurons determined immediately upon gaining access were unaffected.

* p=0.0475

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

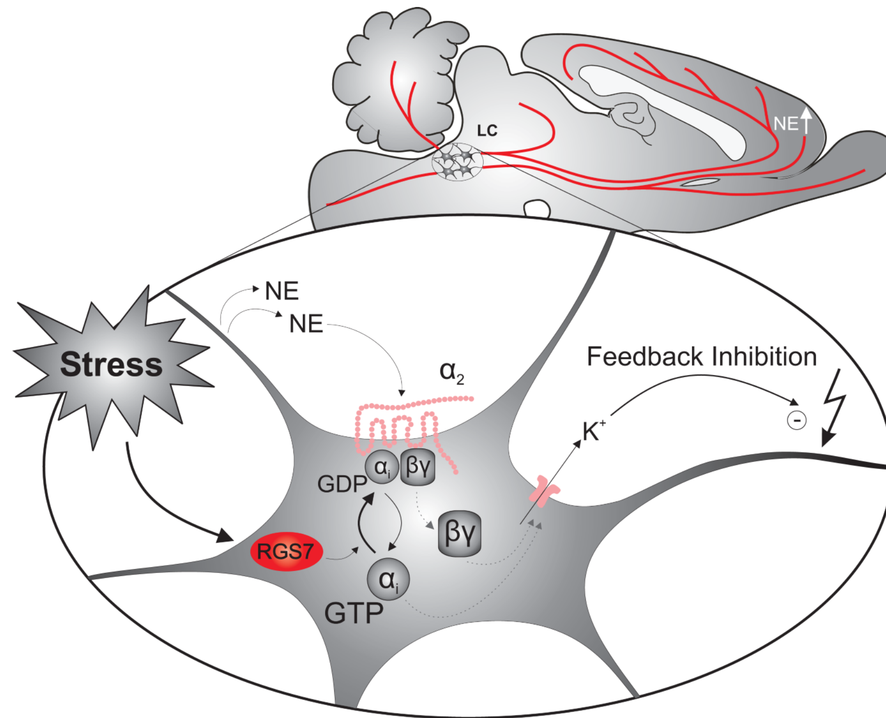

Figure 6.

Norepinephrine released by LC neurons contributes to the feedback inhibition of activity via α_2-receptor-mediated opening of potassium conductances. A chronic stress-induced increase in RGS7 expression accelerates the hydrolysis of GTP and leads to a more rapid termination of the effect of noradrenergic autoreceptor stimulation. The resulting reduction of the autoreceptor-mediated feedback inhibition leads to an enhanced activation of LC neurons, particularly upon stimulation, and increased norepinephrine release throughout the central nervous system, which may contribute to increased anxiety-related behaviors.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1

In vivo LC characteristics

	Control	**Cold**	**p-value**
	N=4	**N=6**	
Baseline Firing Rate (Hz)	0.9±0.1	1.7±0.3	0.045
% Spikes in bursts	0.0±0.0	7.7±2.3	0.010
% halothane	2.6±0.4	2.2±0.3	NS
ED_{50} Clonidine (μg/kg)	1.64±0.20	2.40±0.22	0.030

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 2

In vitro LC characteristics

	Control N=17	Cold N=14	p-value
Baseline Firing Rate (Hz)	1.1±0.1	1.6±0.1	0.004
% spikes in bursts	0.1±0.1	0.4±0.2	0.089
ED_{50} Clonidine (nM)	8.6±1.7	14.5±2.3	0.040