

Paroxetine-Induced Increase in Activity of Locus Coeruleus Neurons in Adolescent Rats: Implication of a Countertherapeutic Effect of an Antidepressant

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The concern that antidepressant (AD) drugs, especially selective serotonin reuptake inhibitors and paroxetine (PAR) in particular, can increase suicidality during the early treatment of juvenile patients (children and adolescents) has created a dilemma for clinicians treating depressives. Although preclinical research cannot resolve controversy in this area, our present findings may provide insight into how AD drugs might, under certain conditions, exacerbate rather than ameliorate the depressive state. Both clinical and preclinical evidences indicate that the principal noradrenergic cell group in the brain, the locus coeruleus (LC), is overactive in depressives and that, conversely, effective AD treatments decrease the activity of LC neurons. We report here that short-term (2 and 4 days) administration of PAR produces an increase in the activity of LC neurons (spontaneous firing rate and sensory-evoked responses) in young rats, contrary to the 'therapeutic' decrease in activity typically observed in adult rats. Blood levels of PAR were lower in young rats than in adult rats, although similar low blood levels produced by a lower dose of PAR in adult rats failed to produce an increase in LC activity. In addition, activity of young rats in the swim test was determined to assess depressive-like responses. The same dose/durations of PAR, which produced the largest increases in LC activity in young rats, produced decreases in swim-test activity, indicating that brief administration of PAR in young rats can promote, rather than reduce, the depressive state. These results offer a model that may help screen potential adjunctive treatments to avoid early adverse effects of ADs.

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INTRODUCTION

Approximately 60% of completed suicides occur in people with depression (National Strategy for Suicide Prevention, see Stephens, 2008). Depression is a major risk factor for suicide in adolescents (Shaffer *et al*, 1996; Olfson *et al*, 2003), and suicide is the third leading cause of death in adolescents (Pfeffer *et al*, 1991; Brent *et al*, 1999). Pharmacotherapy for depression with antidepressant (AD) medication should decrease the risk of suicide. Indeed, ample evidence indicates that, with adequate treatment, this is so (eg, Hall *et al*, 2003; Olfson *et al*, 2003; Gibbons *et al*, 2005; Tihihonen *et al*, 2006).

However, concern has grown that some ADs, most notably during the early stages of treatment, can actually

produce an increase in suicidality in certain individuals. This concern has focused on the selective serotonin reuptake inhibitor (SSRI) medications, paroxetine (PAR) in particular, especially when administered to children and adolescents (see Goodman *et al*, 2007). Regulatory agencies in the United States and Great Britain have investigated the risk of AD-induced increase in suicidality and have consequently issued precautionary warnings. In 2004, the US Food and Drug Administration issued a 'Black Box' warning label for all AD drugs indicating they may increase the risk of suicidality in pediatric patients (see Newman, 2004).

This warning, and the instigating concern of increased suicidality, has engendered a heated controversy and a serious dilemma for clinicians treating depressive patients. Since its initiation, numerous meta-analyses, epidemiological studies, observational studies, and reviews of data from clinical trials have been brought to bear on of this issue (see Discussion for references). Although the controversy remains unresolved, many believe that the increased risk may be small and limited to a subgroup of patients, but nevertheless is real.

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By use of preclinical research, it is not possible to resolve an issue involving suicidality in humans. However, we describe here a response to an SSRI in rats that is opposite to the response to ADs that is regularly associated with a therapeutic outcome. This opposite response occurred early in AD drug administration in young or adolescent animals. Potentially, this response could indicate that the AD drug had produced a nontherapeutic, counterproductive effect, one that, if it occurred in people, may be indicative of worsening depressive symptoms and possibly increased suicidality. If this is so, measures to prevent such an undesirable response could be explored in the animal model used here as a precursor to clinical investigation of these measures.

A major goal of our laboratory has been to improve our understanding of neurobiological mechanisms underlying depression. We have previously observed that many symptoms of depression appear in conjunction with an increase in activity of the principal noradrenergic cell group in the brain, the locus coeruleus (LC) (Simson and Weiss, 1988; Weiss *et al*, 1996, 1998, 2005). Evidence of LC hyperactivity in depression has been obtained from clinical observations, such as higher cerebrospinal fluid levels of norepinephrine and its primary metabolite 3-methoxy-6-hydroxyphenylglycol in depressives (Wong *et al*, 2000; Ehnvall *et al*, 2003) and higher levels of tyrosine hydroxylase, the rate-limiting enzyme for norepinephrine synthesis, in LC of suicide victims (Ordway *et al*, 1994; Zhu *et al*, 1999).

Consistent with the view of LC hyperactivity associated with depression, we (and others) have reported that effective AD treatments (including 13 AD drugs tested to date and electroconvulsive shock) reduce the activity of LC neurons (Grant and Weiss, 2001; West *et al*, 2009) and, consistent with this finding, decrease the expression of tyrosine hydroxylase (an activity-dependent enzyme) in LC (eg, Nestler *et al*, 1990). In support of this proposed action of ADs, clinical studies have reported that a common effect of chronic treatment with various classes of AD drugs is to decrease 3-methoxy-6-hydroxyphenylglycol in the cerebrospinal fluid of patients (see Table 1 in Grant and Weiss, 2001).

During the course of an investigation, we observed that the usual decrease in LC activity produced by chronic administration of ADs was, in the case of some ADs, diminished or even absent in younger rats (West *et al*, 2009). Subsequent experiments revealed the SSRI, PAR, when administered for shorter durations, resulted in an unexpected increase, rather than the usual 'therapeutically relevant' decrease, in LC activity.

In this study, we present the exploration of this phenomenon, comparing the electrophysiological responses of LC neurons to PAR across a range of doses and across various durations of drug administration. Importantly, we compare these responses in young rats (45 days old, approximating the adolescent stage of development) and mature adult rats (5–7 months old) with the level of PAR in the blood determined in all cases. Finally, a smaller behavioral study was conducted in parallel with PAR administration to young rats in which we measured activity in the swim test to determine whether reduced swim-test activity (ie, 'depressed' behavior) might also be seen in those instances in which PAR produced an increase in LC activity.

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats (virus/antigen free, bred in our laboratory) were used. The study assessed effects in both: (1) juvenile or 'adolescent' rats (ie, rats nearing or just beyond the age when reproduction becomes possible) about 45 days old; and (2) mature adult rats 5–7 months old. All animals were group-housed two per cage directly on bedding in polycarbonate cages. The animals in any one cage both received the same drug (or vehicle). Food (lab chow) and water were available *ad libitum*. A 12-h light–dark cycle (lights on 0700 h) and temperature of about 21°C was maintained in the colony room. Procedures and animal use described in this paper were approved by the Emory University Institutional Animals Care and Use Committee, and all surgical procedures were conducted under appropriate aseptic conditions and in accord with the NIH Guidelines and the US Department of Agriculture regulations.

Drugs and Administration

The effects of the SSRI AD drug paroxetine HCl (PAR) were examined. PAR was prepared by Dr M Owens as described in McConathy *et al* (2007). Four doses of PAR (0.625, 1.25, 2.5, and 5 mg/kg/day) plus its vehicle (50% DMSO, 25% polyethylene glycol (MW 400), and 25% distilled water) were studied. Drug and vehicle were administered using Alzet Osmotic Minipumps (Model 2ML2; Durect Corporation) to ensure continuous drug administration throughout the duration of administration and at the time of recording or behavioral (swim) testing. Each animal's body weight was used to determine the concentration of drug to be loaded into the minipump for that animal. Minipumps were implanted subcutaneously under isoflurane anesthesia in the dorsal rear flank region and the wound closed with stainless steel clips; details of surgery can be found in West and Weiss (1998). An additional measure to ensure adequate drug delivery, in view of its solubility and vehicle-related issues, involved connecting a 10 cm length of silastic tubing (0.04 i.d. \times 0.085 o.d.; filled with drug or vehicle) to the output of the minipump. The distal end of the tubing was then introduced through a small hole into the peritoneal cavity and secured to the abdominal wall while closing the hole with suture. The skin over the abdominal wound was then closed with stainless steel clips. Following surgery, animals were returned to the home cage and were not disturbed until electrophysiological recording or behavioral testing.

In male rats aged 5–7 months, different doses of AD drug can be delivered through minipump accurately across the duration of administration (maximum of 14 days) in that their body weight (range 550–700 g) has stabilized. In rats aged 45 days, the body weight would increase during the duration of administration. Therefore, the expected body weight at the time of electrophysiological recording or behavioral testing (range 225–300 g) was calculated based on age–weight charts. The dose of PAR to be delivered was then calculated for that expected body weight at the time-of-testing (ie, about 45 days old). For duration of

administration of 14 days in the juvenile animals, the minipump was replaced after 7 days, with the drug concentration adjusted at that time for the increased weight of the animal. This procedure minimized the delivery of high doses during the early portion of the delivery period when the young rats were at their minimal weights. Nevertheless, it may be noted that the 45-day-old groups did receive somewhat higher doses of drug during the early portion of drug delivery than those specified.

PAR (or vehicle) was administered for the following four durations: 2, 4, 8, and 14 days. Note that the lowest dose (0.625 mg/kg/day) of PAR was tested at durations of administration of only 2 and 4 days, the durations in which increases in LC activity were observed in young rats, in order to determine whether this lowest dose of drug would also produce an increase in LC activity. For the behavioral (swim) testing, only a single dose (2.5 mg/kg/day) was test based on the electrophysiological findings (ie, dose of PAR producing the maximal increase in LC activity in juvenile rats).

Electrophysiological Recording

Recording of single-unit electrophysiological activity of LC neurons was performed under chloral hydrate (400 mg/kg, i.p.) anesthesia. After induction of anesthesia to the surgical plane, depth of anesthesia was carefully monitored by frequent testing of hind leg withdrawal to foot pinch, ensuring that all animals were adequately and consistently anesthetized. Supplemental doses of chloral hydrate (10–15% of the initial dose) were administered as needed. Anesthetized rats were mounted in a stereotaxic instrument, where body temperature was continually monitored and maintained. The skull was opened and the dura retracted. Micropipette glass electrodes were pulled from 1.5-mm capillary tubing and filled with 1.8% NaCl, and the tips were broken to 2–3 μ m. The impedance of the electrodes was 2–10 M Ω measured at 135 Hz. Electrical signals were passed through a filtering preamplifier (Fintronics model WDR-420) and fed into an oscilloscope, audio monitor, and computer for display and analysis using a commercial computer program (Experimenter's Workbench, Datawave Technologies).

Recording of LC neurons was verified by criteria described in various studies (Graham and Aghajanian, 1971; Korf *et al*, 1974; Foote *et al*, 1980; Aston-Jones and Bloom, 1981; Simson and Weiss, 1987; Borsody and Weiss, 1996; West *et al*, 2009). These criteria were: (a) long-duration action potential with positive–negative waveform, (b) an IS-SD break notch on the ascending phase of the action potential, (c) moderately regular spontaneous firing rates typically of 0.1–5 spikes/s, and (d) a characteristic burst firing in response to a compression of the contralateral hind paw, followed by reduced firing during the post-stimulus inhibitory period. When a single unit of stable amplitude was isolated, spontaneous firing rate was recorded for 3 min, with the last 2 min used as the spontaneous firing rate for that unit. The magnitude of the sensory-evoked response of the neuron was then measured. The paw was compressed for 1 s between the ends of 13 cm surgical forceps, consistently compressed with each trial to a stop point inserted between the forceps. Paw compression (PC) applied in this manner reliably elicits a burst of spikes in the anesthetized rat and the evoked response shows little or no sensitization or

habituation with repeated trials. To determine the magnitude of the sensory-evoked response by a unit, 10 PCs were applied with each PC spaced at least 10 s apart. Spikes occurring in the first 0.5 s of the PC were counted, limited to this time because almost all sensory-evoked increase in firing rate for both drug- and vehicle-treated animals occurred within the first 0.5 s of the PC. The amount of sensory-evoked firing of a unit was determined by using the average of the 10 PC trials. In all cases, 10 LC units were recorded from each animal.

Swim Test

The swim-test procedure is a modification of the one used extensively in our laboratory. It uses a Plexiglas cylinder (62 cm high and 30 cm in diameter) as a swim tank in which depth of water (19–21 cm for 45-day-old rats, water at room temperature) is adjusted for the size of the animal to be tested such that the animal's feet cannot touch the bottom of the tank whereas the animal's head remains above water. The distal end of the animal's tail can reach the bottom of the tank, thereby enabling the animal to float without sinking. The tank is surrounded by a black screen except for a 30 cm opening at the front and is illuminated by a 25-W lamp suspended 25 cm above the center. For testing, after the specified duration of drug (or vehicle) administration, the animal was brought to the testing room from its home cage and was dropped into the tank from a height of 20 cm. During the 15-min test, two behaviors were timed: (1) struggling, defined as all four limbs in motion with the forelimbs breaking the surface of the water; and (2) floating, defined as all limbs motionless in the water. These behaviors are recorded by trained observers, with reliability coefficients for scoring by different observers regularly greater than $r = 0.90$. After testing, the animal was removed from the tank, gently dried by towel, and returned to the home cage.

Measurement of Blood Levels of Drug

To ascertain the level of PAR in circulation at the time of electrophysiological recording or swim testing, blood levels of PAR were measured. After testing, animals were decapitated while fully anesthetized and trunk blood was collected. Serum samples were frozen at -80°C until analyzed. PAR was measured using a UPLC-ms/ms assay (Ritchie *et al*, 2009). The assay was fully validated, has a sensitivity of 0.2 ng/ml, and is capable of measuring nine of the newer ADs and their active metabolites in a 5 min run.

Statistical Analysis

Statistical analysis, which was performed on the activity of individual units, was conducted primarily by using one-way analysis of variance (ANOVA). If a significant main effect of treatment ($p < 0.05$) was observed, the significance of the differences between any dose of drug and the control (vehicle) condition was then determined using Dunnett's test. For swim-test scores (struggling and floating), significance of differences between drug and vehicle scores for the equivalent duration of administration was determined by independent t -test (two tailed).

RESULTS

Spontaneous Firing Rate of LC Neurons

Figure 1 shows the spontaneous firing of LC neurons in adult and young rats in response to administration of PAR in different doses (ranging from 0.625 to 5.0 mg/kg/day) with electrophysiological recording conducted after different durations of drug administration (ie, after 2, 4, 8,

and 14 days). Effects seen in adult rats are shown in the left half of the figure. As can be seen, PAR administration to adult rats produced, in relation to vehicle, only significant decreases in spontaneous firing rate; when significant decreases were not seen, smaller decreases or no effect on spontaneous firing was observed. Regarding statistical analysis, a one-way ANOVA performed at each duration of drug administration revealed a statistically significant effect of group (ie, dose of drug) for durations of

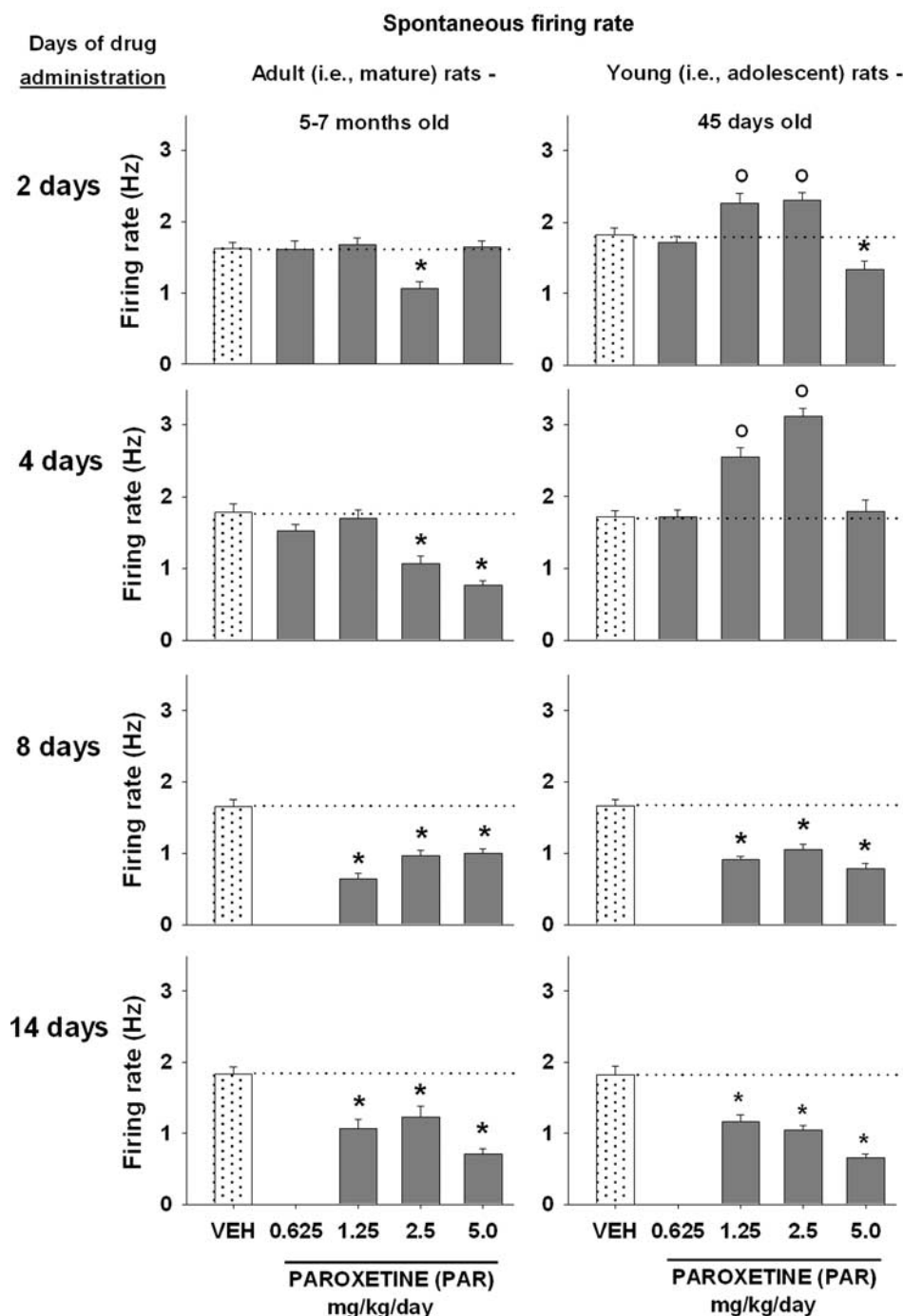


Figure 1 Spontaneous firing rate of LC neurons (spikes/s (Hz)) after administration of different doses of paroxetine (PAR) to adult (5–7 months old) or young (45 days old) male rats for various durations (2, 4, 8, and 14 days). Doses of PAR of 0.625, 1.25, 2.5, and 5.0 mg/kg/day or vehicle were delivered by minipump through intraperitoneal tubing. Means and SEM are shown. For each bar, mean is based on 50 neurons, $n = 5$ animals, 10 neurons per animal. *Significantly (at least $p < 0.05$) lower than vehicle treated for the same duration of administration. $○$ = significantly (at least $p < 0.05$) higher than vehicle treated for the same duration of administration.

drug administration of 2 ($F(4,245)=7.1$, $p<0.001$), 4 ($F(4,245)=19.0$, $p<0.001$), 8 ($F(4,245)=28.0$, $p<0.001$), and 14 ($F(4,245)=10.1$, $p<0.001$) days. Subsequent comparisons of each dose to the vehicle condition (by Dunnett's test) indicated that spontaneous firing rates were significantly decreased with doses of 2.5 mg/kg/day at 2 days, 2.5 and 5 mg/kg/day at 4 days, and 1.25, 2.5, and 5.0 mg/kg/day at 8 and 14 days of PAR administration.

Effects seen in young rats are shown in the right half of Figure 1. In contrast to what was seen in adult rats, relative to vehicle, significant increases in spontaneous firing rate were seen with administration of lower doses of PAR (ie, 1.25 and 2.5 mg/kg/day) for the shortest periods of drug administration (ie, for 2 and 4 days). The lowest dose used at these durations (0.625 mg/kg/day, designed to test the limits of the ability of PAR to increase firing rate) had no effect. At longer periods of administration (ie, 8 and 14 days), significant decreases in firing rate were seen with all doses tested as was observed in adult rats. Regarding statistical analysis, a one-way ANOVA performed at each duration of drug administration revealed a statistically significant effect of group (ie, dose of drug) for durations of 2 ($F(4,245)=13.5$, $p<0.001$), 4 ($F(4,245)=29.0$, $p<0.001$), 8 ($F(4,245)=30.1$, $p<0.001$), and 14 ($F(4,245)=29.6$, $p<0.001$) days. Subsequent comparisons at each duration of administration of each dose to the vehicle condition (by Dunnett's test) indicated that spontaneous firing rates were significantly increased with doses of 1.25 and 2.5 mg/kg/day at both 2 and 4 days of PAR administration, and were significantly decreased with doses of 5.0 mg/kg/day at 2 days, and with 1.25, 2.5, and 5.0 mg/kg/day at 8 and 14 days of PAR administration.

Sensory-Evoked Firing Rate of LC Neurons

Figure 2 shows the sensory-evoked firing rate of LC neurons in adult and young rats in response to administration of PAR in different doses; these results parallel the findings shown in Figure 1 for spontaneous firing rate. The findings are similar to those shown in Figure 1. Again, effects in adult rats are shown in the left half of the figure. PAR administration to adult rats produced, in relation to vehicle, only significant decreases in sensory-evoked firing rate; when significant decreases were not seen, smaller decreases or no effect on sensory-evoked firing was observed. Regarding statistical analysis, a one-way ANOVA performed at each duration of drug administration revealed a statistically significant effect of group (ie, dose of drug) for durations of 2 ($F(4,245)=10.8$, $p<0.001$), 4 ($F(4,245)=20.7$, $p<0.001$), 8 ($F(4,245)=28.3$, $p<0.001$), and 14 ($F(4,245)=32.5$, $p<0.001$) days. Subsequent comparisons at each duration of administration of each dose to the vehicle condition (by Dunnett's test) indicated that sensory-evoked firing rates were significantly decreased with doses of 2.5 mg/kg/day at 2 days, 2.5 and 5 mg/kg/day at 4 days, and 1.25, 2.5, and 5 mg/kg/day at 8 and 14 days of PAR administration.

Effects seen in young rats are shown in the right half of Figure 2. Again, in contrast to what was seen in adult rats, relative to vehicle, significant increases in sensory-evoked firing rate of LC neurons were seen with administration of lower doses of PAR (ie, 1.25 and 2.5 mg/kg/day) for the

shortest periods of drug administration (ie, for 2 and 4 days). The lowest dose used at these durations (0.625 mg/kg/day) showed the limits of the ability of PAR to increase LC firing as no effect of this dose was seen. At longer periods of administration (ie, 8 and 14 days), significant decreases in sensory-evoked firing rate were again seen with all doses tested, as was observed in adult rats. Regarding statistical analysis, a one-way ANOVA performed at each duration of drug administration revealed a statistically significant effect of group (ie, dose of drug) for durations of 2 ($F(4,245)=33.3$, $p<0.001$), 4 ($F(4,245)=30.0$, $p<0.001$), 8 ($F(4,245)=29.6$, $p<0.001$), and 14 ($F(4,245)=44.1$, $p<0.001$) days. Subsequent comparisons at each duration of administration of each dose to the vehicle condition (by Dunnett's test) indicated that sensory-evoked firing rates were significantly increased with doses of 1.25 and 2.5 mg/kg/day at both 2 and 4 days of PAR administration. Comparisons also indicated that sensory-evoked firing rates were significantly decreased with doses of 1.25, 2.5, and 5.0 mg/kg/day at 8 and 14 days of PAR administration.

Blood Levels of PAR

Figure 3 shows the blood levels of PAR that were present at the end of the recording sessions for all animals used for LC electrophysiology. It is noteworthy that the young (adolescent) rats had considerably lower blood levels of PAR than did the adult rats. Particularly at 2 and 4 days of administration, doses of PAR that produced increased LC activity in young rats (ie, 1.25 and 2.5 mg/kg/day) resulted in very low blood levels of PAR, much lower levels than were seen in adult rats with these doses.

Swim-Test Activity

As is evident from Figures 1 and 2, the largest increase in LC activity observed in young rats was produced by PAR at a dose of 2.5 mg/kg/day when administered for durations of 2 and 4 days. To examine a behavioral measure that may be indicative of a depressive response and determine whether depressive-like behavior was associated with the electrophysiological findings suggesting this, swim-test behavior was studied in young rats administered with 2.5 mg/kg/day PAR or its vehicle. Results are shown in Figure 4. These results are consistent with the electrophysiological effects in that decreased swim activity (decreased struggling and increased floating) was observed at the same durations of administration when LC activity was increased (ie, 2 and 4 days). When compared with vehicle scores for the same period of administration, *t* tests revealed that PAR administered for 4 days significantly decreased struggling ($t=2.53$, $p<0.05$) and significantly increased floating ($t=3.57$, $p<0.01$). Similar changes in these swim-test measures were seen with a 2-day administration of PAR but were not statistically significant.

DISCUSSION

The summary table describing the consequences of effective AD treatments on the activity of LC neurons in adult rats shown in West *et al* (2009) makes clear that such treatments

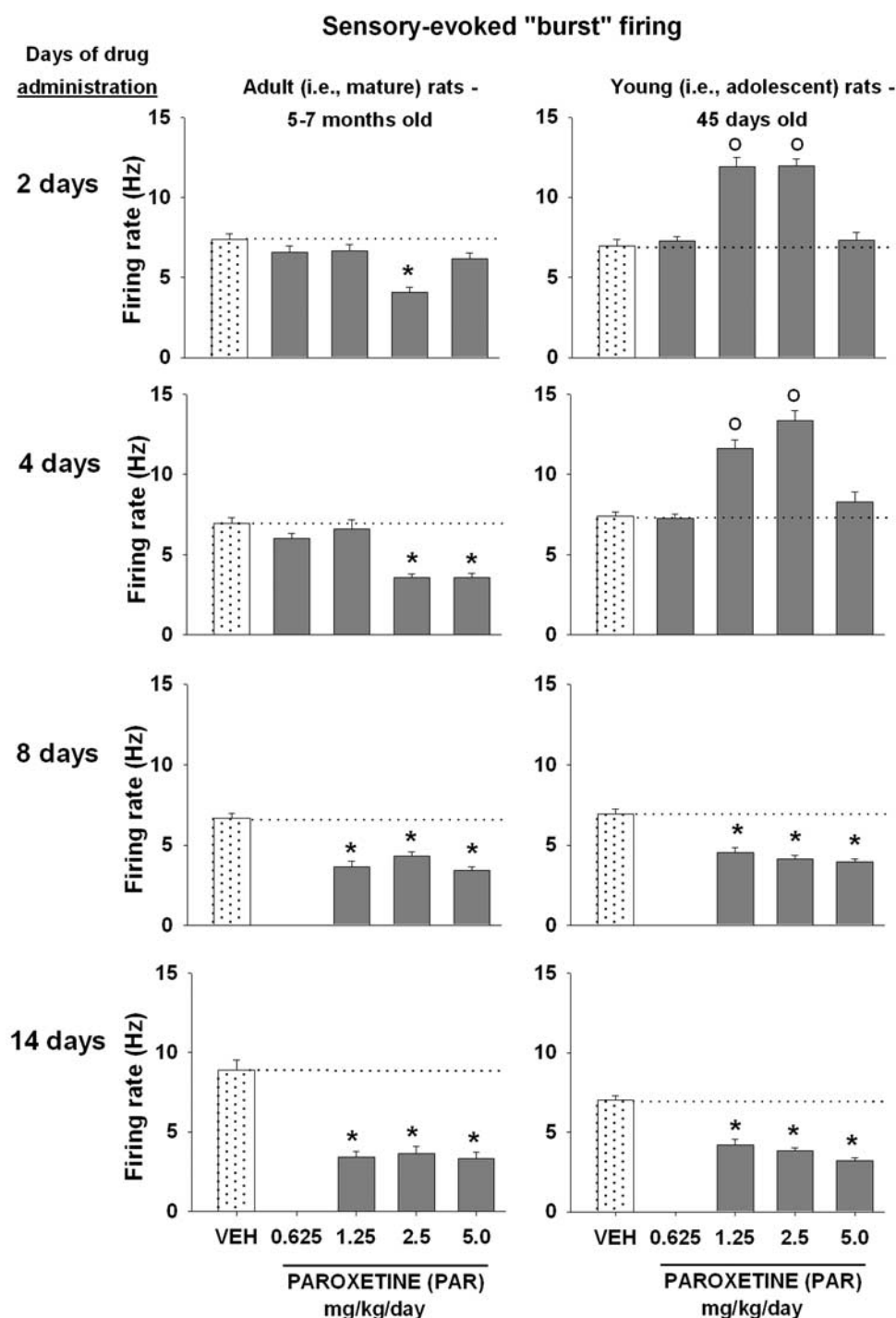


Figure 2 Sensory-evoked firing rate of LC neurons (spikes/s (Hz)) after administration of different doses of PAR to adult (5–7 months old) or young (45 days old) male rats for various durations (2, 4, 8, and 14 days). All other details are as in legend for Figure 1.

consistently cause LC activity to be decreased. In that publication, explanation is provided for the three studies out of sixteen in which clear-cut decreases in LC activity were not observed. Consistent with this, Nestler *et al* (1990) reported that seven drugs representing several classes of ADs plus electroconvulsive shock all decreased levels of tyrosine hydroxylase, an activity-dependent enzyme, in the rat LC. Thus, a reduction in LC activity is associated with a therapeutic effect of AD treatment. In marked contrast to

this effect, we observed that, in young rats approximating adolescents, significant increases in LC activity occurred during the initial days of treatment with PAR (ie, with treatment for 2 or 4 days). This was seen at low-to-moderate doses of PAR. Only at a very low dose, designed to ascertain the lower limit of the LC activity-increasing effect, and at the highest dose tested, did this not occur. The remarkable effect of PAR to increase LC activity in young rats was observed both for spontaneous firing rate and sensory-

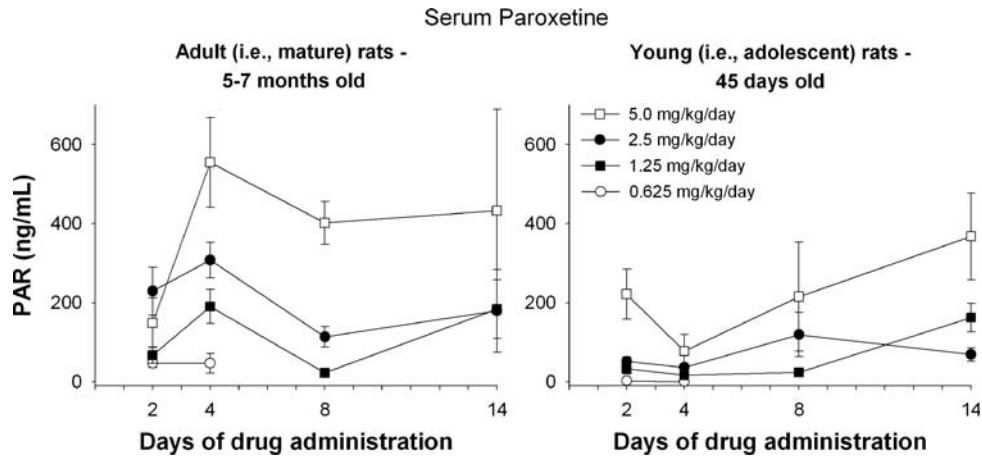


Figure 3 Concentration of PAR found in blood (in ng/ml serum) after administration of different doses of PAR to male rats for various durations (2, 4, 8, and 14 days). Doses of PAR of 0.625, 1.25, 2.5, and 5.0 mg/kg/day were delivered by minipump through intraperitoneal tubing. Means and SEM are shown. For each bar, mean is based on $n=5$ animals. Blood levels shown here were obtained from animals used to obtain data shown in Figures 1 and 2.

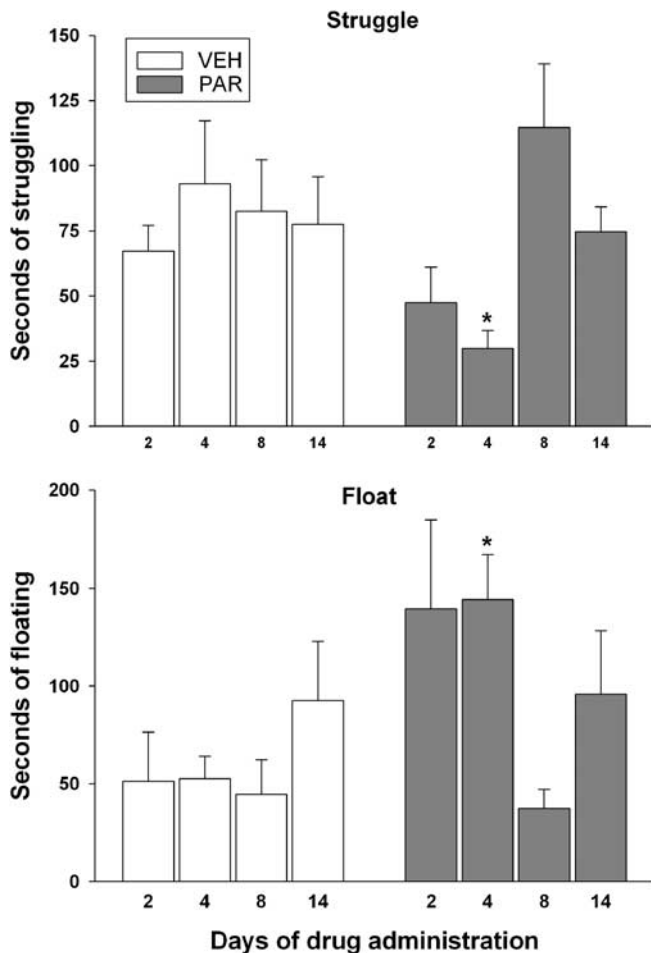


Figure 4 Times spent struggling (top) and floating (bottom) during a 15-min swim test by young male rats (45 days old) after having been administered vehicle (VEH) or PAR (2.5 mg/kg/day) for 2, 4, 8, and 14 days. Means and SEM are shown. Number of animals tested in each condition were, for VEH, $n=6, 8, 6,$ and 10 for the four durations of administration; and, for PAR, $n=8, 8, 10,$ and 10 . *Differs significantly (at least $p<0.05$) from vehicle-treated animals for the same duration of administration.

evoked firing of LC neurons. In over a decade of study of the influence of effective AD treatments on LC activity, this represents the first time that we have observed an increase in LC activity.

A potentially significant correlate of the increased LC activity that occurs in young rats in the early stages of treatment with PAR is the blood level of drug that was present. In comparison to blood levels of drug present in adult rats given the same dose of drug, young rats have levels that are much lower. Apparently, young rats metabolize, and eliminate from circulation, PAR much more efficiently than do adult rats. Note that this may be true for other AD drugs in rats as well because we observed a similar phenomenon for mirtazapine (West *et al*, 2009); and these findings in rats may be relevant to the human condition in that younger people metabolize ADs faster than older adults (Preskorn, 1997). Most relevant here, this has been reported for PAR, with significantly higher plasma concentrations and slower elimination observed in elderly patients compared with younger individuals (Kaye *et al*, 1989; Lundmark *et al*, 1989). Concerning the time/dose points at which young rats showed significantly increased LC activity, with 2 days of 1.25 mg/kg/day PAR administration, young rats had blood levels (in ng/ml) of 33.5 ± 14.2 , whereas adult rats had blood levels of 66.8 ± 20.6 . With 2 days of 2.5 mg/kg/day PAR, young rats had blood levels of 51.7 ± 11.2 , whereas adult rats had blood levels of 229.4 ± 60.4 . Similarly, with 4 days of 1.25 mg/kg/day PAR, young rats had blood levels of 16.5 ± 3.1 , whereas adult rats had blood levels of 190.2 ± 42.8 ; and with 4 days of 2.5 mg/kg/day PAR, young rats had blood levels of 36.6 ± 10.1 , whereas adult rats had blood levels of 307.6 ± 45 .

However, it is equally clear that a low blood level of PAR is not sufficient in and of itself to generate increased LC activity. Both adult and young rats had quite low blood levels of PAR on day 8 of drug administration with a dose of 1.25 mg/kg/day (23.0 ± 7.2 and 23.9 ± 11.3 ng/ml, respectively), and neither age of rat showed increased LC activity at this dose/time point. In addition, adult rats had

low blood levels when given the lowest dose tested (0.625 mg/kg/day) for 2 and 4 days (47.2 ± 11.0 and 46.7 ± 24.7 , respectively), the time points at which young rats showed increased LC activity, and did not show any increase in LC activity at these time points with the low dose/blood level of PAR.

Another point of interest related to low blood levels as a correlate of increased LC activity in young rats is that, as can be seen in Figure 3, the young rats had lower blood levels of PAR on day 4 of drug administration than they had on day 2, and it was on day 4 that the most robust increases in LC activity were seen in young rats. Thus, increased LC activity may be associated not only with low, but also decreasing blood levels of drug. In terms of falling levels of AD drug being important in the clinical situation, Yerevanian *et al* (2004) found a fivefold increase in risk for suicidal behavior after AD discontinuation; and Bogetto *et al* (2002) reported that 42.3% of dysthymic patients showed a discontinuation syndrome appearing 2–5 days after discontinuation of PAR. It has been suggested that lack of adherence to AD drug treatment by adolescents, especially with ADs with a shorter half-life that would result in more rapidly falling blood levels, is an important factor in AD-induced increase in suicidality (Weiss and Gorman, 2005).

In summary, increased LC activity is seen in the young rats in conjunction with low blood levels of PAR, and may be most pronounced with decreasing blood levels, and this occurs early in PAR administration (ie, with drug given for 2 or 4 days). With more prolonged administration of PAR (ie, for 8 or 14 days), increased LC activity was not seen in young rats even with low blood levels. In addition, adult rats do not seem susceptible to showing increased LC activity regardless of blood level and brief duration of PAR administration.

As noted above, various lines of evidence, both preclinical and clinical, indicate that therapeutically effective AD treatments result in a decrease in the activity of LC neurons (see Nestler *et al*, 1990; Ordway *et al*, 1994; Zhu *et al*, 1999; Grant and Weiss, 2001; West *et al*, 2009). For PAR, the time-course data for adult rats in this study indicate that this effect develops over treatment time, perhaps determined by gradual changes in brain receptor function and/or other trophic processes triggered by the drug treatment. This decrease in LC activity with AD treatment is consistent with earlier study in our laboratory indicating that symptoms of depression appear in conjunction with disinhibition and a resultant increase in activity of LC neurons (for summaries, see Weiss *et al*, 1996, 1998, 2005). Our earlier research had pointed to the importance of LC activity in depression based on the observations that depression-related behavioral changes in rats (a) are accompanied by LC hyperactivity (Simson and Weiss, 1988) and (b) can be prevented by manipulations that counteract LC hyperactivity (Simson *et al*, 1986). This would suggest that the PAR-induced increase in LC activity observed here in young rats could be indicative of an increase in depressive symptoms. At least insofar as reduced activity in the swim test accurately reflects responses indicative of depression, the findings from the swim test reported here support this idea. Most apparent with 4 days of administration, the dose of PAR that produced the largest increase in LC activity in young

rats also significantly decreased swim-test activity, indicated by both a decrease in struggling and an increase in floating (see Figure 4). An important aspect of the swim-test findings is that they were obtained in unanaesthetized, awake animals. The LC recording data were obtained under chloral hydrate anesthesia with the possibility, thereby, that the results were affected by the presence of the anesthetic. No anesthetic was present during the swim tests, thus supporting the validity and clinical relevance of the electrophysiological findings.

The response we use in this study to demarcate a depressive-like state, and reduction of a depressive-like state by effective AD treatment, is the activity of LC neurons. A misconception that might arise from this is that we therefore attribute changes in depression to changes in noradrenergic transmission in the brain. As we have come to understand from many years of investigation in this area, the relationship between changes in noradrenergic transmission and changes in depression is not a compelling one. Direct manipulation of brain norepinephrine produces changes in depression-related symptoms that are often small, variable, and dependent on specific testing conditions in order to be seen at all (see Weiss *et al* (1996) for review). Therefore, rather than focusing on the changes in noradrenergic transmission as related to depression, we have directed our attention to how LC activity can affect another catecholaminergic system (ie, dopamine), which seems to have more direct influence on depressive symptoms than does changes in brain norepinephrine. This influence is hypothesized to occur through release of the peptide galanin, which is colocalized in norepinephrine-containing nerve terminals (see Weiss *et al*, 1996, 2005). It is not necessary to describe the details of this hypothesis here; rather, suffice it to say we emphasize in our formulation that the firing rate of LC neurons is important and not the changes in noradrenergic transmission.

The main impetus for the research reported here and its potential clinical significance is the concern that initial treatment with AD drugs, especially use of SSRIs in pediatric and young adult patients, may result in a paradoxical increase in suicidality in at least a portion of these individuals. An early warning of this was issued in 2003 by the Medicine and Healthcare Products Regulatory Agency of the British Department of Health. In review of proprietary data in 2003, the Medicine and Healthcare Products Regulatory Agency of the British Department of Health found an increase in suicidal behavior from 2.5% with placebo to 3.7% with SSRIs. They thereby issued a warning to avoid off-label use of PAR for treating depression in patients 18 years of age or younger, and a similar warning was soon issued by the Food and Drug Administration (see Vitiello and Swedo, 2004). In an interim report by the UK Committee on Safety of Medicines, one concern was that SSRIs, and PAR in particular, may increase suicidal thoughts in the early stages of treatment in patients under the age of 19 (see Jick *et al*, 2004). Independent review of adverse events from data from 24 placebo-controlled trials of AD use in children and adolescents found that suicidality significantly increased with SSRI treatment compared with placebo, leading the Food and Drug Administration to issue a 'black box' warning (see Brent, 2004; Newman, 2004; Goodman *et al*,

2007). Additional studies have likewise found evidence of increased suicidality with use of SSRIs in juveniles, suggesting that there may be an unfavorable risk to benefit ratio for SSRIs (with fluoxetine as a possible exception) (Vitiello and Swedo, 2004; Whittington *et al*, 2004; Cipriani *et al*, 2005; Doggrell, 2005; Fergusson *et al*, 2005; Richmond and Rosen, 2005; Ryan, 2005; Scahill *et al*, 2005; Hammad *et al*, 2006; Laughren, 2006; Mosholder and Willy, 2006; Olfson *et al*, 2006; Tiihonen *et al*, 2006; Wohlfarth *et al*, 2006; Bridge *et al*, 2007; Goodman *et al*, 2007; Bailly, 2008; Olfson and Marcus, 2008; Barbui *et al*, 2009). Many of these and other studies have pointed out difficulties in interpretation of the data and have stated that there is considerable risk for suicidality if pediatric patients are not adequately treated (eg, Mann *et al*, 2006; Bridge *et al*, 2007).

In summary, we report here a neurobiological change that occurs in response to PAR administration early in treatment of young animals (ie, an increase in LC activity), which is opposite to changes in LC activity that we and others have found to be associated with a therapeutic effect of treatments in depression. This PAR-induced change that we report in young animals, at least as indicated by swim-test results, may be associated with a worsening of depressive symptoms. In humans, such an increase in depressive symptoms might be related to an exacerbation of suicidal tendencies in vulnerable individuals. Lending support to the idea that SSRIs can have differential effects in young and older rodents, Oh *et al* (2009) reported that juvenile mice administered with fluoxetine showed an anxiogenic response, unlike the anxiolytic response observed in adult mice. Differential responses in young and adult hamsters were also found in that fluoxetine inhibited play-fighting behavior in adult animals but enhanced it in early-pubertal animals (Taravosh-Lahn *et al*, 2006).

It should be noted here with regard to extrapolating our findings in rats to human depressives, the animals used in this study were normal rats, not a model of depression, other than the fact that all rats underwent a surgical procedure to implant the drug-delivering minipumps, which could be considered stressful. Therefore, the response to PAR could be different in depressed individuals or in an animal model of depression. Whether the presence of a depression-like state in animals can alter the effects of PAR on LC reported here is currently under investigation. However, we note that, when we studied the effects of AD drugs on firing activity of dopaminergic neurons in the ventral tegmentum, we observed increases in firing rate of these neurons in normal animals, but we observed larger and more consistent effects, and with a wider range of AD drugs, in animals representing the model of depression (CHK West and JM Weiss, unpublished observations). Thus, the effects in the 'depressed' condition were more pronounced than in the normal condition. Consequently, we do not believe the effects reported here in normal subjects will not be relevant to depressed subjects. Likewise, the reaction to initial PAR treatment could be different in patients with variable levels of depression; and, indeed, it may be only particularly vulnerable individuals who show an adverse reaction to PAR treatment, potentially explaining why the reported increase in suicidality is observed in only a small subpopulation of patients.

With respect to how the effects described in this paper might be useful for clinical practice, testing of drugs and/or procedures that will block the increase in LC activity seen early in PAR treatment might be helpful for screening candidates for adjunctive therapies during the early period of treatment with PAR. Blocking the electrophysiological effect described here (ie, PAR-induced increase in LC activity seen in young animals) by coadministration of a test drug could be a first step toward determining that drug's potential utility for avoiding countertherapeutic effects. Of course, it must be emphasized that we have no way to know at this time whether the biological effect that we report here (a) occurs in humans, or (b) has any relationship to suicidal ideation and/or tendencies. Nevertheless, availability of a simple procedure with animals to screen potentially useful compounds for adjunctive treatment seems to be worthy of further investigation.

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DISCLOSURE

The authors declare no conflict of interest.

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