

Voluntary exercise and clomipramine treatment elevate prepro-galanin mRNA levels in the locus coeruleus in rats

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Abstract

Exercise exerts antidepressant effects in humans and rodent models of affective disorders. These effects may be mediated by the upregulation of endogenous factors that exert antidepressant actions. The physiological functions and behavioral actions of the neuropeptide galanin (GAL) suggest antidepressant activity. Previous studies have shown that various modes of exercise elevate GAL gene expression in the locus coeruleus (LC) in rats. The present experiments examined the interaction between voluntary exercise and antidepressant pharmacotherapy. Male Sprague–Dawley rats were provided access to activity wheels (exercise condition) or inoperative wheels (sedentary condition) for 28 days. Rats in each group were injected with clomipramine (10 mg/kg/day) or vehicle throughout this period (for 3 weeks). Prepro-GAL mRNA in the LC was measured by *in situ* hybridization histochemistry. Exercise and clomipramine treatment significantly elevated GAL gene expression, though prepro-GAL mRNA levels in rats receiving both interventions did not differ from sedentary controls that received vehicle. Prepro-GAL mRNA levels were significantly correlated with running distance. The results further implicate a role for GAL in the antidepressant effects of exercise and pharmacotherapy, though the mechanisms through which these treatments influence GAL gene expression appear to differ significantly.

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An accumulating body of evidence consistently reveals that physical exercise exerts antidepressant effects in humans [3,4,8]. Research from our laboratory using different rodent models of depression supports this finding. Specifically, we have reported that voluntary physical activity in rats restores the deficits in appetitively motivated behavior that occur in two, distinct rodent models of depression [2,16]. These and other experiments have also revealed potential neural mechanisms for the antidepressant effects of exercise. For example, the expression of various neurotrophins, such as brain-derived neurotrophic factor (BDNF) [12,15], and the neuropeptide galanin (GAL) [10,15] increases after exercise. Both BDNF [7,13] and GAL [9] have been shown to exert antidepressant-like actions in rodent models, suggesting their role in the mitigation of depression symptoms through exercise.

Considering the putative role that the forebrain noradrenergic system plays in affective disorders, the locus coeruleus (LC)

is a good candidate for examining the neural mechanisms for the antidepressant effects of exercise, pharmacotherapy, and the potential interaction between these two treatments. Our previous research has focused primarily on exercise-induced upregulation of GAL in the LC, where GAL is densely co-localized with noradrenergic neurons. Prior studies have demonstrated that various experimental modes of exercise in rats increase galanin (GAL) gene expression in the LC. We have thus reported that 6 weeks of treadmill training in Fischer rats increased prepro-GAL mRNA levels in the LC without influencing tyrosine hydroxylase [10]. Three weeks of voluntary wheel running similarly increased GAL gene expression in the LC of Long-Evans rats [15].

The present experiment was designed to control for a potentially confounding variable inherent in our previous experiments, and it provides further evidence supporting the generalizability of exercise-induced regulation of GAL. Our previous report of GAL upregulation after voluntary, activity wheel exercise in Long-Evans rats [15] involved a fear-conditioning paradigm subsequent to the exercise condition. The increased GAL we observed may, therefore, have resulted from an interaction between the stress of the learning paradigm and the exercise

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condition. The present experiments involved no such behavioral intervention. Sprague–Dawley rats served as subjects in order to determine whether previous effects observed in Long-Evans rats generalize to this strain.

The present experiment was also designed to examine the effect of antidepressant therapy on GAL gene expression in the LC. Rats thus received either saline or the tricyclic antidepressant clomipramine. We hypothesized that chronic clomipramine treatment would elevate GAL mRNA in the LC similarly to exercise. The potential interaction between the exercise manipulation and drug treatment was thereby assessed.

All experiments were approved by the University of Georgia Animal Care and Use Committee and followed the guidelines of the NIH Guide for the Care and Use of Laboratory Animals. Male Sprague–Dawley rats weighing 225–250 g at the beginning of the exercise condition were group housed in 30 cm × 60 cm polycarbonate cages in a temperature and humidity-controlled vivarium for a 1-week acclimation period. Lighting was maintained on a 12/12 h light/dark cycle with lights off at 07:00 h. Following acclimation, rats were individually housed in the same size cages equipped with 105 cm circumference Minimitter activity wheels fitted with electromagnetic revolution counters. Rats were randomly assigned to either exercise or sedentary conditions. Wheels were locked in place for the sedentary condition. Rats in both exercise and sedentary conditions were randomly assigned to drug treatment conditions of vehicle (0.9% saline) or clomipramine (10 mg/kg/day, IP), yielding a 2 × 2 factorial design (exercise × drug). Injections began on the 8th day of introducing the activity wheel and continued with a single, daily injection for 21 days at 18:00–20:00 h. Running distance was measured daily and calculated by multiplying the number of revolutions by the circumference of the wheel. All rats were weighed daily. Rats were killed between 07:00 and 11:00 h on the final day of the exercise condition (approximately 12 h after their final drug injection). Brains were removed, frozen on dry ice, and stored at –80 °C.

Brains were cut in 12 μm sections through the LC (Plates 57–59 of Paxinos and Watson atlas [11]) using a Microm cryostat and prepared for in situ hybridization histochemistry as previously described. Briefly, sections were fixed in 4% formaldehyde in 0.12 M sodium phosphate-buffered saline (PBS, pH 7.4), dehydrated using a series of ethanol and acetic anhydride washes, delipidated in chloroform, and allowed to dry. An oligonucleotide probe (Oligos Etc.) complementary to bases 115–153 of porcine prepro-GAL mRNA transcript was labeled at the 3'-end with [35S]-dATP (Perkin-Elmer), terminal deoxynucleotidyl transferase (25 units/ml; Roche), and tailing buffer. Column separation was used to separate unincorporated nucleotides from the probes. Sections were hybridized with radiolabeled probes in solution containing 50% formamide, 600 mM NaCl, 80 mM Tris–HCl, 4 mM EDTA, 0.1% sodium pyrophosphate, 0.2% SDS, 0.2 mg/ml heparin sulfate, and 10% dextran sulfate. Brain sections were incubated with the hybridization solution for 20 h in a humid chamber at 37 °C. Sections were then subjected to a series of washes to reduce non-specific binding (M sodium citrate and 50% formamide at 40 °C) and were exposed to autoradiographic film (BioMax MR, Eastman Kodak).

Autoradiographic films were analyzed using a computerized image analysis system to determine optical density (OD) within the LC (Image 1.38 software, Rasband, 1995, National Institute of Mental Health).

The LC was analyzed by taking the average OD reading using an 8 × 10 pixel oval placed in the dorsal portion of the nucleus, in each section, of each animal. The investigator was unaware of subject condition at the time of quantification.

Based on our previous studies we expected to demonstrate a 1 standard deviation effect for the 2 (exercise versus sedentary) × 2 (drug versus saline) interaction for GAL mRNA. A sample size of 11 animals per cell was deemed necessary to provide a statistical power of 0.80 at an α of $p < 0.05$. Therefore, 11 animals were assigned to each of the experimental conditions. Running distance and body weight data were analyzed by two-way ANOVA with weeks as a repeated measure. Huynh-Feldt was used to adjust degrees of freedom for violations of sphericity in the repeated measure. Autoradiographic film data were analyzed by two-way ANOVA followed by post hoc contrasts. Data were missing for three animals each in the drug and sedentary groups. Outliers (two runners in the saline group and one runner in the drug group) were identified using Grubb's criterion [6]. The relation between running distance and prepro-GAL mRNA levels was examined using multiple linear regression analysis, including drug group and body weight as covariates.

Consistent with our previous studies, running distance increased over the course of the exercise condition ($F(3,48) = 22.9$, $p < 0.01$, $\eta^2 = 0.59$). The increase was best described by a quadratic trend ($F(1,16) = 21.2$, $p < 0.01$), with increases diminishing after week 3. Differences in running distances between saline-treated and clomipramine-treated rats, and the group-by-time interaction, were not significant ($p > 0.40$). By week 3 and 4, mean running distances for both the saline and clomipramine-treated rats were comparable (25.0 km/week in saline-treated rats and 25.4 km/week in clomipramine-treated rats during week 4). The overall average daily running distance was approximately 30% lower than that observed in our previous study of the effects of activity wheel running on GAL mRNA in the LC [15]. Body weights increased linearly over the 4 weeks of the study ($F(3,105) = 210.7$, $p < 0.01$, $\eta^2 = 0.86$). In addition, a group-by-time interaction was observed ($F(9,105) = 5.9$, $p < 0.01$, $\eta^2 = 0.34$). Sedentary rats treated with clomipramine exhibited a lower rate of weight gain than the other groups. Sedentary rats treated with saline had the highest weights by week 4 (mean = 297.7 g). Mean body weights for all other groups ranged between 276 and 279 g.

Analysis of the in situ hybridization data for prepro-GAL mRNA levels revealed a significant interaction between exercise and drug treatment ($F(1,31) = 6.03$, $p = 0.02$, $\eta^2 = 0.16$; see Fig. 1). The mean prepro-GAL mRNA levels in the LC in exercising rats that received saline and sedentary rats that received clomipramine were one standard deviation higher than that of sedentary rats treated with saline ($p = 0.04$). Linear regression analyses revealed a significant, positive relation between running distance during week 4 and prepro-GAL mRNA in the LC that was independent of effects of drug treatment and body weight (partial $r = 0.65$, $p < 0.01$; see Fig. 2).

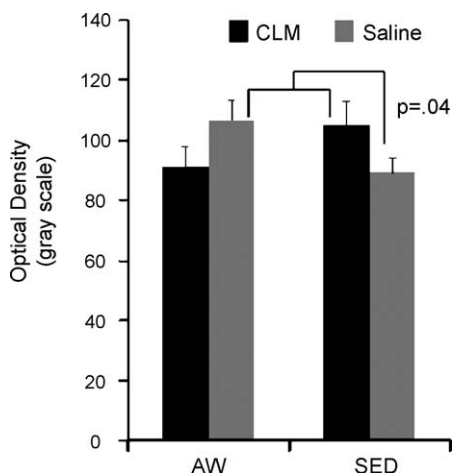


Fig. 1. The effects of activity wheel (AW) running and chronic clomipramine (CLM) treatment on prepro-galanin mRNA levels in the locus coeruleus measured by in situ hybridization. Prepro-galanin mRNA levels were significantly elevated in AW + saline and in SED + CLM rats, compared to SED + saline rats ($p = 0.04$).

The results confirm and extend our previous finding of exercise-induced upregulation of prepro-GAL in the LC. Though voluntary wheel-running distances were lower than those observed in our previous study [15], the exercise-induced increases in GAL gene expression were comparable between the two studies (approximately one standard deviation increase in the present study versus approximately 1.5 in the prior experiment). The significant correlation between running distance and prepro-GAL mRNA suggests that the effect of exercise on GAL gene expression is “dose-dependent”, so it is reasonable to conclude that differences in the magnitude of GAL gene expression observed across experiments are directly related to differences in running distance. The lower running distance observed in the present study was most likely the result of mild stress inherent in daily handling and injections. Though Long-Evans rats served as subjects in the previous experiments, strain differences are probably not a significant factor in the effects of voluntary running on GAL gene expression. Experiments with Sprague–Dawley rats currently underway in our laboratory demonstrate higher rates of voluntary running compared to our prior work with Long-Evans

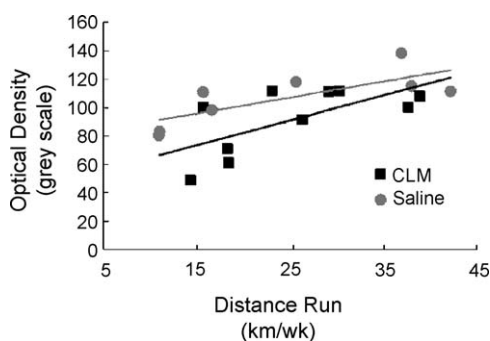


Fig. 2. Scatter plot depicting the relation between running distance and prepro-galanin mRNA levels in the locus coeruleus. Prepro-galanin mRNA was significantly correlated with running distance independently of body weight or drug treatment ($p < 0.01$).

rats [in preparation]. That exercise increases GAL gene expression in both strains suggests that exercise-induced upregulation of GAL is a general phenomenon in rats.

The present findings resolve a potential confound inherent in our previous study. In that study [15], rats were killed immediately after testing in a fear-conditioning paradigm, which involved measure of defensive freezing behavior conditioned to electric footshocks. It was therefore possible that the increased GAL gene expression we observed resulted from an interaction between stress and exercise. The present experiments involved no significant stressor other than daily handling and IP injections, and the nature and time course of this mild stressor clearly differs from that of fear-conditioning. Rats were injected daily for 21 days, offering the opportunity for stressor habituation, and the final injection was given approximately 12 h prior to brain collection, diminishing the potential impact of any acute stress response that may have occurred to the injection. Further supporting the conclusion that exercise-induced GAL gene expression does not require an interaction with stress, a previous, unpublished experiment from our laboratory found no effect of wheel running on LC GAL in rats that were injected daily but were killed three to five days after activity wheels were removed. In order to determine whether our failure to detect exercise-induced changes in GAL in this previous, unpublished experiment was due to the delay between exercise and brain harvesting rather than the influence of daily injections, the present experiments employed daily injections as before but advanced the time point for euthanasia. Though we have not systematically evaluated this possibility, it appears that elevated GAL gene expression may diminish within days after exercise has ceased.

Our results are consistent with a previous report of elevations in GAL gene expression in the LC consequent to chronic antidepressant therapy. In this experiment [9], prepro-GAL mRNA was measured by real time PCR after fluoxetine was administered daily for 14 days. This previous study also found increased GAL gene expression after sleep deprivation and electroconvulsive therapy. Our data revealed no additive effects of exercise and chronic clomipramine treatment. Rather, a negative interaction was observed, suggesting that exercise and pharmacotherapy alter GAL gene expression through distinct and possibly interfering processes. Taken together, the present and previous results suggest that upregulation of endogenous GAL may function as a common mediator of antidepressant effects for a variety of interventions, though the precise mechanisms by which these interventions alter GAL gene expression may differ significantly. Considering the well established antidepressant effects of exercise in humans [3,4,8], and previous findings of similar effects in rat models of depression [1,2,5,16], the present findings implicate GAL in the exercise-induced amelioration of depression symptoms.

References

- [1] A. Bjornebekk, A.A. Mathe, S. Brene, The antidepressant effect of running is associated with increased hippocampal cell proliferation, *Int. J. Neuropsychopharmacol.* 8 (2005) 357–368.

- [2] H.O. Chambliss, J.D. Van Hoomissen, P.V. Holmes, B.N. Bunnell, R.K. Dishman, Effects of chronic activity wheel running and imipramine on male copulatory behavior after olfactory bulbectomy, *Physiol. Behav.* 82 (2004) 593–600.
- [3] A.L. Dunn, M.H. Trivedi, J.B. Kampert, C.G. Clark, H.O. Chambliss, Exercise treatment for depression: efficacy and dose response, *Am. J. Prev. Med.* 28 (2005) 1–8.
- [4] A.L. Dunn, M.H. Trivedi, H.A. O'Neal, Physical activity dose-response effects on outcomes of depression and anxiety, *Med. Sci. Sports. Exerc.* 33 (2001) S587–S597.
- [5] B.N. Greenwood, T.E. Foley, H.E. Day, J. Campisi, S.H. Hammack, S. Campeau, S.F. Maier, M. Fleshner, Freewheel running prevents learned helplessness/behavioral depression: role of dorsal raphe serotonergic neurons, *J. Neurosci.* 23 (2003) 2889–2898.
- [6] F.E. Grubbs, G. Beck, Extension of sample sizes and percentage points for significance test of outlying observations, *Technometrics* 14 (1972) 847–854.
- [7] B.A. Hoshaw, J.E. Malberg, I. Lucki, Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects, *Brain Res.* 1037 (2005) 204–208.
- [8] D.A. Lawlor, S.W. Hopker, The effectiveness of exercise as an intervention in the management of depression: systematic review and meta-regression analysis of randomised controlled trials, *BMJ* 322 (2001) 763–767.
- [9] X. Lu, A.M. Barr, J.W. Kinney, P. Sanna, B. Conti, M.M. Behrens, T. Bartfai, A role for galanin in antidepressant actions with a focus on the dorsal raphe nucleus, *Proc. Natl. Acad. Sci. U.S.A.* 102 (2005) 874–879.
- [10] H.A. O'Neal, J.D. Van Hoomissen, P.V. Holmes, R.K. Dishman, Preprogalanin mRNA levels are increased in rat locus coeruleus after treadmill exercise training, *Neurosci. Lett.* 299 (2001) 69–72.
- [11] G. Paxinos, C. Watson, *The Rat Brain Stereotaxic Coordinates*, Academic Press, Orlando, FL, 1986.
- [12] A.A. Russo-Neustadt, R.C. Beard, Y.M. Huang, C.W. Cotman, Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus, *Neuroscience* 101 (2000) 305–312.
- [13] J.A. Siuciak, D.R. Lewis, S.J. Wiegand, R.M. Lindsay, Antidepressant-like effect of brain-derived neurotrophic factor (BDNF), *Pharmacol. Biochem. Behav.* 56 (1997) 131–137.
- [15] J.D. Van Hoomissen, P.V. Holmes, A.S. Zellner, A.M. Poudevigne, R.K. Dishman, The effect of B-adrenergic blockade during chronic exercise on contextual fear conditioning and mRNA for galanin and brain-derived neurotrophic factor, *Behav. Neurosci.* 118 (2004) 1378–1390.
- [16] H.S. Yoo, R.L. Tackett, B.N. Bunnell, J.B. Crabbe, R.K. Dishman, Antidepressant-like effects of physical activity vs. imipramine: neonatal clomipramine model, *Psychobiology* 28 (2000) 540–549.