Interaction Between the Forced Swimming Test and Fluoxetine Treatment on Extracellular 5-Hydroxytryptamine and 5-Hydroxyindoleacetic Acid in the Rat

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ABSTRACT
We used in vivo microdialysis to examine extracellular levels of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the striatum and the lateral septum during the forced swimming test, (FST) a behavioral test conducted in rats that is commonly used to predict the effect of antidepressant drugs. The forced swimming test consisted of a 15-min pretest swim and a 5-min test swim 24 hr later. The antidepressant fluoxetine (20 mg/kg s.c.) or saline was administered 23.5, 5 and 1 hr before the test swim. In the striatum, the pretest swim increased 5-HT in both treatment groups. On the second day, the test swim had no effect on 5-HT in saline-treated rats but slightly decreased striatal 5-HT in fluoxetine-treated rats. In the lateral septum, the pretest swim decreased 5-HT in both treatment groups. On the second day, the test swim had no effect on 5-HT in saline-treated rats but decreased lateral septum 5-HT in fluoxetine-treated rats. Ratings of behavior showed that fluoxetine treatment increased swimming behavior and decreased immobility during the test swim. Immobility was positively correlated and swimming was negatively correlated with changes in extracellular 5-HT in the lateral septum but not in the striatum. Therefore, fluoxetine treatment altered adaptation of the regional response of extracellular 5-HT ordinarily produced in the FST, reversing the 5-HT response to the initial swim in the striatum and restoring the response to the initial swim in the lateral septum.

The FST is a behavioral test used frequently to evaluate the potential efficacy of prospective antidepressant drugs in rats or mice (Porsolt et al., 1977, 1978). As typically used, rats are exposed to a 15-min pretest swim period and followed the next day with a 5-min test swim. Immersion of rodents in the water for an extended period of time produces a characteristic behavior called immobility, in which the rat makes only those movements necessary to keep its head above water. When antidepressant drugs are administered between the pretest and test periods, usually three times within 24 hr, the behavioral immobility is selectively decreased by a variety of classes of antidepressant drugs (for review, see Borsini and Meli, 1988). Furthermore, antidepressants reduce immobility at doses that either do not change or even decrease motor behavior in open field tests. Recently, a time-sampling behavioral scoring procedure was introduced that can discriminate the effects of antidepressants that selectively alter noradrenergic or serotonergic transmission (Detke et al., 1995). The FST is also relatively selective for antidepressant drugs because few other psychoactive drugs elicit similar effects. Although this test has been used extensively as a drug screen for potential antidepressant compounds, relatively little is known about the neurochemical or neuroanatomic substrates that contribute to the pharmacological selectivity of the test.

In previous work from this laboratory, we have used in vivo microdialysis to investigate the effects of both acute and repeated forced swimming on extracellular 5-HT. Microdialysis is ideally suited for the study of neurochemical changes produced by the FST because it allows the experimenter to follow the time course and magnitude of neurochemical changes produced by pharmacological and/or environmental manipulations in an individual subject (for a review, see Ungerstedt, 1984; Young, 1993). Microdialysis also allows the experimenter to examine the relationship between behavior and neurochemistry. In comparing the effects of forced swimming with a number of other environmental stressors, such as forced locomotion, cold, immobilization and tail pinch, forced swimming produced the largest changes in extracellular 5-HT (Kirby et al., in press). In a detailed analysis of the effects of forced swimming in different brain regions,
forced swimming was shown to produce distinct, regionally specific changes in extracellular 5-HT: an increase of 5-HT in the striatum, a decrease in 5-HT in the amygdala and lateral septum and no change in 5-HT in the frontal cortex or hippocampus (Kirby et al., 1995). Most recently, the effect of repeated exposure to forced swimming for 15 min was examined on extracellular 5-HT in two brain regions that showed opposite neurochemical responses to acute forced swimming: the striatum and the lateral septum. There was rapid adaptation to the effects of repeated forced swimming of both types of responses of extracellular 5-HT, both the increased concentrations in the striatum and the decreased concentrations in the lateral septum. However, changes in the behavioral responses produced by repeated swimming tests were correlated with changes in extracellular 5-HT measured in the lateral septum but not in the striatum (Kirby and Lucki, submitted).

The purpose of the current study was to examine the interaction between changes in extracellular 5-HT produced during the FST, as it is traditionally conducted with a 15-min pretest swim followed 24 hr later by a 5-min test swim, and treatment with fluoxetine, an antidepressant drug that is a 5-HT-selective uptake inhibitor (Fuller and Wong, 1977). Although treatment with fluoxetine would be expected to increase extracellular concentrations of 5-HT in several brain regions (Kreiss and Lucki, 1995; Perry and Fuller, 1992; Rutter and Auerbach, 1993), its effects have not been examined when given in the subchronic regimen (i.e., three injections within 24 hr) typically used in FST studies. Extracellular 5-HT concentrations were measured in the striatum or lateral septum, regions in which forced swimming behavior was shown to increase or decrease extracellular 5-HT, respectively (Kirby et al., 1995). Repeated exposure to forced swimming for 15 min has been shown to result in adaptation of the 5-HT response in the striatum and lateral septum (Kirby and Lucki, submitted), but this effect was not measured under conditions typically used to measure antidepressant drug effects in the FST. Importantly, the results of this study revealed an interaction between the effects of fluoxetine and forced swimming on extracellular 5-HT that was not predicted by either treatment alone. In addition, because conditions were identical to those used to test antidepressant behavioral effects in the FST, the relationship was examined between behavioral responses produced by treatment with fluoxetine (Detke et al., 1995) and corresponding changes in extracellular 5-HT measured in the lateral septum and the striatum during the FST.

Materials and Methods

Subjects. Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing between 250 and 300 g were housed two or three per cage on a 12-hr light/dark schedule in a temperature-controlled (22°C) colony room. Rats were given access to ad libitum food and water.

Probe implantation and sample collection. Custom concentric-style dialysis probes were constructed similar to a design that was described by Parry et al. (1990). Dialysis membranes were made from hollow cuprammonia rayon fibers with 224-μm outer diameter and 35,000 molecular weight cutoff (C-series; Terumo Corp., Somerset, NJ). The dialysis fiber was inserted into a 25-mm piece of 25-gauge thin-wall stainless steel tubing (Small Parts, Miami, FL) and secured with cyanoacrylate gel so that a 2- or 3-mm surface area was exposed for lateral septum or striatal placement, respectively. The open end of the dialysis fiber was sealed with a 1-mm epoxy plug. A 1-mm length of 21-gauge stainless steel tubing was secured with epoxy to the probe body 19 mm behind the membrane to accommodate the length of the guide cannula (Plastics One, Roanoke, VA). A threaded plastic cap (Plastics One) was placed over the 1-mm tubing so the probe could later be screwed in place when inserted into the guide cannula. Inflow and outflow tubes were 35 and 30 cm in length, respectively, and were made of polyimide-coated fused silica tubing (Polymicro Technologies, Phoenix, AZ). Inflow and outflow tubes were inserted into the open end of the stainless-steel tube and secured with cyanoacrylate. Polyethylene PE 60 tubing (Clay Adams, Parsippany, NJ) was secured with cyanoacrylate gel to the back of the probe body and extended to cover most of the exposed inflow and outflow tubes. PE 50 tubing was glued over PE 10 tubing to connect the inlet tube to the 22-gauge needle of a syringe placed in a syringe pump (Instech Laboratories, Plymouth Meeting, PA) and the outlet tube to the liquid swivel of the microdialysis apparatus (Instech Laboratories).

Filtered artificial cerebrospinal fluid (147 mM NaCl, 2.3 mM CaCl₂, 0.9 mM MgCl₂, and 4.0 mM KCl, with unadjusted pH 6.3–6.5) was pumped through the probe at 0.8 μl/min with a syringe pump. The recovery characteristics of individual probes were determined before use in an experiment by placing them into a standard solution of 5-HT (10 nM) and 5-HIAA (120 nM) at room temperature. Probe recoveries for 5-HT and 5-HIAA ranged between 20% and 35%.

Each rat was placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), and guide cannulae were implanted under surgical anesthesia (sodium pentobarbital, 40 mg/kg) into the ventral striatum (0.2 mm anterior to bregma, 2.8 mm lateral to midline and 5.0 mm ventral from dura) or the lateral septum (0.5 mm anterior to bregma, 0.8 mm lateral to midline and 4.0 mm ventral from dura) (Paxinos and Watson, 1986). The guide cannula was secured to the skull with three screws and dental cement, and the incision was closed with wound clips. After surgery, the rat was handled and allowed to recover from surgery for 1 week before microdialysis experiments. On the day before the experiment, the rat was placed into a 37.5-cm-high, clear polycarbonate cylindrical microdialysis apparatus with a counterbalance arm holding a liquid swivel and spring tether (Instech Laboratories). The probe was gently inserted into the guide cannula and continuously perfused with artificial cerebrospinal fluid at the rate of 0.8 μl/min through the liquid swivel. The collection of dialysate samples began 21 to 23 hr after probe insertion. Samples (8 μl) were collected at 10-min intervals into polypropylene microcentrifuge vials (Fisher Scientific, Pittsburgh, PA) and stored at −80°C until analysis. Samples were collected over a 2-day period, and only data from animals that successfully completed both days of the experiment were used. On each day, the 5-HT and 5-HIAA base line was determined by averaging seven samples before exposure to the stressor. Samples were then collected during the swimming session and for 2 hr after swimming onset. On the first day, baseline samples were collected immediately before the pretest swim. On the second day, baseline samples were collected 5 hr before the test swim to accommodate the injection protocol (fig. 1).

Analysis of dialysate samples. Dialysates were automatically injected into a Bioanalytical Systems 460 high performance liquid chromatograph (HPLC) (BAS, West Lafayette, IN) by a CMA/200 Refrigerated Microsampler (CMA, Stockholm, Sweden) set to a 6.5-μl injection volume. The HPLC mobile phase (0.67 mM EDTA, 0.43 mM sodium octyl sulfate, 0.36 mM NaH₂PO₄, 12–20% acetonitrile, pH 4.0) was pumped through a reverse-phase column (C₁₈, BAS) at a flow rate of 90 μl/min (Kreiss et al., 1993).

5-HT and 5-HIAA from chromatograms of dialysate samples were identified by comparing their elution times with those of reference standards. The amount of 5-HT and 5-HIAA in each dialysate sample was quantified from their respective peak heights using a linear
regression analysis of the peak heights obtained from a series of reference standards. The detection limit, defined as the sample amount producing a peak height twice the height of background noise, was 0.5 fmol. This sensitivity was sufficient to measure baseline levels of 5-HT with 10-min resolution without the need to add a 5-HT uptake inhibitor to the perfusion medium.

**Histological analysis.** On completion of each experiment, green ink was pumped through the dialysis probes to mark their location, animals were killed and their brains were removed. Brains were sectioned with a refrigerated cryostat and stained with cresyl violet, and the tissue examined for the location of the dialysis probe. Only data from animals with $\geq 75\%$ of the probe membrane located in the targeted brain regions were used.

**Forced swimming test.** Rats were placed in a cylindrical glass tank (46 cm tall $\times$ 20 cm in diameter) of 21°C to 22°C water filled to a depth of 30 cm for a 15-min pretest. Injections (saline or fluoxetine) were given 23.5, 5 and 1 hr before a 5-min test swim (fig. 1). The water depth of 30 cm allowed the rats to swim or float without their hindlimbs touching the bottom of the tank. Control animals (untreated: no injections; fluoxetine: three injections as described above) were not subjected to forced swimming on either day.

**Drugs.** Fluoxetine hydrochloride was obtained from Eli Lilly and Co. (Indianapolis, IN). Fluoxetine was administered subcutaneously in a volume equivalent to 2 ml/kg at a dose of 20 mg/kg, calculated by the weight of the base, and was dissolved in deionized water. Saline (0.9%) was also administered subcutaneously in a volume equivalent to 2 ml/kg.

**Behavioral analysis.** The swimming session on each day was videotaped for behavioral analysis. Behavior during the test swimming session was scored using a time-sampling method (Detke et al., 1995) modified from the method traditionally used in the FST. Every 5 sec, one of three behaviors was recorded. Immobility was scored when the animal was making the minimum movements necessary to stay afloat. Swimming was scored when the animal actively swam around the tank, making movements greater than those necessary to stay afloat. Climbing was scored when the animal made vigorous thrashing movements with its forepaws, usually directed against the sides of the tank. Behavioral results are shown as the total number of counts for each behavioral category of a maximum of 60.

**Data analysis.** Baseline values of 5-HT and 5-HIAA were determined for each rat from the mean of dialysate samples collected before exposure to the swim. Values were corrected for individual probe recoveries. The effect of day, swim and drug on mean baseline levels of 5-HT and 5-HIAA in each brain region were assessed by a three-way analysis of variance (ANOVA). Separate one-way ANOVAs were calculated to determine the effects of day, swim or drug on baseline levels of 5-HT and 5-HIAA. Individual values were compared using post hoc Dunnett’s two-tailed test. The effect of drug and time on 5-HT and 5-HIAA levels were assessed by two-way ANOVA. The effect of time on 5-HT and 5-HIAA were assessed for each drug treatment group by one-way ANOVA. The values at individual time points were compared with base-line values using post hoc Dunnett’s two-tailed test.

The effect of fluoxetine treatment on mean behavior counts were assessed for each behavior scored during the test session of the FST by one-way ANOVA. The values of fluoxetine-treated animals were compared with saline-treated animals by post hoc Dunnett’s two-tailed test. The relationship between behaviors during the test swim and extracellular 5-HT, calculated as maximal effect scores for individual subjects and expressed as a percent of base line, was assessed by simple regression analysis.

**Results**

**Microdialysis Experiments**

**Baseline levels of 5-HT and 5-HIAA.** Table 1 shows the baseline levels of 5-HT in the striatum or lateral septum for all experimental groups. A three-way ANOVA demonstrated a significant effect of day $[F(1,56) = 6.30, P < .05]$, drug $[F(1,56) = 3.35, P < .05]$ and day $\times$ drug interaction $[F(1,56) = 9.47, P < .01]$ but no significant effects of group $[F(1,56) = 3.07, P > .05]$ or interaction terms involving group $[P > .05]$ on baseline striatal 5-HT. Similarly, ANOVA demonstrated a significant effect of day $[F(1,58) = 9.51, P < .01]$, drug $[F(1,58) = 15.10, P < .01]$ and day $\times$ drug interaction $[F(1,58) = 15.25, P < .01]$ on baseline lateral septum 5-HT but no significant effects of group $[F(1,58) = 1.42, N.S.]$ or interaction terms involving group $[P > .05]$. Rats treated with fluoxetine between days 1 and 2 showed significantly higher

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**TABLE 1**

Baseline extracellular 5-HT

<table>
<thead>
<tr>
<th>Region</th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline $\pm$ SE</td>
<td>Fluoxetine $\pm$ SE</td>
</tr>
<tr>
<td>Striatum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swim</td>
<td>4.6 $\pm$ 0.6</td>
<td>5.5 $\pm$ 1.2</td>
</tr>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 9)</td>
</tr>
<tr>
<td>Control</td>
<td>5.6 $\pm$ 0.8</td>
<td>3.2 $\pm$ 1.0</td>
</tr>
<tr>
<td></td>
<td>(n = 6)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>Lateral septum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swim</td>
<td>6.4 $\pm$ 1.4</td>
<td>4.3 $\pm$ 0.9</td>
</tr>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Control</td>
<td>6.6 $\pm$ 1.6</td>
<td>8.6 $\pm$ 2.3</td>
</tr>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 7)</td>
</tr>
</tbody>
</table>

Values are mean ($\pm$ S.E.) baseline striatal and lateral septum dialysate content of 5-HT (fmol/0.5 $\mu$l of sample) in the swim group and no swim controls treated with saline or fluoxetine (20 mg/kg) on day 1 and 2 of the FST.

*$P < .05$, $^aP < .01$ vs. corresponding value on day 1, according to Dunnett’s test.

*$^bP < .05$, $^dP < .01$ vs. corresponding saline control, according to Dunnett’s test.
baseline values for striatal 5-HT on day 2 than on day 1 in both the swim and no swim (P < .05) groups and significantly higher baseline values than saline-treated animals on day 2 in the swim group (P < .05). Baseline 5-HT values in the lateral septum on day 2 were significantly increased by fluoxetine treatment in both the swim and no swim groups (P < .01) and were significantly higher on day 2 than on day 1 in the swim group (P < .01).

Table 2 shows the baseline levels of striatal or lateral septum 5-HIAA for all experimental groups on days 1 and 2 of the FST. A three-way ANOVA demonstrated a significant effect of drug × group interaction [F(1,56) = 15.11, P < .01] but no significant effects of drug [F(1,56) = 3.64, P > .05], day [F(1,56) = .84, N.S.], or interaction terms involving day [P > .05] on baseline striatal 5-HIAA. A three-way ANOVA demonstrated no significant main effects or interaction terms [P > .05] on baseline lateral septum 5-HIAA. Fluoxetine treatment reduced baseline striatal 5-HIAA values on day 2 from day 1 in the swim (P < .05) and no swim (P < .01) groups and lowered baseline striatal 5-HIAA on day 2 in the no swim group (P < .05). Baseline striatal 5-HIAA was significantly higher in the swim group than the no swim controls in fluoxetine-treated animals on both days (P < .01).

Striatal 5-HT and 5-HIAA: Effect of fluoxetine treatment in the FST and no swim controls. Figure 2 shows the effect of the pretest (fig. 2A) and test (fig. 2B) swim on striatal 5-HT in saline or fluoxetine-treated animals. As shown in figure 2A, in the saline group, extracellular 5-HT was elevated 20 min after the onset of the pretest swim, peaked 60 min later at a maximum of 105% above baseline and remained elevated throughout the session. In the fluoxetine group, extracellular 5-HT peaked 30 min after the onset of the pretest swim at a maximum of 63% above baseline and returned to baseline 50 min later. An overall two-way ANOVA of striatal 5-HT during the pretest swim demonstrated a significant effect of time [F(11,187) = 3.52, P < .01] but no significant effect of drug [F(1,17) = .12, N.S.] or interaction [F(11,187) = 1.72, P > .05]. An analysis of simple main effects on striatal 5-HT during the pretest swim indicated a significant effect of time in the saline [F(9,110) = 2.82, P < .01] and fluoxetine groups [F(8,99) = 2.43, P < .05].

As shown in figure 2B, the test swim reduced extracellular 5-HT slightly in the fluoxetine after 30 min to a minimum of 25% below baseline and returned to baseline 30 min later. The test swim did not alter extracellular 5-HT in the saline group. An overall two-way ANOVA of striatal 5-HT during the test swim demonstrated a significant effect of drug [F(1,17) = 7.94, P < .05] and time [F(11,187) = 2.73, P < .01] but no significant interaction [F(11,187) = 1.09, N.S.]. Analysis of simple main effects during the test swim revealed a significant effect of time in the fluoxetine [F(8,99) = 1.96, P < .05] but not the saline group [F(9,110) = 1.36, N.S.].

No swim control animals showed no change in striatal 5-HT over time in either treatment group on day 1 or day 2 (data not shown). An overall two-way ANOVA of striatal

### TABLE 2

Baseline extracellular 5-HIAA

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Fluoxetine</td>
</tr>
<tr>
<td>Striatum Swim</td>
<td>4.8 ± 1.2</td>
<td>7.5 ± 0.8$^d$</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 9)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Control Swim</td>
<td>7.1 ± 1.7</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(n = 7)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Lateral septum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Striatum Swim</td>
<td>6.9 ± 1.3</td>
<td>7.2 ± 0.9</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>(n = 10)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>Control Swim</td>
<td>6.7 ± 1.7</td>
<td>5.9 ± 1.1</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>(n = 7)</td>
<td>(n = 8)</td>
</tr>
</tbody>
</table>

Values are mean (± 1 S.E.) baseline striatal and lateral septum dialysate content of 5-HIAA (pmol/6.5 μl of sample) in the swim group and no swim controls treated with saline or fluoxetine (20 mg/kg) on day 1 and 2 of the FST.

$^a$P < .05, $^b$P < .01 vs. corresponding value on day 1, according to Dunnett’s test.

$^c$P < .05 vs. corresponding saline control, according to Dunnett’s test.

$^d$P < .01 vs. corresponding no swim control, according to Dunnett’s test.
5-HT demonstrated no significant main effects or interaction term on either day 1 or day 2.

Figure 3 shows the effect of the pretest (fig. 3A) and test (fig. 3B) swim on striatal 5-HIAA in saline or fluoxetine-treated animals. As shown in figure 3A, in the saline group, extracellular 5-HIAA was reduced immediately after the pretest swim onset to a minimum 20 min later of 45% below baseline and returned to baseline 30 min later. In the fluoxetine group, extracellular 5-HT was reduced immediately after the pretest swim onset to a minimum 20 min later of 44% below baseline and returned to baseline 70 min later. An overall two-way ANOVA of striatal 5-HIAA during the pretest swim indicated a significant effect of time \( F(11,187) = 25.67, P < .01 \) but no significant effect of drug \( F(1,17) = 1.82, \text{N.S.} \) or interaction \( F(11,187) = 1.56, \text{N.S.} \). An analysis of simple main effects on striatal 5-HIAA during the pretest swim indicated a significant effect of time in both the saline \( F(9,110) = 15.75, P < .01 \) and fluoxetine groups \( F(8,99) = 11.49, P < .01 \).

As shown in figure 3B, in the saline group, extracellular 5-HIAA was reduced immediately after the test swim onset to a minimum of 29% below baseline, returning to baseline 10 min later. In the fluoxetine group, extracellular 5-HIAA was reduced immediately after the test swim onset to a minimum 20 min later of 37% below baseline and returned to baseline 90 min later. An overall two-way ANOVA of striatal 5-HIAA during the test swim demonstrated a significant effect of time \( F(11,187) = 11.61, P < .01 \) and interaction \( F(11,17) = 2.98, P < .01 \) but no significant effect of drug \( F(1,17) = .74, \text{N.S.} \). Analysis of simple main effects on striatal 5-HIAA during the test swim indicated a significant effect of time in the saline \( F(9,110) = 8.13, P < .01 \) and fluoxetine groups \( F(8,99) = 6.36, P < .01 \).

No swim control animals showed no change in striatal 5-HIAA over time in either treatment group on day 1 or day 2 (data not shown). An overall two-way ANOVA of striatal 5-HIAA on day 1 demonstrated a significant effect of drug \( F(1,11) = 6.08, P < .05 \) and interaction \( F(11,121) = 2.12, P < .05 \) but no significant effect of time \( F(11,121) = .88, \text{N.S.} \). An analysis of simple main effects on striatal 5-HIAA on day 1 indicated no significant effect of time in the untreated \( F(5,66) = 1.52, \text{N.S.} \) or fluoxetine-treated group \( F(6,77) = .79, \text{N.S.} \). An overall two-way ANOVA of striatal 5-HIAA on day 2 demonstrated a significant effect of drug \( F(1,11) = 9.34, P < .05 \) but no significant effect of time \( F(11,121) = .19, \text{N.S.} \) or interaction \( F(11,121) = .42, \text{N.S.} \). An analysis of simple main effects on striatal 5-HIAA on day 2 indicated no significant effect of time in the untreated \( F(5,66) = .23, \text{N.S.} \) or fluoxetine-treated group \( F(6,77) = 1.07, \text{N.S.} \).

**Lateral septum 5-HT and 5-HIAA: Effect of fluoxetine treatment in the FST and no swim controls.** Figure 4 shows the effect of the pretest (fig. 4A) and test (fig. 4B) swim on lateral septum 5-HT in saline or fluoxetine-treated animals. As shown in figure 4A, in the saline group, extracellular 5-HT was reduced 30 min after the pretest swim onset to a minimum of 36% below baseline and returned to baseline 50 min later. In the fluoxetine group, extracellular 5-HT was reduced 30 min after the pretest swim onset to a minimum of 49% below baseline and then spiked 10 min later over baseline in response to the first injection. An overall two-way ANOVA of lateral septum 5-HT during the pretest swim indicated a significant effect of time \( F(11,176) = 2.53, P < .01 \) but no significant effect of drug \( F(1,16) = .04, \text{N.S.} \) or interaction \( F(11,176) = 1.18, \text{N.S.} \). An analysis of simple main effects on lateral septum 5-HT during the pretest swim indicated a significant effect of time in the fluoxetine \( F(9,110) = 2.28, P < .05 \) but not the saline \( F(7,88) = 1.01, \text{N.S.} \) group.

As shown in figure 4B, the test swim reduced extracellular 5-HT in the fluoxetine group, starting immediately after onset to a minimum 20 min later of 42% below baseline and returning to baseline 40 min later but not in the saline group. An overall two-way ANOVA of lateral septum 5-HT during the test swim demonstrated a significant effect of drug \( F(1,16) = 4.76, P < .05 \) and time \( F(11,176) = 2.27, P < .05 \) but no significant interaction \( F(11,176) = 1.76, P > .05 \). An analysis of simple main effects on lateral septum 5-HT during the test swim indicated a significant effect of time in the fluoxetine \( F(9,110) = 2.46, P < .01 \) but not the saline group \( F(7,88) = .73, \text{N.S.} \).

No swim controls showed no significant change in lateral septum 5-HT over time in either treatment group on day 1 or day 2 (data not shown). An overall two-way ANOVA of lateral septum 5-HT on day 1 demonstrated no significant main effect or interaction term. An overall two-way ANOVA of lateral septum 5-HT on day 2 demonstrated a significant effect of drug \( F(1,13) = 11.32, P < .01 \) but no significant
effect of time $F(11,143) = 5.77, \text{N.S.}$ or interaction $F(11,142) = 1.16, \text{N.S.}$.

Figure 5 shows the effect of the pretest (fig. 5A) and test (fig. 5B) swim on lateral septum 5-HIAA in saline or fluoxetine-treated animals. As shown in figure 5A, in the saline group, extracellular 5-HIAA was reduced 20 min after the pretest swim onset to a minimum of 39% below baseline and returned to baseline 30 min later. In the fluoxetine group, extracellular 5-HIAA was reduced immediately after the pretest swim onset to a minimum 30 min later of 46% below baseline and returned to baseline 80 min later. An overall two-way ANOVA of lateral septum 5-HIAA during the pretest swim demonstrated a significant effect of time $F(11,176) = 17.49, P < .01$ and interaction $F(11,176) = 2.84, P < .01$ but no significant effect of drug $F(1,16) = .56, \text{N.S.}$. Analysis of simple main effects on lateral septum 5-HIAA during the pretest swim indicated a significant effect of time in the saline $F(7,88) = 16.15, P < .01$ and fluoxetine groups $F(9,110) = 6.36, P < .01$.

As shown in figure 5B, in the saline group, extracellular 5-HIAA was reduced 20 min after the test swim onset to a minimum of 17% below baseline, returning to baseline 30 min later. In the fluoxetine group, extracellular 5-HIAA was reduced immediately after the test swim onset to a minimum 20 min later of 50% below baseline and remained below baseline throughout the session. An overall two-way ANOVA of lateral septum 5-HIAA during the test swim demonstrated a significant effect of time $F(11,176) = 10.57, P < .01$ and interaction $F(11,176) = 3.85, P < .01$ but no significant effect of drug $F(1,16) = 4.23, P > .05$. Analysis of simple main effects on lateral septum 5-HIAA during the test swim indicated a significant effect of time in the saline $F(7,88) = 5.34, P < .01$ and fluoxetine groups $F(9,110) = 9.93, P < .01$.

In no swim controls, on day 1 there was a slight increase in 5-HIAA in the fluoxetine but not the untreated group immediately after the first injection to a maximum 20 min later of 14% above baseline, returning to baseline 30 min later (data not shown). An overall two-way ANOVA of lateral septum 5-HIAA on day 1 demonstrated a significant effect of time $F(11,143) = 2.03, P < .05$ and interaction $F(11,143) = 2.70, P < .01$ but no significant effect of drug $F(1,13) = .90, \text{N.S.}$. Analysis of simple main effects on lateral septum 5-HIAA on day 1 indicated a significant effect of time in the fluoxetine $F(6,77) = 3.75, P < .01$ but not the untreated group $F(7,88) = .93, \text{N.S.}$. There were no significant changes in lateral septum 5-HIAA over time in either group on day 2 (data not shown).

Behavioral Effects of FST

Figure 6 shows that the effect of fluoxetine treatment on mean counts of immobility, swimming and climbing during the test swim of animals with probes implanted in the striatum (fig. 6A) or lateral septum (fig. 6B) was similar. Figure 6A shows that in the striatum group, fluoxetine treatment
reduced immobility by 35% \(F(1,18) = 5.82, P < .05\) and increased swimming by 84% \(F(1,18) = 15.42, P < .01\) but did not change climbing. Similarly, figure 6B shows that in the lateral septum group, fluoxetine treatment reduced immobility by 21% \(F(1,17) = 5.53, P < .05\) and increased swimming by 56% \(F(1,17) = 15.90, P < .01\) and did not change climbing.

**Relationship between Behavior and Changes in Extracellular 5-HT in the FST.** Figure 7 shows the relationship among immobility (fig. 7A), swimming (fig. 7B) and climbing (fig. 7C) behaviors shown during the test swim and changes in extracellular 5-HT in the striatum for saline and fluoxetine-treated animals. Changes in 5-HT were calculated as maximal effect scores for individual subjects and expressed as a percent of baseline. There was no relationship between striatal 5-HT and either immobility \(r(19) = .26, \text{N.S.}\), swimming \(r(19) = -0.38, \text{N.S.}\) or climbing \(r(19) = .07, \text{N.S.}\).

Figure 8 shows the relationship among immobility (fig. 8A), swimming (fig. 8B) and climbing (fig. 8C) behaviors in the test swim and changes in extracellular 5-HT in the lateral septum. There was a significant positive correlation between lateral septum 5-HT and immobility \(r(18) = .49, P < .05\) and a significant negative correlation between lateral septum 5-HT and swimming \(r(18) = -0.62, P < .01\) but no relationship between lateral septum 5-HT and climbing \(r(18) = .18, \text{N.S.}\).

**Discussion**

We examined the interaction between changes in extracellular 5-HT produced during the FST, as it is traditionally conducted to detect the behavioral effects of antidepressant
drugs, and treatment with fluoxetine, an antidepressant drug that selectively inhibits 5-HT uptake. Fluoxetine treatment produced higher baseline levels of 5-HT on the second day of the FST in both the swim and no swim groups, as expected from previous studies (Kreiss and Lucki, 1995; Perry and Fuller, 1992; Rutter and Auerbach, 1993). In the lateral septum, extracellular 5-HT was decreased during the pretest swim but not during the test swim. In the striatum, the pretest swim produced an increase in extracellular 5-HT, but no changes in striatal 5-HT were produced by the test swim. These effects in this study are also consistent with our previous reports showing that forced swimming produces divergent effects on extracellular 5-HT in different brain regions (Kirby et al., 1995) and that the 5-HT responses to swimming adapted rapidly when the animals were exposed 24 hr later to another swimming session (Kirby and Lucki, submitted).

The most important finding of this study is that fluoxetine treatment altered the normal adaptation of the distinct response patterns of 5-HT in the lateral septum and the striatum produced during the two swimming sessions of the FST. In contrast to saline treatment, rats treated with fluoxetine showed a decrease in extracellular 5-HT in the lateral septum during both the pretest and test swimming sessions. After the test swim, rats treated with fluoxetine showed a decrease in extracellular 5-HT in the striatum as well, even though the response to the pretest swim in this region was to increase extracellular 5-HT. Thus, after treatment with fluoxetine, the response of 5-HT in the lateral septum during the pretest swim of the FST reappeared during the test swim and the response of striatal 5-HT during the initial swim was reversed from an increase to a decrease. The adaptation of the regionally specific changes in 5-HT to the FST may indicate that the pretest swim alters 5-HT synthesis, release, uptake mechanisms, autoreceptor function or some combination of these mechanisms in such a manner that they cause extracellular 5-HT to be less responsive to a subsequent test swim. Alternatively, the adaptation of the 5-HT responses may be due to modification of the regulation of 5-HT transmission by facilitatory or inhibitory afferents, either at the raphe nuclei or in terminal regions.

The results of this study indicate that there also was an interaction between fluoxetine treatment and the FST for the response of 5-HIAA in both brain regions. In both brain regions, the pretest swim produced a decrease in extracellular 5-HIAA that was diminished during the second test swim. After treatment with fluoxetine, however, the decrease in extracellular 5-HIAA after the test swim was similar in magnitude to the pretest swim. The initial exposure to forced swimming may reduce the levels of 5-HIAA by reducing the rate of intracellular metabolism of 5-HT into 5-HIAA by monoamine oxidase. Fluoxetine treatment, in general, produced a reduction in baseline levels of 5-HIAA on the second day of the FST by blocking the uptake of 5-HT and reducing 5-HT synthesis, resulting in lower levels of intracellular 5-HT available for metabolism into 5-HIAA by monoamine oxidase, as reported previously (Perry and Fuller, 1992). The magnitude of this effect may be enhanced by the combined influence of fluoxetine and swimming. The results of repeated exposure to forced swimming from saline-treated animals in this study differ from our previous report, which showed no change in magnitude of the response of 5-HIAA in the striatum and the lateral septum after exposure to two 15-min swimming sessions 24 hr apart (Kirby and Lucki, submitted). However, this difference is probably due to the use of only a 5-min test in the current study, mandated by procedures commonly used in the FST.

The use of microdialysis to measure changes in 5-HT during the FST allowed comparison of the behavioral effects of fluoxetine with neurochemical changes in extracellular 5-HT. Treatment with fluoxetine produced significant changes in behavior in the FST; immobility was reduced and swimming
behavior was increased, although there was no effect on climbing behavior. This behavioral pattern after fluoxetine treatment in the FST is similar to that reported previously using these methods (Detke et al., 1995). There was a significant positive correlation between 5-HT and immobility and a negative correlation between 5-HT and swimming behavior in the lateral septum, but no correlation between 5-HT and climbing behavior. In contrast, none of the correlations between striatal 5-HT and any of the behaviors were statistically significant. Thus, there appears to be a relationship between changes in extracellular concentrations of 5-HT in the lateral septum and shifts in behavioral responses during the FST that may underlie its utility as an animal model of depression. Specifically, reductions in lateral septum 5-HT appear to coincide with lower levels of immobility and higher levels of swimming behavior. The pretest swim of the FST has been suggested to act as an inescapable stressor that elicits escape-oriented behaviors and, subsequently, behavioral immobility (Lahmame and Armario, 1996). The ability of forced swimming to reduce extracellular 5-HT in the lateral septum may be related to the emission of swimming as a coping response during inescapable forced swimming. In the FST, the subsequent test swim is often a behavioral assay for the development of behavioral immobility from the pretest swimming session (Porsolt et al., 1978). The inability to reduce extracellular 5-HT in the lateral septum during forced swimming may thus be related to the development of behavioral immobility. In the present study, treatment with the antidepressant drug fluoxetine restored the behavioral and neurochemical responses observed during the pretest swim during the test session. Fluoxetine treatment prevented the development of behavioral immobility, increased the emission of swimming behavior and restored the ability of forced swimming to reduce extracellular 5-HT in the lateral septum. The correlation found in this study is similar to the correlations observed between behavioral responses and changes in 5-HT concentrations in the lateral septum to repeated forced swimming in our previous report (Kirby and Lucki, submitted). These correlations, although indicative of a potentially causal relationship between changes in lateral septum 5-HT and behavior, cannot distinguish between the possibility that the neurochemical changes drive the behavioral changes, or vice versa.

The behavioral effects of fluoxetine in the FST are shared by other 5-HT-selective uptake inhibitors, but a different behavioral pattern results from treatment with tricyclic antidepressant drugs. Although both classes of antidepressants decrease behavioral immobility, 5-HT-selective uptake inhibitors increase swimming behavior, and tricyclic compounds increase climbing behavior during the test swim (Detke et al., 1995). If the changes in lateral septum 5-HT underlie the observed behavioral changes in the FST, then it would be predicted that treatment with other selective 5-HT uptake inhibitors would produce similar changes in neurochemistry and behavior. However, it is not clear whether tricyclic antidepressants would be expected to share the neurochemical responses produced by fluoxetine because they produce a different pattern of behavioral effects in the FST. Furthermore, reducing 5-HT levels in the lateral septum during the test session should produce antidepressant effects in the FST. Antidepressant effects in the FST have been demonstrated shortly after administration of the 5-HT$_{1A}$ agonist 8-hydroxy-2-di-n-propylaminotetralin (8-OH-DPAT) into the dorsal raphe nucleus (Schreiber and De Vry, 1993). 8-OH-DPAT injected into this cell body region would stimulate inhibitory autoreceptors, resulting in a decrease of 5-HT synthesis and release in terminal regions such as the lateral septum. Other studies have implicated the lateral septum in the FST. For example, forced swimming activates mRNA for the immediate early gene c-fos in several brain regions, including the lateral septum (Cullinan et al., 1995), and increases glucose utilization in the lateral septum, which is reversed by pretreatment with imipramine, a tricyclic antidepressant (Duncan et al., 1993). Local administration of the 8-OH-DPAT in the lateral septum produced antidepressant-like behavioral effects in the FST (Schreiber and De Vry, 1993). This particular finding is not consistent with the simple conceptualization that decreased lateral septum 5-HT is associated with antidepressant effects in the FST, because in this region, 8-OH-DPAT would act as an agonist at postsynaptic 5-HT$_{1A}$ receptors. However, it is still possible that administration of 8-OH-DPAT into the lateral septum might alter the release of 5-HT in this region in response to stress, possibly due to a long feedback loop involving afferents to the raphe nuclei. Such a result would be consistent with the more general model proposed by this study: that the ability of the lateral septum to neurochemically respond to stress is associated with coping behaviors while its inability to respond to stress is associated with depression-like behaviors in the FST.

Other studies have implicated reductions of 5-HT in terminal regions in the learned helplessness paradigm, another animal model of depression. Antidepressant effects in the learned helplessness model have been demonstrated by administration of benzodiazepines into the dorsal raphe nucleus, which would have an inhibitory effect on the firing rate of 5-HT neurons, resulting in reduced synthesis and release of 5-HT in terminal regions (Maier et al., 1994). Furthermore, some studies have specifically identified the lateral septum as a neuroanatomical substrate of the learned helplessness model. Local administration of 5-HT into the lateral septum reversed the development of learned helplessness (Sherman and Petty, 1980), and there was a decrease in in vitro 5-HT release from slices of lateral septum of rats previously exposed to learned helplessness that was reversible by chronic imipramine pretreatment (Sherman and Petty, 1982). Therefore, there may be a unique role of the lateral septum in the production of behaviors that are sensitive to antidepressant treatment in the FST and possibly other animal models of depression.

It is possible that the adaptation exhibited by 5-HT neurons projecting to the lateral septum and striatum after repeated swim exposures is due to a short-term change in the responsiveness of local mechanisms controlling extracellular 5-HT (i.e., synthesis, release, uptake or autoreceptor function). It is also possible that this adaptation is secondary to a change in another neuronal circuit that regulates 5-HT release by projections to serotonin cell body regions. For example, the initial swim exposure may alter another neuronal system that is an afferent input to the raphe nuclei, dampening the responsiveness of the cell bodies to the subsequent swim exposure. It is possible that antidepressant treatment alters this afferent input in such a way as to remove its influence on the raphe nuclei, thus restoring the responsiveness of the raphe to the second swim exposure. Most studies
have favored the involvement of postsynaptic 5-HT1A receptors in the actions of antidepressant drugs in the FST (see Lucki et al., 1994, for review). However, it is possible that the activation of postsynaptic receptors alters other neuronal circuits that project to serotonin cell bodies and restores the ability of raphe neurons to respond to the swim exposure (i.e., reducing endogenous 5-HT in projection regions such as the lateral septum).

Few reports exist of neurochemical effects of antidepressants during animal models of depression. For example, Rossetti et al. (1993) demonstrated that a 40-min swim session resulted in a decrease in extracellular striatal dopamine that was partially reversed by chronic pretreatment with imipramine. These authors concluded that the dopamine depletion is associated with the depressive state or “behavioral despair” produced by the inescapable swim. Petty et al. (1992) demonstrated that rats exhibiting learned helplessness showed a decrease in cortical 5-HT that was reversed, along with the behavioral depression, by chronic imipramine treatment. Our results add to these reports, implicating changes in lateral septum 5-HT with behavioral changes in the FST and their reversal by antidepressant drug treatment.

In summary, this study used the technique of in vivo microdialysis to examine the interaction between fluoxetine treatment and exposure to the FST on extracellular 5-HT in two brain regions: the striatum and the lateral septum. The test swim in the FST produced an adaptation of the region-specific, bidirectional 5-HT response to the initial prettest swim. Fluoxetine treatment altered adaptation of the neurochemical response in each brain region, resulting in a unique pattern of neurochemical responses that was distinct from either treatment alone. Furthermore, the behavioral response of individual rats to forced swimming was correlated with changes in extracellular 5-HT in the lateral septum but not the striatum. Therefore, these changes of 5-HT in lateral septum might underlie those behaviors in the FST that are characteristically vulnerable to alteration by antidepressant drug treatment. In the future, it will be important to determine whether these observed neurochemical effects are replicated in other animal models of depression. Furthermore, it would be interesting to test the behavioral and neurochemical effects of other antidepressant compounds in the FST, especially other classes of antidepressants, such as tricyclic antidepressants, that do not alter extracellular 5-HT (Kreiss et al., 1995) but are behaviorally active in the FST (Detke et al., 1995). It is possible that some of the symptoms of human depression result from an adaptation of the 5-HT response to life stresses. Successful antidepressant treatment may depend on the ability of those drugs to restore the plasticity of a normal serotonergic stress response in the brain.

References


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