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An automated analysis of rat behavior in the forced swim test

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Abstract

The Porsolt forced swim test (FST) is a commonly used paradigm to evaluate antidepressant activity of drugs. This test is based on visual measurement of the rat's floating time (FT) in a tank filled with water. Here, we present an automated, accurate and faster method for estimating FT by the distance moved (DM) by the animal via the use of the Ethovision software in three separate experiments. Experiment 1 investigated the effect of varying delays (24-h and 7-day) between pretest and test on FT and DM. Experiment 2 aimed at examining the effects of a 2-day withdrawal period in rats sensitized to amphetamine and cocaine, on FT and DM. Finally, Experiment 3 looked at the effects of desipramine and fluoxetine on FT and DM. The results of these experiments show that increasing the delay between pretest and test reduced FT during subsequent exposure (test). In addition, rats sensitized to and then withdrawn from either amphetamine or cocaine did not differ in FT or DM compared with control rats. Finally, both desipramine and fluoxetine reduced FT and increased DM. Furthermore, DM was consistently significantly negatively correlated with FT. These results support the use of an automated method for the evaluation of rat behavior in FST. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

The forced swim test (FST), introduced by Porsolt in 1977 (Porsolt et al., 1977b) has been extensively used to investigate the effects of new drugs with potential antidepressant activity (Borsini et al., 1991; Bourin, 1990; Healy et al., 1999; Wong et al., 2000). FST has also proven its usefulness for further characterizing in rats, neurochemical (Connor et al., 2000) or behavioral (Detke et al., 1995; Redrobe et al., 1998) effects of drugs with known beneficial outcomes in the treatment of human depression (for a review, see Borsini and Meli, 1988). In addition, FST has been exploited for assessing animal models of depression induced by pharmacological manipulation (Kokkinidis et al., 1986) or breeding selection (Overstreet et al., 1995; Tizabi et al., 2000) and as a model for the negative symptoms of schizophrenia (Corbett et al., 1999; Noda et al., 2000).

FST consists of placing a rat into a tank filled with water. The procedure is generally divided into a preexposure session (pretest) lasting 15 min, followed 24 h later by a 5-min test session. Following an initial period of vigorous struggling, the animal adopts a typical posture, performing only those movements necessary to keep its head above water (i.e., floating). The total amount of time the animal demonstrates this behavior is then measured. The pretest session induces in naïve rats a prolonged floating time (FT) during the test session. This prolonged FT during the second exposure to the tank has been interpreted by Porsolt as reflecting the animal's state of despair (Porsolt et al., 1977b, 1978a), elicited by the inescapable nature of the tank, that was learned during pretest. Whilst this learned helplessness/despair interpretation has been questioned (Borsini et al., 1986; De Pablo et al., 1989; Nishimura et al., 1988), increased FT during the test session has been repeatedly and consistently reduced by a large variety of drugs known for their efficacy in successfully treating human depression. This has led to the use of FT measurement for assessing the efficacy of antidepressant agents in the FST.

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Because the measurement of FT is performed visually by human observers, it requires training and observer objectivity. Even when the observer is trained and objective, there still remains a problem of behavioral definition: FT is also referred to as immobility, but to remain afloat the animal must make slight movements and is therefore not strictly immobile. In addition, the measurement of FT is time consuming and is not readily compatible with experiments requiring large groups of animals and the testing of many drugs. In an attempt to overcome these limitations, two studies have reported automated measurement of mobility (i.e., the inverse of FT) in the FST. Shimazoe et al. (1987) used tremor sensors surrounding the tank to record water vibrations during rat swimming, and De Pablo et al. (1989) measured the variations in the frequency of the natural electromagnetic field of water induced by rat movements. These methods, although appropriate for the FST, require complex and dedicated equipment and one such set-up per animal. Here, we introduce a new automated method for measuring the rat's behavior in the FST. This is based on the determination of the rat's distance moved (DM) within the tank. We reasoned that if we could demonstrate that DM was closely correlated with FT, then DM could be used as the dependent measure in the FST. This would confer several advantages. The method is rapid and objective, and extends the use of the existing Noldus Ethovision Software to the FST. In addition to the gain in objectivity and rapidity, a further advantage of this method is that it uses a software that is already widely employed in laboratories. Indeed, the same software can be used in tasks such as the open field and therefore to identify compounds that induce false positive results in FST due to psychomotor effects (Plaznik et al., 1985; Porsolt et al., 1977b; Tizabi et al., 1999; West et al., 1999). In order to test its validity, this new application of Noldus software was investigated and compared against FT in different experiments to measure (1) the hypothesized depressive-like state of rats during the withdrawal from repeated amphetamine treatment and (2) the known antidepressant-like activity of fluoxetine and desipramine.

Withdrawal of psychostimulants (cocaine or amphetamine) following their repeated intermittent administration has been shown to induce depressive-like symptoms in humans (Jittiwutikan et al., 1997; Kosten et al., 1998; Schildkraut et al., 1971; Watson et al., 1972). In addition, several lines of evidence suggest that withdrawal from repeated amphetamine treatment in rodents induces an anhedonia- or more generally, a disphoria-like state as well as other symptoms resembling human depression. The former includes reduction of intracranial self-stimulation (Borowski and Kokkinidis, 1992; Paterson et al., 2000; Wise and Munn, 1995); the latter include nocturnal hypoactivity (Paulson et al., 1991; Paulson and Robinson, 1996), lower locomotor activity in a novel environment (Persico et al., 1995), and impaired sexual behavior (Barr et al., 1999). Furthermore, Kokkinidis et al. (1986) have reported increased duration of immobility in the FST in mice chronically treated with amphetamine. However, these behavioral effects were observed in animals receiving large doses of amphetamine, ranging from 1 to 12 mg/kg either via chronic repeated injections or via continuous delivery from subcutaneous osmotic minipumps (Paterson et al., 2000).

The first set of experiments of the present study aimed at investigating the effects of the withdrawal period from repeated injections of doses of amphetamine or cocaine that have been shown to produce behavioral sensitization (Hedou et al., 2001) on FT and DM in the FST. In order to assess the feasibility of administering sensitization-inducing treatments between pretest and test, we first had to validate the procedure of using a prolonged delay of 7 days between pretest and test as compared with the 24-h delay of the original description of the procedure (Experiment 1). Behavioral sensitization was induced by repeated, intermittent injections of 1.5 mg/kg amphetamine or 20 mg/kg cocaine for 5 days, and rat behavior in the FST was then measured following a 48-h withdrawal period (Experiment 2). In Experiment 3, DM and FT in the FST were measured following treatment with repeated injections of the classical antidepressant desipramine and the selective re-uptake inhibitor (SSRI) fluoxetine. These antidepressants are known for their efficacy in decreasing FT in FST (Detke et al., 1995; Page et al., 1999) as well as in alleviating depressive symptoms in humans (Schatzberg, 2000).

2. Materials and methods

2.1. Subjects

Male Wistar [Zur:WIST(HanIbm)] and Sprague–Dawley [Zur:SD(Crl:CD (SD)BR)] rats (ETH Research Unit, Schwerzenbach, Switzerland) weighing 250–300 g were group housed in a temperature- ($21 \pm 1.0°C$) and humidity- ($55 \pm 5\%$) controlled room. They had free access to food (Nafag, 9431, Nafag Ecossan, Gossau, Switzerland) and water and were kept on a reversed 12-h light/dark cycle (lights on at 7:00 p.m.). Daily care was provided to the animals by in-house animal technicians. This included changing and cleaning soiled cages twice weekly, providing food and water, and monitoring the general health of all animals. Wistar (Wis) rats were used in Experiments 1 and 2 and Sprague–Dawley (SD) rats were chosen for Experiment 3. SD rats have been reported to be more sensitive to antidepressants than Wis rats (Porsolt et al., 1978b) and are the most commonly used strain for testing the effects of desipramine and fluoxetine (Detke et al., 1995; Page et al., 1999). All the procedures and experiments were conducted in accordance with Swiss federal regulations for animal

experimentation and approved by the Veterinary Office of the canton of Zurich.

2.2. FST procedure

We used the FST as described in Porsolt's original paper (Porsolt et al., 1977b) with some modifications. Briefly, the rats experienced a pretest session followed by a test session either 24 h (Experiments 1 and 3) or 7 days later (Experiments 1 and 2). For both the pretest and the test sessions, conducted under low illumination (12 lx), the rats were placed in a plastic cylindrical tank (44 cm high by 32 cm in diameter) filled with tap water at 22°C, to a depth of 28 cm, so that the rat's hindlimbs could not reach the tank's floor. In all the experiments, the pretest was carried out for 15 min and the test for 5 min in the same tank. The apparatus was kept as clean as possible by changing the water following two 15-min or six 5-min sessions and feces were removed after each session. Following either pretest or test sessions, rats were dried with a towel and kept warm on a heating pad for 30 min in their home cage. All experimental groups were composed of eight rats. Four rats were monitored at the same time following a counterbalancing protocol for both treatments and tanks.

2.3. Behavioral analysis

Both sessions (pretest and test) were videotaped for visual and automated quantitative analysis of the FT and DM, respectively. All analyses were performed by one person (GH), after reaching an intraobserver reliability of $r = .88$. The measurement of FT was based on the original functional definition of Porsolt et al. (1977b), that is the time during which the rat performed "only those movements necessary to keep its head above water." To elaborate on this definition, any slight movements of the tail, body, limbs or head, with the animal otherwise in a quiet state, were interpreted as floating. Therefore, floating was clearly differentiated from those behaviors that were escape attempts. Escape included the behaviors of vigorous movements of the whole body, climbing, swimming, diving; it also included head movements which appeared to indicate that the rat was searching for an escape, even when the rat's body remained in the same position within the tank. We expressed total FT in seconds and DM in centimeters for the first 5 min of each session. Sessions were recorded by a SONY digital monochrome CCD IRIS camera connected to a SONY black and white SSM-930 CE monitor and a SONY SVT 1000 P videocassette recorder. The videocassette recorder was coupled with a Compaq Deskpro 4100 computer equipped with the Noldus software (Ethovision version 1.90, Noldus Information Technology, Wageningen, the Netherlands) for data acquisition. The software allowed the measurement of total DM (in centimeters) by track tracing the animal displacement in a defined arena. Images of these displacements were acquired twice per second.

2.4. Drug treatments

Cocaine and amphetamine, dissolved in 0.9% saline solution at 20 and 1.5 mg/ml, respectively, were freshly prepared prior to the injection and injected intraperitoneally (ip) in a final volume of 1.0 ml/kg (Experiment 2). The sensitization procedure was performed as follows: drugs were administered between pretest (Day 1) and test (Day 8). Rats were exposed to pretest at Day 1, and received intraperitoneal injections of saline (0.9%), amphetamine (1.5 mg/kg), or cocaine (20 mg/kg) once per day for 5 days (Days 2 to 6). The test session was performed following a 48-h withdrawal period.

Fluoxetine hydrochloride (20 mg/kg) and desipramine hydrochloride (15 mg/kg) were diluted in 0.9% saline for an injection volume of 2 ml/kg. These doses have been shown to produce effective antidepressant effects in the FST (Detke et al., 1995; Duncan et al., 1996; Kirby and Lucki, 1997; Lopez-Rubalcava and Lucki, 2000). The doses were calculated as base weight. Fluoxetine and desipramine were injected subcutaneously (sc) three times per subject, with fresh solutions prepared just prior to the injection. These injections were performed 23.5, 5, and 1 h before the test (Experiment 3).

Amphetamine sulfate, cocaine hydrochloride, and desipramine hydrochloride were purchased from Sigma (St. Louis, MO) and dissolved in 0.9% saline at 1.5, 20, and 7.5 mg/ml, respectively. Fluoxetine was a generous gift from Dr. G. Higgins (F. Hoffmann-La Roche, Basel, CH) and dissolved at 10 mg/ml in saline.

2.5. Statistics

In Experiment 1, a three-way analysis of variance (ANOVA) was used, with a between-subjects factor of delay (24 h vs. 7 days) and within-subject factors of session (pretest vs. test) and time (min bins 1–5). In Experiment 2, a three-way ANOVA was used with a between-subjects factor of treatment (amphetamine vs. cocaine vs. vehicle) and within-subject factors of session (pretest vs. test) and time (min bins 1–5). Results of Experiment 3 were analyzed by a three-way ANOVA with a between-subjects factor of treatment (fluoxetine vs. desipramine vs. vehicle) and within-subject factors of session (pretest vs. test) and time (min bins 1–5). In addition, a comparison was conducted between the naïve rats of Experiment 1 in the 24-h delay condition and the saline-injected rats of Experiment 3 in order to determine possible strain differences (Wis vs. SD, respectively). This analysis compared performance in terms of both FT and DM. A three-way ANOVA was used with a between-subjects factor of strain (Wis vs. SD) and within-subject factors of session (pretest vs. test) and time (min bins 1–5).

Following confirmation of main effects or interactions by the overall analysis, post hoc t tests based on the error terms derived form the appropriate overall ANOVA were

performed for all the results of the present report. Statistical significance was set at $P<.05$. The relationship between visually scored FT and automated measurement of DM was analyzed by using simple linear regression analyses.

3. Results

3.1. Experiment 1: Comparison of the effects of a 24-h vs. 7-day delay between pretest and test sessions on FT and DM in naïve rats

In this experiment, either 24 h or 7 days separated pretest and test sessions. In both conditions, FT and DM were measured and compared (Fig. 1).

3.1.1. Floating time

FT increased rapidly after the first minute of immersion in both the pretest session and the test session of the 24-h and the 7-day delays, and reached a plateau 3 min after the onset of the session (Fig. 1A). An overall ANOVA on FT with a between-subjects factor of delay (24 h vs. 7 days) and repeated measurements factors of session (pretest vs. test) and time (1-min bins over the first 5 min) revealed main effects of delay [$F(1,14)=4.7$, $P<.05$], session [$F(1,14)=25.9$, $P<.0005$], and time [$F(4,56)=63.4$, $P<.0001$], as well as a significant Session × Time interaction [$F(4,56)=11.6$, $P<.0001$], and a nonsignificant trend toward a Delay × Session × Time interaction [$F(4,56)=2.1$, $P=.098$]. FT during the test session increased when compared with FT during pretest in both delay conditions. *t* test

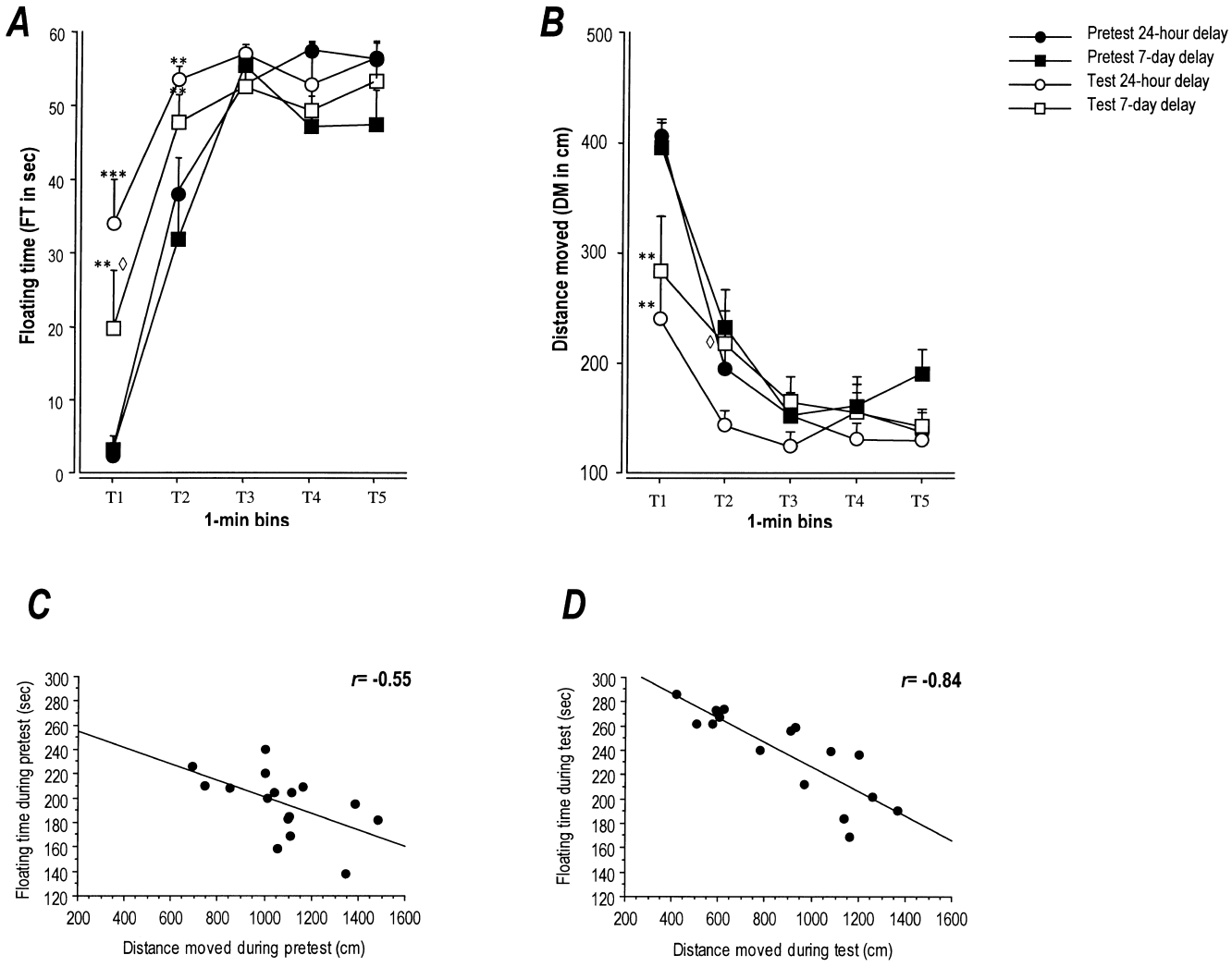

Fig. 1. Effects of either a 24-h or a 7-day delay on FT (A) and DM (B) during the pretest and test sessions of the FST. Values are expressed as means ± S.E.M. The FT during pretest was increased compared with FT during test for the first 2 min (A). This effect was confirmed by further *t* tests conducted on the error terms given by the ANOVA. In addition, FT in the 7-day delay was reduced compared with FT in the 24-h delay condition. During test, the DM was significantly reduced compared with pretest (B). This effect reached significance for Minute 1 as revealed by *t* test comparisons. ** $P<.01$, *** $P<.001$ for pretest vs. test, and ◊ $P<.05$ for tests in the 24-h vs. 7-day condition. (C) and (D) present correlation analyses between total DM and total FT over the 5 min for both the pretest (C) and test (D). FT was significantly negatively correlated with DM during both the pretest: $r=-.55$ ($y=268.53-0.068\,x$), and the test: $r=-.84$ ($y=327-0.101\,x$).

analysis was conducted separately on both the 24-h and the 7-day delays. This revealed a significant increase in the test FT when compared with pretest for the first minute [$t(14)=6.51$, $P<.001$ and $t(14)=3.41$, $P<.01$ for the 24-h and 7-day delays, respectively] and the second minute [$t(14)=3.18$ and $t(14)=3.25$, $P<.01$ for both delays] of the experiment. FT during test in the 7-day delay condition was compared with FT during test in the 24-h delay condition. *t* test analysis based on the error terms derived from the ANOVA revealed that in the first minute of the test only FT in the 7-day condition was significantly shorter than in the 24-h delay condition [$t(14)=2.92$, $P<.02$].

These analyses demonstrate that rats floated more during the test session compared with the pretest session. This difference reached significance for the first 2 min. However, a greater delay between pretest and test decreased this effect; that is to say that although significantly longer compared with FT in pretest, FT during Minute 1 of the test session was shorter in the 7-day delay condition than in the 24-h delay condition.

3.1.2. Distance moved

DM decreased rapidly over the 5-min period of both the pretest and test sessions (Fig. 1B). An overall ANOVA conducted on DM, with a between-subjects factor of delay (24-h vs. 7-day) and repeated measurements factors of session (pretest vs. test) and time (1-min bins over the first 5-min period) revealed significant main effects of session [$F(1,14)=18.7$, $P=.001$] and time [$F(4,56)=64.0$, $P<.0001$] as well as a significant Session × Time interaction [$F(4,56)=12.1$, $P<.0001$] and a trend toward a significant Delay × Session × Time interaction [$F(4,56)=2.1$, $P=.088$].

DM was lower during the test session as compared with the pretest session in both delay conditions. *t* test comparisons based on the error terms derived from the ANOVA revealed that this effect reached significance for the first minute only, in both the 24-h condition [$t(14)=6.56$, $P<.001$] and the 7-day condition [$t(14)=4.43$, $P<.001$]. Test DM in the 7-day condition was compared with test DM in the 24-h condition using *t* test comparisons. These analyses revealed a significantly greater DM in the 7-day condition, only for the second minute of the test session when compared with the 24-h condition [$t(14)=2.92$, $P<.02$].

Thus, rats moved a shorter distance during the test session compared with the pretest session. This difference reached significance for the first minute in both the 24-h and the 7-day conditions. However, a greater delay between pretest and test decreased this effect. Although significantly reduced compared with DM in pretest, DM during the second minute of the test session was significantly higher in the 7-day delay condition than in the 24-h delay condition.

3.1.3. Relationship between FT and DM

Regression analyses were conducted in order to estimate the relationship between FT and DM (Fig. 1C and D). These analyses were performed separately for the pretest and test sessions with the total FT and total DM over the entire sessions. In both cases (pretest and test), FT was negatively and significantly correlated with DM. The calculated *r* value for pretest was $-.55$ [$F(1,14)=6.13$, $P<.05$] and for test $r=-.84$ [$F(1,14)=34.32$, $P<.0001$].

3.2. Experiment 2: Effect of withdrawal from amphetamine and cocaine on FT and DM

In this second set of experiments pretest was performed on Day 1, 24 h prior to the induction of behavioral sensitization. The sensitization procedure used in the present study reliably induced behavioral sensitization in former experiments (Hedou et al., 2001). It consisted of repeated, intermittent injections of amphetamine or cocaine. From Day 2 onward, rats received one injection per day for the next 4 days (five injections in total from Day 2 to Day 6). Following a 48-h withdrawal period, the test session was performed and FT and DM were measured (Fig. 2).

3.2.1. Floating time

An overall ANOVA was conducted on FT with a between-subjects factor of treatment (amphetamine vs. cocaine vs. vehicle), and repeated measurements factors of session (pretest vs. test) and time (1-min bins over the first 5-min period). There was no effect of treatment either as a main effect or an interaction, for either pretest or test sessions. The analysis did reveal main effects of session [$F(1,20)=14.29$, $P<.005$] and time [$F(4,80)=102.92$, $P<.0001$], and a significant Session × Time interaction [$F(4,80)=2.74$, $P<.05$]. *t* test comparisons of FT between the pretest and test sessions revealed that rats floated more during test as compared with pretest. This was true for all treatments for Minute 1 [vehicle: $t(14)=3.05$, $P<.01$; amphetamine: $t(12)=2.20$, $P<.05$; and cocaine: $t(14)=2.71$, $P<.02$] and, for cocaine only, also for Minute 2 [$t(14)=2.72$, $P<.02$].

3.2.2. Distance moved

An overall ANOVA was conducted on DM with a main factor of treatment (amphetamine vs. cocaine vs. vehicle) and repeated measurements factors of session (pretest vs. test) and time (1-min bins over the first 5-min period). There was no effect of treatment either as a main effect or an interaction for either pretest or test sessions. The ANOVA did reveal main effects of session [$F(1,21)=33.40$, $P<.0001$] and time [$F(4,84)=130.19$, $P<.0001$], and a significant Session × Time interaction [$F(4,84)=13.96$, $P<.0001$]. *t* test comparisons based on the error terms derived from the ANOVA yielded a significant decrease in DM during test session compared with pretest. This was true for all the treatment groups for Minute 1 [vehicle: $t(14)=5.35$, $P<.001$; amphetamine: $t(14)=4.29$, $P<.001$; and cocaine: $t(14)=5.90$, $P<.001$] and for vehicle and

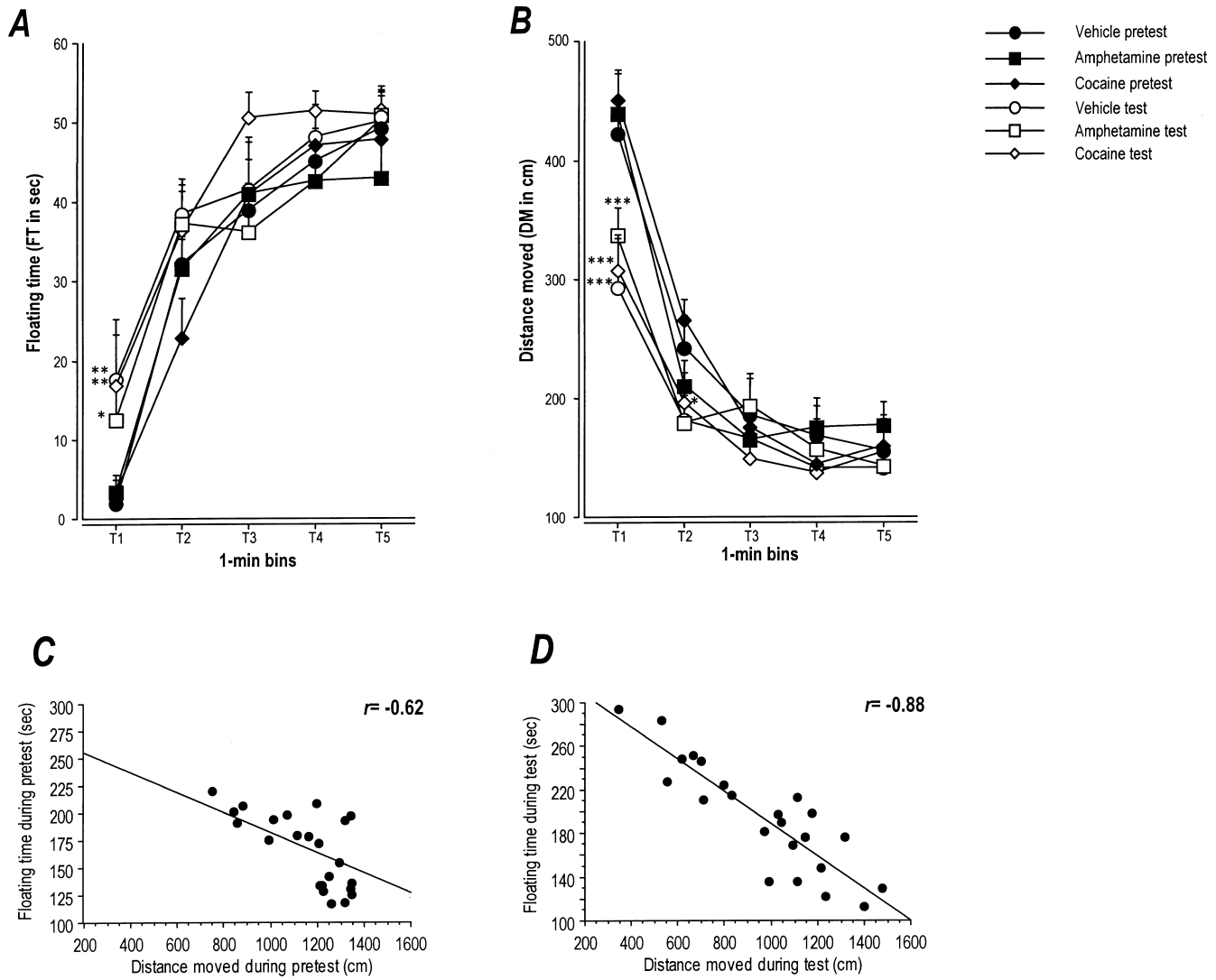

Fig. 2. Effect of a 2-day withdrawal period from the repeated, intermittent administration of either amphetamine (1.5 mg/kg ip) or cocaine (20 mg/kg ip) on FT (A) and DM (B) in the FST. FT was increased during test compared with pretest (A). *t* test comparisons showed that this effect was statistically significant for Minute 1. No statistical differences between psychostimulant- and vehicle-treated rats at any time point were observed during the test. (B) shows DM during pretest and test for vehicle and sensitized rats. In both treatment regimens, DM was significantly reduced during the test compared with the pretest. This difference reached significance for Minute 1 as shown by *t* test comparisons. No statistically significant differences were seen between treatment groups. * $P<.05$, ** $P<.01$, *** $P<.001$. Values are expressed as means ± S.E.M. Correlation analyses between total FT and total DM for both the pretest (C) and test (D) over the 5-min sessions show that FT was significantly negatively correlated with DM: pretest $r=-.62$ ($y=273.87-0.092x$), test $r=-.88$ ($y=336.91-0.148x$).

cocaine groups for Minute 2 [vehicle: $t(14)=2.56$, $P<.05$ and cocaine: $t(14)=2.88$, $P<.02$].

3.2.3. Relationship between FT and DM

Regression analyses were conducted in order to study the relationship between FT and DM under different drug treatments (Fig. 1C and D). These analyses were performed separately for the pretest and test for the total FT and the total DM measured over the entire session. In both cases (pretest and test), FT was negatively and significantly correlated with DM. For pretest, the calculated *r* value was $r=-.62$ [$F(1,22)=13.38$, $P<.005$] and for test $r=-.88$ [$F(1,22)=72.95$, $P<.0001$].

3.3. Experiment 3: Effect of desipramine and fluoxetine on FT and DM

In this experiment, rats were exposed to the test session 24 h following the pretest. They received subcutaneously vehicle, desipramine (15 mg/kg), or fluoxetine (20 mg/kg) in three boli administered 23.5, 5, and 1 h before test. FT and DM were measured during both the pretest and the test sessions (Fig. 3).

3.3.1. Floating time

An overall ANOVA on FT was conducted with a between-subjects factor of treatment (fluoxetine or

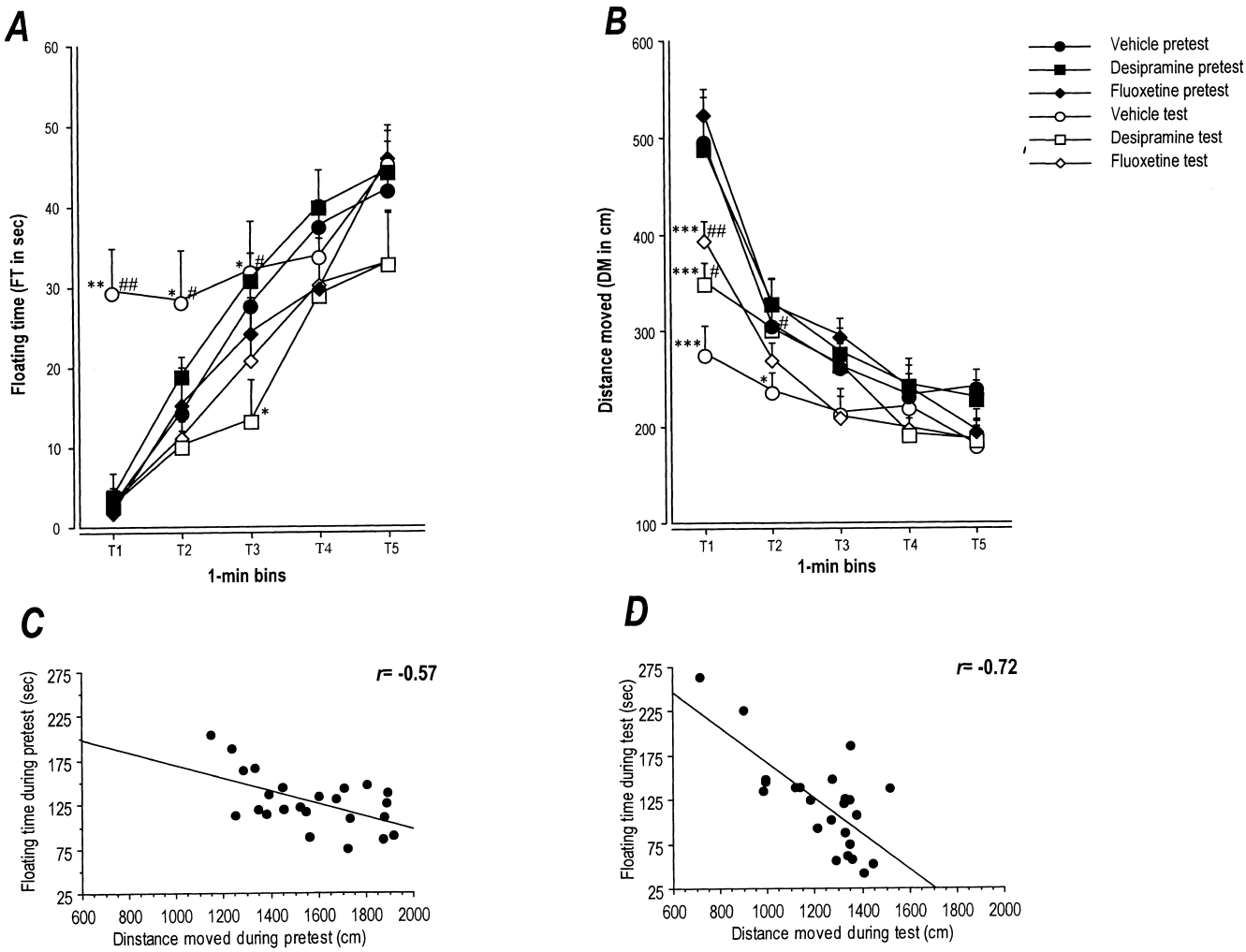

Fig. 3. Effect of fluoxetine (20 mg/kg) and desipramine (15 mg/kg) on FT (A) and DM (B) during the pretest and test sessions of the FST. (A) shows the effects of both antidepressants on FT during the pretest and test. FT was significantly increased in the test compared with pretest in vehicle-treated rats, whereas FT in test of desipramine- as well as of fluoxetine-treated rats did not differ from pretest (except Minute 3 for desipramine-treated rats $P<.05$). In addition, at test vehicle-treated rats exhibited a significantly increased FT compared with antidepressant-treated rats. During test session, vehicle-treated rats showed a decreased DM compared with pretest (B). This effect reached significance for the first 2 min. Treatment with either desipramine or fluoxetine led to a reduction in DM during Minute 1 of the test session compared with pretest. However, although significantly different from pretest DM, this reduction was significantly smaller than that observed for vehicle-treated rats. Values are expressed as means ± S.E.M. * $P<.05$, ** $P<.01$, and *** $P<.001$ for pretest vs. test, $^{\#}$ $P<.05$, $^{\#\#}$ $P<.01$ for desipramine and fluoxetine vs. saline during test session. Correlation analyses of total FT and total DM for both the pretest (C) and test (D) show that in all cases FT was significantly and negatively correlated with DM: pretest $r=-.57$ ($y=242.65-0.073\,x$), test $r=-.72$ ($y=365.89-0.2\,x$).

desipramine vs. saline) and repeated measurements factors of session (pretest vs. test) and time (1-min bins over the 5-min session). This analysis revealed an almost significant trend toward a main effect of treatment [$F(2,21)=3.38$, $P=.053$], a significant main effect of time [$F(4,84)=82.29$, $P<.0001$], and significant Treatment × Session [$F(2,21)=9.73$, $P<.002$] and Session × Time [$F(4,84)=4.67$, $P<.002$] interactions. t test comparisons of FT between pretest and test revealed that vehicle-treated rats floated more during test as compared with pretest. This difference reached significance for Minute 1 [$t(14)=4.12$, $P<.01$] and Minute 2 [$t(14)=2.13$, $P<.05$]. Treatment with desipramine reversed the increased FT during test, with FT of treated rats during the test being almost equal to FT during pretest. This was revealed by the lack of significant difference between pretest and test for Minutes 1, 2, 4, and 5, FT for the third minute of the test being even significantly lower than FT in the pretest [$t(14)=2.73$, $P<.02$]. Fluoxetine also reversed the increase in FT during test compared with the pretest. This was revealed by the lack of significant differences at any time point between pretest and test.

The effect of each antidepressant treatment was compared with vehicle separately using minute-by-minute t test analyses. This revealed a significant increase in FT in the vehicle compared with desipramine-treated rats for the first, second, and third minutes of the experiment [$t(14)=3.66$, $P<.01$; $t(14)=2.79$, $P<.02$; and $t(14)=2.88$, $P<.02$, respectively].

The same was true in comparison with fluoxetine-treated rats for the first 2 min [$t(14)=4.08$, $P<.01$ and $t(14)=2.61$, $P<.05$].

Altogether, these analyses confirmed that antidepressants can produce a reversal of increased FT during the test session compared with control animals, with the FT of treated rats approximating that observed during the pretest session.

3.3.2. Distance moved

An overall ANOVA on DM was performed with a between-subjects factor of treatment (fluoxetine or desipramine vs. saline) and repeated measurements factors of session (pretest vs. test) and time (1-min bins over the 5-min session). This analysis failed to yield a treatment effect [$F(2,21)=0.643$, $P=.536$]. However, the ANOVA revealed significant main effects of session [$F(2,21)=52.71$, $P<.0001$] and time [$F(4,84)=140.62$, $P<.0001$], and significant Treatment × Time [$F(8,84)=2.55$, $P<.05$] and Session × Time [$F(4,84)=12.38$, $P<.0001$] interactions. *t* test comparisons of DM between pretest and test were conducted for each treatment group separately. These analyses revealed that vehicle-treated rats moved less in test compared with pretest. This observation reached significance for Minute 1 [$t(14)=7.74$, $P<.001$] and Minute 2 [$t(14)=2.43$, $P<.05$]. During test session, rats treated with desipramine showed a significantly reduced DM compared with pretest at Minute 1 only [$t(14)=4.88$, $P<.001$]. However, DM of desipramine-treated rats was significantly higher at test than that of saline-treated rats for the first and second minutes [$t(14)=2.73$, $P<.02$ and $t(14)=2.27$, $P<.05$]. Finally, rats treated with fluoxetine exhibited a decreased DM during test compared with pretest for Minute 1 [$t(14)=4.55$, $P<.001$]. In addition, fluoxetine-treated rats showed an increased DM during Minute 1 of test compared with vehicle-treated rats [$t(14)=4.15$, $P<.001$].

Thus, DM of all rats during test was lower than that of pretest at least at the beginning of the session. In addition, both desipramine- and fluoxetine-treated rats increased DM at an earlier phase of the test session than their vehicle counterparts.

3.3.3. Relationship between FT and DM

The relationship between FT and DM was investigated by performing regression analyses for both pretest and test sessions for the total FT and the total DM measured over the entire session. In all cases FT was significantly and negatively correlated with DM for each of the drug treatments (Fig. 3C and D). The correlation coefficient between FT and DM for pretest was $r=-.57$ [$F(1,22)=10.42$, $P<.005$] and for test $r=-.72$ [$F(1,22)=23.51$, $P<.0001$].

3.4. Comparison between Wis and SD performance in the FST

A comparison between Wis and SD rats in the development of FT and DM was performed. Although rats used in this analysis were involved in different experiments (Experiment 1 vs. Experiment 3), and received different treatments (naïve rats vs. saline-injected rats), the otherwise comparable experimental conditions allowed a direct comparison between the two strains. FT and DM were compared between naïve rats in the 24-h delay condition of Experiment 1 (Wis) and saline-treated rats of Experiment 3 (SD).

3.4.1. Floating time

An overall ANOVA on FT was conducted with a between-subjects factor of strain (Wis vs. SD) and repeated measurements factors of session (pretest vs. saline) and time (1-min bins over the 5-min session). This analysis revealed main effects of strain [$F(1,14)=31.87$, $P<.0001$], session [$F(1,14)=26.29$, $P<.0005$], time [$F(4,56)=54.55$, $P<.0001$] and significant Time × Strain [$F(4,56)=7.47$, $P<.0001$] and Session × Time [$F(4,56)=15.942$, $P<.0001$] interactions. Separate *t* test comparisons of FT be-tween pretest and test revealed that Wis rats floated more than SD rats during both pretest [$t(14)=3.25$, $P<.01$] and test [$t(14)=3.39$, $P<.01$]. This difference for the pretest reached significance for Minutes 2, 3, 4, and 5 [$t(14)=4.7$, $P<.001$; $t(14)=4.9$, $P<.001$; $t(14)=3.95$, $P<.01$; and $t(14)=2.82$, $P<.05$, respectively], and for the test for Minutes 2, 3, 4, and 5 [$t(14)=5.06$, $P<.001$; $t(14)=5.0$, $P<.001$; $t(14)=3.77$, $P<.01$; and $t(14)=2.26$, $P<.05$, respectively].

Altogether, these results show that Wis rats have an overall tendency to float more than rats belonging to the SD strain.

3.4.2. Distance moved

A similar ANOVA performed for DM yielded significant main effects of strain [$F(1,14)=15.95$, $P<.005$], session [$F(1,14)=42.86$, $P<.0001$], and time [$F(4,56)=60.79$, $P<.0001$] and a significant Session × Time [$F(4,56)=26.40$, $P<.0001$] interaction. Separate *t* test comparisons of DM between pretest and test revealed that Wis rats moved significantly less than SD rats during both the pretest [$t(14)=4.62$, $P<.001$] and the test [$t(14)=2.91$, $P<.05$]. For the pretest this difference reached significance for Minutes 1, 2, 3, 4, and 5 [$t(14)=4.00$, $P<.01$; $t(14)=4.87$, $P<.001$; $t(14)=4.85$, $P<.001$; $t(14)=4.48$, $P<.001$; $t(14)=4.9$, $P<.0019$, respectively] and for the test for Minutes 2, 3, and 4 [$t(14)=4.15$, $P<.001$; $t(14)=3.99$, $P<.01$; $t(14)=2.86$, $P<.05$].

These results corroborate the results presented in the previous section showing that Wis rats float more than SD rats.

4. Discussion

The present study introduces a new automated method for the measurement of rat behavior in the FST. This measurement is based on the DM by the rat in the swim tank

calculated by the Noldus Ethovision software instead of the usual visual assessment of rat FT. The usefulness of our method was demonstrated in three experiments. In all these experiments, DM was negatively correlated with the established measure of FT. The first experiment showed that the increase in FT during the test session was smaller when pretest and test were 7 days apart compared with a 24-h delay. However, even in the extended delay condition, FT during test was still significantly reduced compared with pretest. The automated measurement of DM in the same animals during test showed that data for the 7-day delay and the 24-h delay were similar. Experiment 2 revealed that 2 days of withdrawal from the repeated, intermittent administration of either amphetamine or cocaine over a 5-day period did not increase FT or decrease DM compared with saline-treated animals. Finally, Experiment 3 demonstrated that both fluoxetine and desipramine increased DM and reduced FT at test.

While Wis rats were used in Experiments 1 and 2, the rats used in Experiment 3 were from the SD strain. The results of the present study revealed differences in the FST performance between the two strains, i.e., Wis float more (or move less) than SD rats. These results reproduce those of a recent study showing that Wistar–Kyoto (WKY) rats were more prone to stay immobile than SD rats (Lopez-Rubalcava and Lucki, 2000). These authors further demonstrated that desipramine reduced immobility both in WKY and SD rats while fluoxetine reduced immobility only in SD rats. Thus, despite the highest rate of immobility in WKY rats, which would have facilitated detection of a drug-induced antidepressant effect, the very fact that SD rats respond to both fluoxetine and desipramine make them a suitable strain to test the effects of these drugs. Furthermore, the vast majority of studies aimed at investigating the effects of known (e.g., Detke et al., 1995; Duncan et al., 1996; Page et al., 1999; Porsolt et al., 1979; Reneric and Lucki, 1998; West and Weiss, 1998; Wieland and Lucki, 1990), or putative antidepressants (e.g., Healy et al., 1999) or to model depression-like behavior (e.g., Alonso et al., 1991; Connor et al., 1998, 2000; Drugan et al., 1989; Nishimura et al., 1988; Tizabi et al., 1999; Zangen et al., 1999) have been hitherto conducted on SD rats. Thus, in the present report, the effects of desipramine and fluoxetine were investigated using SD rats. In contrast, previous sensitization studies to the locomotor stimulant effects of amphetamine (Hedou et al., 2001; H. Russig, C. Murphy, J. Feldon, unpublished observations) and cocaine (Heidbreder et al., 1995, 1996; Murphy et al., 2001) have been consistently conducted in our laboratory using Wis rats. These studies have shown reliably that the injection schedule used here induced behavioral sensitization to the locomotor effects of both cocaine and amphetamine. However, given the relatively low rate of immobility shown by SD rats in the naïve and saline-treated conditions, this rat strain would perhaps have been more appropriate to investigate the hypothesized depression-like effects induced by withdrawal from repeated administration of cocaine or amphetamine. Future studies investigating this issue should be conducted on rats from the SD strain.

The results of the present study support the contention that the automated measurement of DM can be readily employed to assess rat behavior in the FST. In addition, this method considerably shortens the duration of the analysis because of its simplicity and the possibility of using the same setup for testing several animals simultaneously. Furthermore, the present method does not require more than the standard material commonly used to assess rat activity, and above all, it reduces the subjectivity of the measure.

To the best of our knowledge, only two previous studies have investigated automated measurements of rat behavior in the FST for the profiling of drugs with potential antidepressant activity. Shimazoe et al. (1987) used ''minor-tremor pickup'' sensors surrounding the tank wall and connected to an amplifier and transducer to record water vibrations induced by the rat's movements. De Pablo et al. (1989) used a measurement of the rat's mobility by means of a sensory unit detecting the ''variations produced by rat's swimming activity on the standard frequency of the electromagnetic field of the sensory unit.'' Both methods require the use of a specific setup to record rat's behavior in FST. In addition, these methods do not allow reanalysis of the rat behavior at the end of the experiment. With our method, the Noldus Ethovision software permits the reanalysis of videotape recordings of the FST sessions, thus making the analysis more flexible. Moreover, the assessment of the antidepressant-like activity of any compound is dependent upon its influence on the general locomotor activity (LMA) of the animal (Dalvi and Lucki, 1999). For example, whereas amphetamine decreases FT in FST (Borsini et al., 1989; Shimazoe et al., 1987; West and Weiss, 1998; Wieland and Lucki, 1990), it also increases LMA in the open field. A correct interpretation requires a combination of both tests. It is therefore important to distinguish compounds inducing a false positive outcome in the FST by further monitoring their effect on LMA. The Noldus Ethovision software is commonly employed to monitor LMA of rodents in a confined area like an open field using the same track tracing method as described above. Using the same setup for monitoring the animal's behavior in both FST and open field LMA serves to reduce potential bias that could originate from the use of different apparatus for assessing different behaviors, simplifies the data analysis, and renders the preclinical profiling of new potential antidepressants more economical. In the second experiment of the present report we demonstrated that withdrawal from the repeated, intermittent administration of either amphetamine or cocaine did not produce decreased DM (or increased FT) during the test session. This negative outcome is unlikely to be due to the lack of sensitization of the rats to the behavioral-activating effects of cocaine and amphetamine. Indeed, these treatments have been successfully used in the past in our laboratory and have reliably led to behavioral sensitization to amphetamine (Hedou et al., 2001; H. Russig, C. Murphy,

J. Feldon, unpublished observations) and cocaine (Heidbreder et al., 1995, 1996; Murphy et al., 2001). Although these negative results might suggest that rats do not present a depression-like state during withdrawal, it opposes the general assumption that amphetamine withdrawal induces behavioral markers of depression-like states in the rat (Cassens et al., 1981; Kokkinidis et al., 1986; Paterson et al., 2000; Paulson et al., 1991; Pulvirenti and Koob, 1993; Wise and Munn, 1995). Extensive research of the literature in this field failed to identify any report using the same schedule of injection as that used here. In addition, the authors cited above used large doses of amphetamine (ranging from 1 to 12 mg/kg) over very long administration schedules (from 4 days up to 6 weeks). These treatments may induce severe behavioral, neurochemical, and anatomical adaptations leading to a more pronounced withdrawal syndrome, including measurable depression-like behavioral changes. In addition, among these authors, Kokkinidis et al. (1986) was the only one to use FST to reveal a depression-like effect of amphetamine withdrawal and this experiment was conducted in mice. The authors of the other studies mentioned above used mainly the intracranial self-stimulation paradigm (Cassens et al., 1981; Leith and Barrett, 1976; Paterson et al., 2000; Wise and Munn, 1995) and locomotor activity (Paterson et al., 2000; Paulson et al., 1991) in order to reveal anhedonia and locomotor retardation, respectively, both of which have been associated with withdrawal-induced depression-like states. These differences in methodology may account for the negative outcome of our study. However, other results from our laboratory (H. Russig, C. Murphy, J. Feldon, unpublished observations) have revealed the same negative results using both escalating (1 to 5 mg/kg, three times a day) and intermittent doses (1.5 mg/kg) of amphetamine over a 6-day injection period in both learned helplessness and FST paradigms. Again, in this case doses of amphetamine and duration of the treatment phase differed from the present study. Thus, the critical dosage and treatment duration above which a persistent and consistent withdrawal syndrome is detectable by the FST requires further investigation.

In the present study we have shown that increasing the delay from 24 h to 7 days between pretest and test in naïve animals reduced the magnitude of the FT increase at test. However, FT was significantly longer in the test than in the pretest session at both delays. This excludes the possibility of having obtained false negative results due to a ceiling effect of FT in the test session. The very fact that the 7-day delay decreased FT during test compared with the 24-h delay allowed the animals in withdrawal from psychostimulants to float more than the saline-treated counterparts, which would have indicated a ''depression-like state.'' This was actually not the case. In addition, no differences were seen between saline- and psychostimulant-treated rats with either the visual or the automated methods. This makes it reasonable to conclude that the treatment regimen used in the present study did not produce a depression-like state in the FST and/or that FST was not sensitive enough to reveal it.

The results of the last experiment indicated that both fluoxetine and desipramine, when injected three times between pretest and test, induced increased DM and decreased FT during the test session. This is in line with previous studies indicating that treatment with antidepressants decreased immobility time in the FST (Connor et al., 2000; Page et al., 1999; Plaznik and Kostowski, 1987; Porsolt et al., 1977a,b, 1978a; Sanchez and Meier, 1997). False positive results due to an increased locomotor activity by either fluoxetine or desipramine administration have been excluded in previous studies (Detke et al., 1995; Redrobe and Bourin, 1998; Wieland and Lucki, 1990), showing rather a decreased locomotor activity in fluoxetine- or desipramine-treated rats relative to control rats. Furthermore, Armario et al. (1988) have suggested that struggling, the behavior that accounts for a large part of the DM by rats in the FST, seems a ''less subjective and more reliable measure of antidepressant action than does immobility in the forced swimming test.''

Taken together, the results of the present study suggest that the use of the Noldus Ethovision software is a reliable, accurate, and rapid method for the measurement of rat behavior in the FST. However, this method is subject to the same limitations as the visual method, i.e., the possibility of obtaining false positive results in the FST and the necessity of using complementary paradigms to further confirm the antidepressant profile of a drug. This method may not only allow the testing of more antidepressant drugs in larger groups but also reduce the duration of time-consuming preclinical studies. Future refinements of this method will focus on the differentiation of behavioral categories differentially influenced by either serotonin or norepinephrine re-uptake inhibitors (Detke et al., 1995).

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