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Effects of Antidepressant Drugs on Rats Bred for Low Activity in the Swim Test

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WEST, C. H. K. AND J. M. WEISS. *Effects of antidepressant drugs on animals bred for low activity in the swim test.* PHARMACOL BIOCHEM BEHAV **61**(1) 67–79, 1998.—To determine responsivity to antidepressant medication of Sprague–Dawley rats bred for low activity in the swim test [Swim Low-Active (SwLo) rats], these animals were given different antidepressant drugs via subcutaneously implanted minipumps for 1, 12, or 26 days, and then were tested for activity in the swim test and 2 days later in the open field. Antidepressant drugs given were amitriptyline, imipramine, desipramine (tricyclics), phenelzine (monoamine oxidase inhibitor (MAOI)], fluoxetine [selective serotonin reuptake inhibitor (SSRI)], venlafaxine, and bupropion (atypical). To assess specificity of response, the nonantidepressant drugs amphetamine, caffeine, and haloperidol were also tested. For comparison, several drugs were also tested in rats bred for high activity in the swim test [Swim High-Active (SwHi) rats]. When administered for 14 and/or 28 days (but not for 1 day), imipramine, desipramine, venlafaxine, phenelzine, and bupropion significantly increased struggling behavior of SwLo rats in swim test. No nonantidepressant drug significantly elevated struggling activity. Long-term administration of phenelzine and bupropion also significantly decreased floating behavior in the swim test, although amphetamine also had this effect at all times of administration. No significant effects of antidepressants were seen in SwHi rats. Amitriptyline and fluoxetine were ineffective in altering either struggling or floating in SwLo rats; however, a high dose of an SSRI (sertraline) did reduce floating, but this type of effect is probably not indicative of antidepressant action. Behavior in the open field was not consistently affected by any drug type. It is concluded that, based on pharmacological response profile in the swim test, SwLo rats represent depression that is responsive to potent norepinephrine reuptake-blocking antidepressants and also MAOIs; atypical depression may fit this profile.

Animal model Rat model Depression Atypical depression Drug Antidepressant Swim test Open field

THE previous article described the selective breeding of animals for high vs. low activity in a swim test, showing that lines of high-active (SwHi) and low-active (SwLo) rats were generated by this procedure (27). By the 18th generation, SwHi and SwLo rats showed no overlap in either struggling or floating activity in a 15-min test in a swim tank. An important impetus for the development of these animals was the possibility that the SwLo rat might be a better test subject for screening of antidepressant drugs in the swim test than is the randomly bred Sprague–Dawley rat. The previous article presented results showing that two different types of antidepressants—the tricyclic antidepressant desipramine (DMI), and the monoamine oxidase inhibitor phenelzine (PHE)—both increased swim-test activity of SwLo rats as would be expected of drugs having antidepressant action. The activity-increasing effect of DMI occurred when SwLo animals took the drug orally or had it delivered continuously by a subcutaneously implanted osmotic minipump. The magnitude of positive response to DMI delivered by minipump was related to the dose given. Thus, initial results were consistent with the possibility that SwLo rats might constitute a useful subject for assessing the effectiveness of antidepressant drugs.

However, the results presented in the initial article are insufficient to indicate that using SwLo rats has any advantage over randomly bred subjects. To make an appropriate assessment of the potential usefulness of SwLo rats, a more extensive screening of drugs is required. The present experiment reports testing of a wide range of antidepressants, expanding the number of drugs previously tested to include 1) other tricyclics in addition to DMI, 2) a selective serotonin reuptake inhibitor (SSRI), 3) a mixed norepinephrine and serotonin uptake inhibitor (venlafaxine), and 4) an antidepressant that

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potently blocks dopamine reuptake (bupropion). In addition, the present experiment tested a phenothiazene (haloperidol) as well as drugs that are not antidepressants but have activating effects and consequently produce "false positives" in the standard Porsolt swim test (amphetamine, caffeine) [see (17)]. In addition to behavior in the swim test, activity in the open field was also examined. Finally, the effects of several drugs were tested on the SwHi rats to permit comparison to effects seen in the SwLo subjects.

METHOD

Animals

Male albino Sprague–Dawley rats from the 16th and 17th generations (S16 and S17) of the Swim Low-Active (SwLo) and Swim High-Active (SwHi) lines were used. Derivation of these lines has been described previously (27). The animals were maintained in laminar-flow enclosed racks also described previously, and were group housed in cages containing two to four rats of the same line. Animals received standard laboratory chow and water ad lib. The colony was maintained on a 12 h L:12 h D cycle, the lights coming on at 0700 and going off at 1900 h. Subjects were 3–4 months old when experimental procedures were begun.

Drugs

Drug was delivered to each animal from an Alzet osmotic minipump (Alza Corp.) model 2ML2 (total capacity 2.0 ml, delivery rate 5.0 μl/h) that was implanted subcutaneously; the 2ML2 pump is designed to deliver fluid for 15–17 days. As noted previously (27), osmotic minipumps were used for drug delivery to avoid repeated handling and injection of the animals. Daily handling and injection of rats to deliver drug has been shown to cause elevated release of norepinephrine in the brain as measured by microdialysis (E. Abercrombie, unpublished data; personal communication) and increased growth of stress-sensitive tumors (8). In that repeated handling and injection of rats for drug delivery appears to be stressful in and of itself, delivery of drug without repeated handling and injection would seem to allow more unambiguous assessment of the effects of the drugs. All drugs except SSRIs were dissolved in sterile distilled water immediately before loading into pumps. Due to low solubility in water, fluoxetine (main study) and sertraline (use described in Discussion) was dissolved in a vehicle of 75% polyethylene glycol (PEG, m.w. 400) and 25% water. Although preliminary studies indicated that swim-test activity did not differ in animals having minipumps that delivered water vs. the PEG/water vehicle, an equal-sized group was given PEG/water vehicle in addition to the group that received the distilled water vehicle. The seven antidepressant drugs and doses were: amitriptyline HCl, 10 mg/kg/day (tricyclic); desipramine HCl, 10 mg/kg/day (tricyclic); imipramine HCl, 10 mg/kg/day (tricyclic); bupropion HCl, 20 mg/kg/day (atypical); venlafaxine HCl, 20 mg/kg/day (atypical); fluoxetine HCl, 10 mg/kg/day (SSRI); and phenelzine sulfate, 5 mg/kg/day (monoamine oxidase inhibitor, MAOI). The three nonantidepressant drugs were: *d*-amphetamine sulfate, 2 mg/kg/day free base (stimulant); caffeine, 20 mg/kg/day (stimulant); and haloperidol, 0.5 mg/kg/day (phenothiaxene antipsychotic). All drugs were purchased from Sigma (St. Louis, MO), with the exception of the following drugs that were generously provided by the indicated pharmaceutical companies: fluoxetine (Lilly, Indianapolis, IN), venlafaxine (Wyeth-Ayerst, Princeton, NJ), bupropion (Burroughs Wellcome, Research Triangle Park, NC) and sertraline (Pfizer, Groton, CT). The doses used were initially selected on the basis of previous reports of effective doses that had been given by bolus (IP injection) administration [i.e., (16,21)], employing the amount that had been given previously (in mg/kg) and administering that amount of drug over the period of a day via minipump (i.e., mg/kg/day). These doses have now been shown to be behaviorally effective in other studies that have assessed antidepressant activity of these drugs (28). The numbers of animals that were employed in each group are shown in Table 1.

Osmotic Pump Surgical Implantation

All surgical procedures were conducted under aseptic conditions and in accord with NIH Guidelines and U.S. Department of Agriculture regulations (Laboratory Animal Welfare Acts, P.L. 89-544, as amended P.L. 94-279 and 99-198). Animals were anesthetized with inhalation of halothane (3%). The lower back area was shaved and washed with surgical disinfectant, a 2–3 cm incision made in this region, the minipump containing the desired drug inserted through the incision into a subcutaneous pocket, and the wound then closed. This surgical procedure normally was completed in 5–10 min. At the conclusion of surgery, animals were removed from the anes-

TABLE 1

NUMBER OF RATS THAT RECEIVED DIFFERENT DRUGS FOR EACH OF THREE DURATIONS OF DRUG ADMINISTRATION (I.E., 1, 12, OR 26 DAYS) BEFORE BEING GIVEN THE SWIM TEST

	Day*	VEH (dist H_2O)	VEH (peg+H_2O)	AMI	IMI	DMI	VEN	FLU	PHE	BUP	CAF	AMP	HAL
SwLo	1	6	6	6	6	6	6	6	6	6	6	6	6
	12	8	8	8	8	8	8	13	6	6	8	8	8
	26	8	8	8	8	8	8	8	6	6	14	8	10
SwHi	1												6
	12	6	6	6		6		6				6	10
	26	9	4	6		6		6				9	8

Drugs administered were: vehicle [distilled water or distilled water and polyethylene glycol] (VEH), amitriptyline (AMI), imipramine (IMI), desipramine (DMI), venlafaxine (VEN), fluoxetine (FLU), phenelzine (PHE), bupropion (BUP), caffeine (CAF), amphetamine (AMP), and haloperidol (HAL).

*Swim test given after this number of days of drug administration. Open-field test was carried out on the same rats 2 days after the swim test.

thesia and returned to their colony cage after they had regained consciousness. For all animals that were given drug for 26 days prior to the swim test, the original minipump was removed after 14 days and replaced by a new minipump. In this case, the surgical procedure described above was repeated 14 days after the first procedure, the original minipump removed, and a new minipump containing the same drug inserted subcutaneously; for this procedure, care was taken to place the new minipump in a different subcutaneous location from that of the first pump, as pilot studies indicated that drug perfusion was improved if the second minipump was not placed in the same subcutaneous pocket as the first had occupied. Except for the surgical procedure to implant the minipump, animals remained undisturbed in the home case until testing.

Swim Test

After animals had received drug or vehicle for either 1, 12, or 26 days, they were given the swim test. The swim test was carried out as described in the preceding article (27). Briefly, each animal was taken to the testing room, a lightweight plastic flotation bubble was taped onto its back, and the animal was then dropped into the tank, which was a Plexiglas cylinder 62 cm high by 30 cm in diameter filled with water at 25°C to a depth of 48 cm. During the test, which lasted 15 min, an observer timed the duration of the following behaviors: struggling, which was defined as all four limbs in vigorous motion with the forelimbs breaking the surface of the water; and floating, which was defined as all four limbs being motionless in the water together with the absence of head movement. Also, number of dives made was counted, a "half-dive" being scored whenever the animal placed its head in a downward direction beneath the surface of the water, and a "full dive" scored whenever the animal's head was sufficiently submerged that it was below the rest of its body. At the conclusion of the swim test, the animal was removed from the tank, dried by a towel, and returned to its home cage. All swim tests were conducted between 0800 and 1300 h.

Open-Field Test

Two days after animals were given the swim test, they were tested in the open field. Details of open-field procedure are given in the preceding article (27). Briefly, the open-field apparatus was a large open enclosure measuring 107 × 107 cm (40 × 40 in.) with walls 50 cm (19 in.) high; the floor and inside walls were painted white. The floor area was divided into 25 equal-sized squares [5 × 5, each square 20 × 20 cm (8 × 8 in.)] by black lines painted on the floor. Testing was conducted in a darkened, quiet room lit by a 25-watt light bulb suspended 90 cm above the center of the open field. Each rat was gently placed onto the middle square of the open-field apparatus facing away from an observer who sat on a stool positioned above one corner of the apparatus to be able to view the entire enclosure, and the observer then traced the animal's movements in the field. The duration of the test was 10 min. Ambulation was subsequently quantified by counting the number of squares that the rat entered (outer squares and inner squares); rearing responses (wall rears and free rears) were also counted. At the end of the session, the presence or absence of urine on the floor was noted and the number of fecal boli was counted. After each animal was tested, the enclosure was cleaned with a solution of 5% acetic acid to eliminate odors for the next test. All open field testing was conducted between 0900–1200 h.

Statistics

Each measure was analyzed by one-way analyses of variance (ANOVA) that were conducted separately on the results obtained at each time of drug administration (i.e., day 1, day 12, or day 26 in the swim test, and day 3, day 14, or day 28 in the open-field test). The type of drug received was the group factor in these analyses. When a statistically significant (p at least < 0.05) main effect attributable to group (i.e., drug type) was found in an analysis, comparison of each individual drug-treated group with the vehicle-treated group was then made using Dunnett's test. Significant differences reported in the text and shown in the figures are based on the results of the Dunnett's tests (one tailed). For measures that were compared by contrasting the percentage of animals in a group that showed a response (i.e., diving in the swim test), the effect of each drug was compared with the vehicle-treated condition by a Fisher's Exact Test.

RESULTS

SwLo Rats

Vehicle groups. As explained above, the vehicle condition consisted of equal-sized groups that received either distilled water or the PEG/water vehicle. To assess whether these different vehicles might have had an effect on any of the behaviors examined in either the swim test or the open-field test, the results for each behavioral measures were subjected to the following two-way analysis of variance: type of vehicle (water vs. PEG/water) × day of testing (day 1, 12, and 26 for swim-test measures, or 3, 14, and 28 for open field measures). None of the six ANOVAs that were carried out yielded a significant main effect of vehicle type, although in some cases a significant interaction was found. Because significant interactions could arise because small but consistent differences resulted from testing of particular animals on a given day, but any overall difference between the vehicles then dissipated when combined with results from testing on other days as evidenced by the lack of any significant main effect in any analysis, the results from water and PEG/water vehicle-treated animals were therefore combined on each day to simplify data presentation and analysis.

Swim test. Figures 1 and 2 show the results obtained from testing SwLo rats in the swim test. Regarding vehicle-treated animals (data shown at far left in the figures), these animals showed the usual pattern of activity shown by SwLo rats—considerable time spent floating (i.e., approximately 700 of the 900 s of the test were spent floating) and very little struggling behavior. Also, it can be seen in the figures that administration of vehicle via minipump for different periods of time (i.e., 1, 12, or 26 days) did not affect swim-test behavior of the SwLo rats.

Effects on struggling behavior. Several of the antidepressant drugs elevated struggling behavior of the SwLo rats (shown in top part of the figures). Increased struggling was produced by imipramine (IMI), desipramine (DMI), venlafaxine (VEN), phenelzine (PHE), and bupropion (BUP) in comparison to the vehicle-treated animals. The groups that showed such effects all received drug for either 12 or 26 days; the analysis of struggling activity seen after 1 day of drug administration failed to show a significant main effect attributable to drug, thus indicating that the drugs were not effective when given for 1 day. None of the nonantidepressant drugs [caffeine (CAF)], amphetamine (AMP), or haloperidol (HAL)] significantly affected struggling behavior of SwLo animals, al-

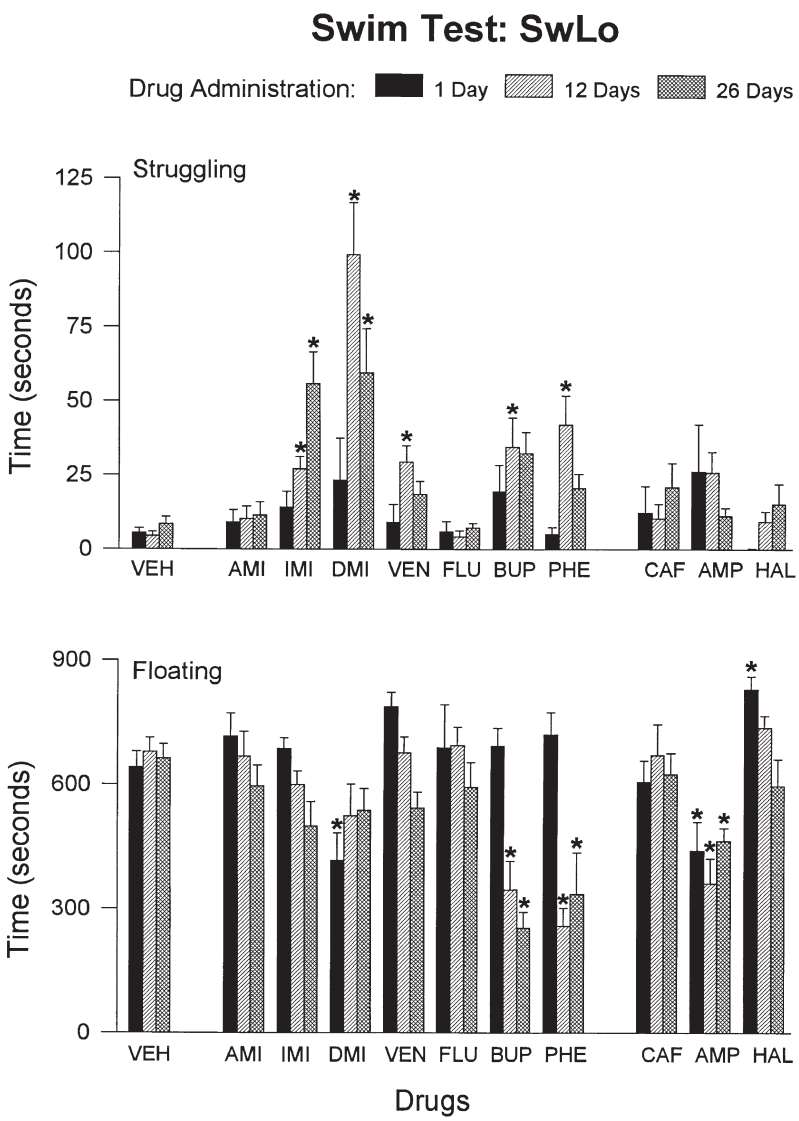
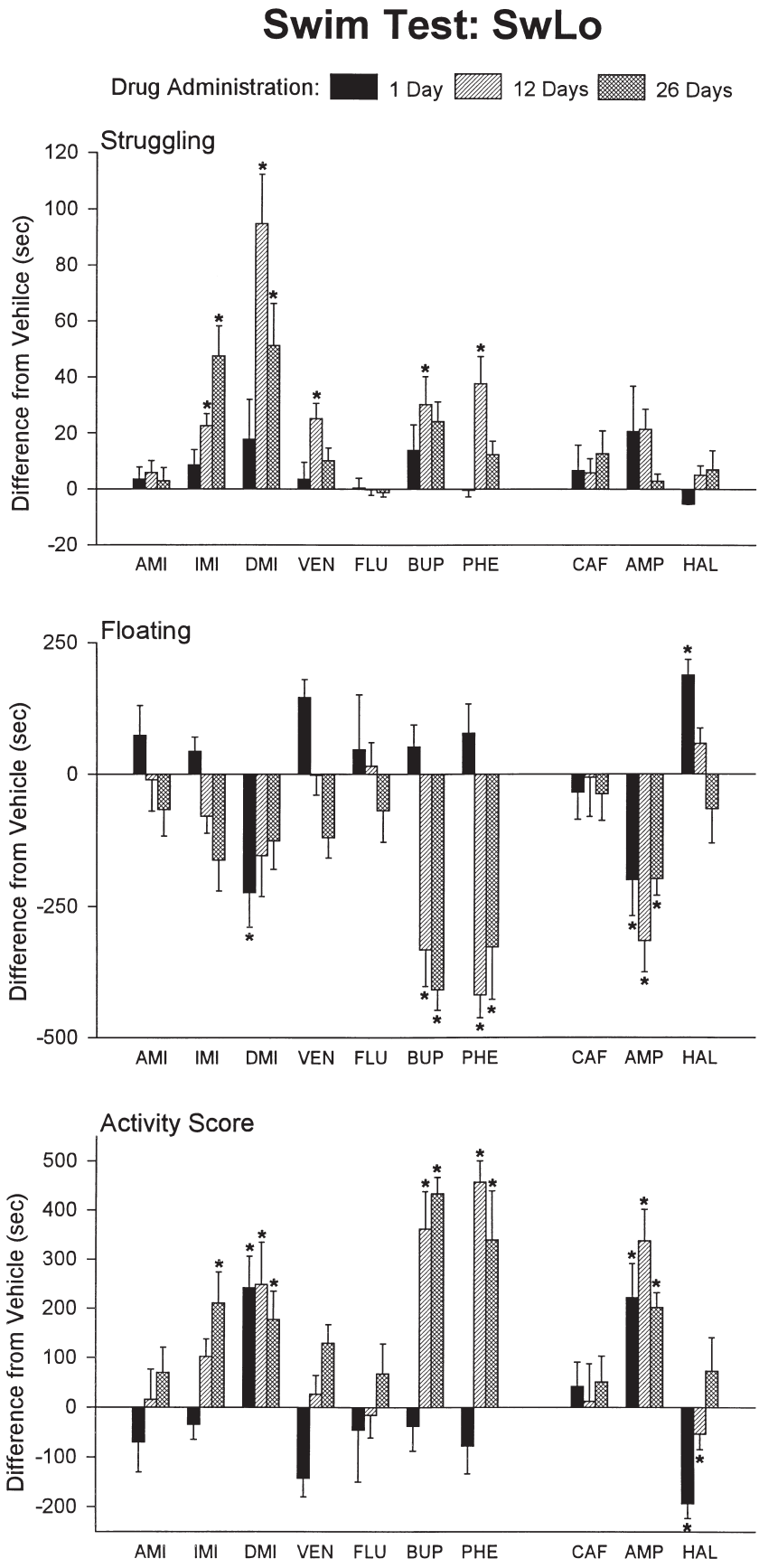


FIG. 1. Swim-test activity of male SwLo rats that received different antidepressant and nonantidepressant drugs. Drugs were administered via subcutaneous minipump, and testing took place either 1, 12, or 26 days after onset of drug administration. Animals received either vehicle (VEH), or the antidepressant drug amitriptyline (AMI; 10 mg/kg/day), imipramine (IMI; 10 mg/kg/day), desipramine (DMI; 10 mg/kg/day), venlafaxine (VEN; 20 mg/kg/day), fluoxetine (FLU; 10 mg/kg/day), phenelzine (PHE; 5 mg/kg/day), or bupropion (BUP; 20 mg/kg/day). Nonantidepressant drugs given were caffeine (CAF; 20 mg/kg/day), amphetamine (AMP; 2 mg/kg/day), or haloperidol (HAL; 0.5 mg/kg/day). At top is shown the mean (and standard error) time spent struggling (seconds) and at bottom is shown the mean (and standard error) time spent floating (seconds) in a 15-min swim test. *Significantly different (at least $p < 0.05$) from vehicle-treated animals tested on the same day after drug administration commenced.

FIG. 2. Swim-test activity of SwLo rats that received various antidepressant and nonantidepressant drugs, shown as the difference (in seconds) from vehicle-treated animals that were tested on the same day after drug administration commenced. Differences from vehicle-treated animals are shown for struggling (at top), floating (at middle), and activity score (i.e., floating time minus struggling time) (at bottom). All groups and drug doses are given in legend for Fig. 1. Means (and standard errors) are shown. *Differs significantly (at least $p < 0.05$) from vehicle-treated animals tested on the same day after drug administration commenced.


Swim Test: SwLo
Drug Administration: 1 Day 12 Days 26 Days
Struggling
Difference from Vehilce (sec)
120
100
80
60
40
20
0
-20
AMI IMI DMI VEN FLU BUP PHE CAF AMP HAL
Floating
Difference from Vehicle (sec)
250
0
-250
-500
AMI IMI DMI VEN FLU BUP PHE CAF AMP HAL
Activity Score
Difference from Vehicle (sec)
500
400
300
200
100
0
-100
-200
AMI IMI DMI VEN FLU BUP PHE CAF AMP HAL

though acute (1-day) AMP produced the largest change seen from these drugs.

Effects on floating behavior. Some of the antidepressant drugs also affected floating behavior (shown below struggling in the figures). The most marked effects were produced by prolonged treatment (i.e., 12 or 26 days) with PHE and BUP; these drugs decreased floating behavior considerably. DMI also decreased floating significantly when administered for 1 day. Regarding the nonantidepressant drugs, AMP decreased floating at all durations of administration. Also, despite the large amount of floating that SwLo animals normally show, acute (i.e., 1 day) HAL increased floating still further.

Effects on activity score. Activity score (i.e., time spent struggling minus time spent floating; shown in bottom part of fig. 2) was increased by several of the antidepressant drugs as would be expected from the results described in the two sections directly above. Long-term administration (i.e., 12 or 26 days) of IMI, DMI, VEN, PHE, and BUP produced significant increases in this score. The only antidepressant drug that showed an effect of 1 day of administration was DMI; this resulted primarily from decreased floating described above. Regarding nonantidepressant drugs, AMP increased activity score at all three times of administration while HAL decreased activity score when administered for 1 day, changes that again were largely due to effects of these drugs on floating behavior.

Effects of diving. As reported previously (27), SwLo rats rarely dive during the swim test. Consistent with this, vehicle-treated SwLo rats showed virtually no diving; of the total of 44 vehicle-treated animals tested in this study, only two animals, or 4.5% of the group, showed any diving at all. Four drugs significantly increased the likelihood of diving by SwLo rats; these drugs were DMI, IMI, BUP, and PHE. The percentage of animals in these groups that showed diving (12 and 26 day drug administration combined) were, respectively, 43.7, 31.2, 50.0, and 41.6%.

Open-field test. Figure 3 shows the findings for the two major categories of behavior quantified in the open field—number of squares entered (ambulation) and number of rearing responses. Although both inner and outer squares entered were counted separately (and also analyzed separately) as were different types of rearing (i.e., wall rears and free rears), these differentiations did not show different effects, and so the categories are combined for presentation here (i.e., total squares entered and total rears).

Total squares entered. Although the ANOVAs carried out for each of the 3 days of testing (i.e., day 3, 14, and 28) yielded a significant overall effect of group for day 3 and day 28 (no overall effect attributable to group was found on day 14), few of the comparisons of individual drug-treated groups with the vehicle-treated animals yielded a significant difference on either day 3 or day 28. HAL significantly decreased the number of squares entered on day 3 and day 28, but no other comparison with vehicle-treated animals reached significance. Inspection of the results (see Fig. 3) reveals that DMI tended to increase the number of squares entered when administered for 14 and 28 days, and BUP, AMP, and CAF also tended to have a similar effect at 3 and 14 days, but none of these effects reached statistical significance; neither did the decreases in squares entered produced by FLU, AMI, VEN, and BUP when administered for 28 days or HAL when administered for all durations.

Total rearing. The most marked effect on number of rearing responses was that this behavior was decreased by HAL; the effect was significant on day 3 and 28. FLU also decreased

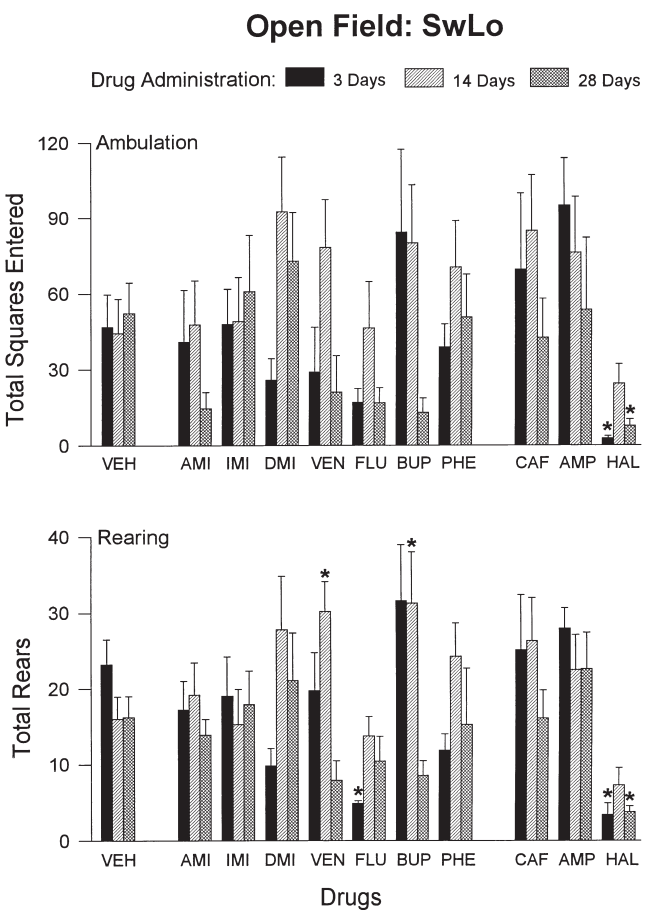


FIG. 3. Open-field ambulatory activity and rearing behavior of SwLo rats that received different antidepressant and nonantidepressant drugs. All details are given in legend to Fig. 1. Animals were tested 2 days after the swim test.

rearing on day 3. In contrast, BUP increased rearing, which was significant on day 14 of administration. VEN also increased rearing when administered for 14 days.

Defecation and urination. Amount of defecation in the open field was not affected by any of the drugs administered. Vehicle-treated animals produced a mean of 3.7 boluses (all durations of administration combined), and similar amounts of defecation were seen in each drug group. For urination, the percentage of animals that urinated in the open field was 69% in the vehicle-treated condition. The only difference for this measure was that fewer fluoxetine-treated rats urinated (44%), a difference that approached, but did not reach, statistical significance ($p < 0.06$).

SwHi Rats

Vehicle groups. As for SwLo rats, separate equal-sized groups of animals received distilled water or the PEG/water vehicle prior to testing. Two-way ANOVAs [i.e., vehicle type (distilled water and PEG/water) × duration of administration (12 and 26 days for measures in the swim test, and 14 and 30 days for measures in the open field)] were conducted for all behavioral measures examined. Of the six ANOVAs carried out, none showed a significant main effect or a significant interac-

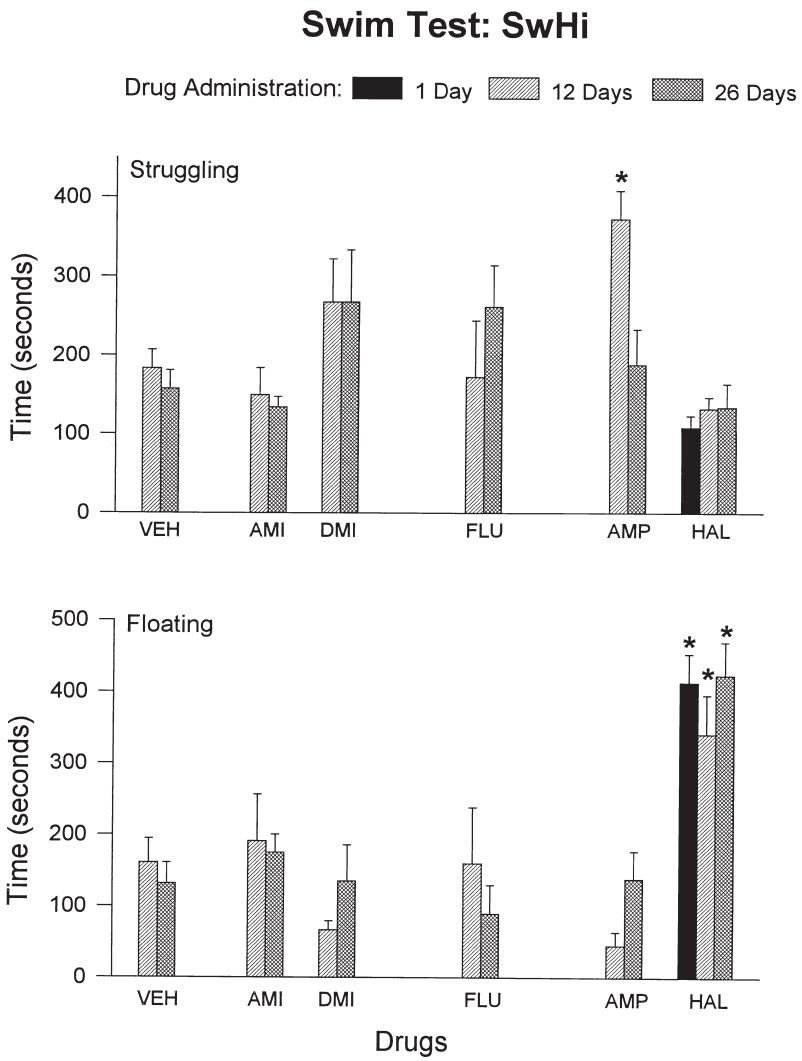


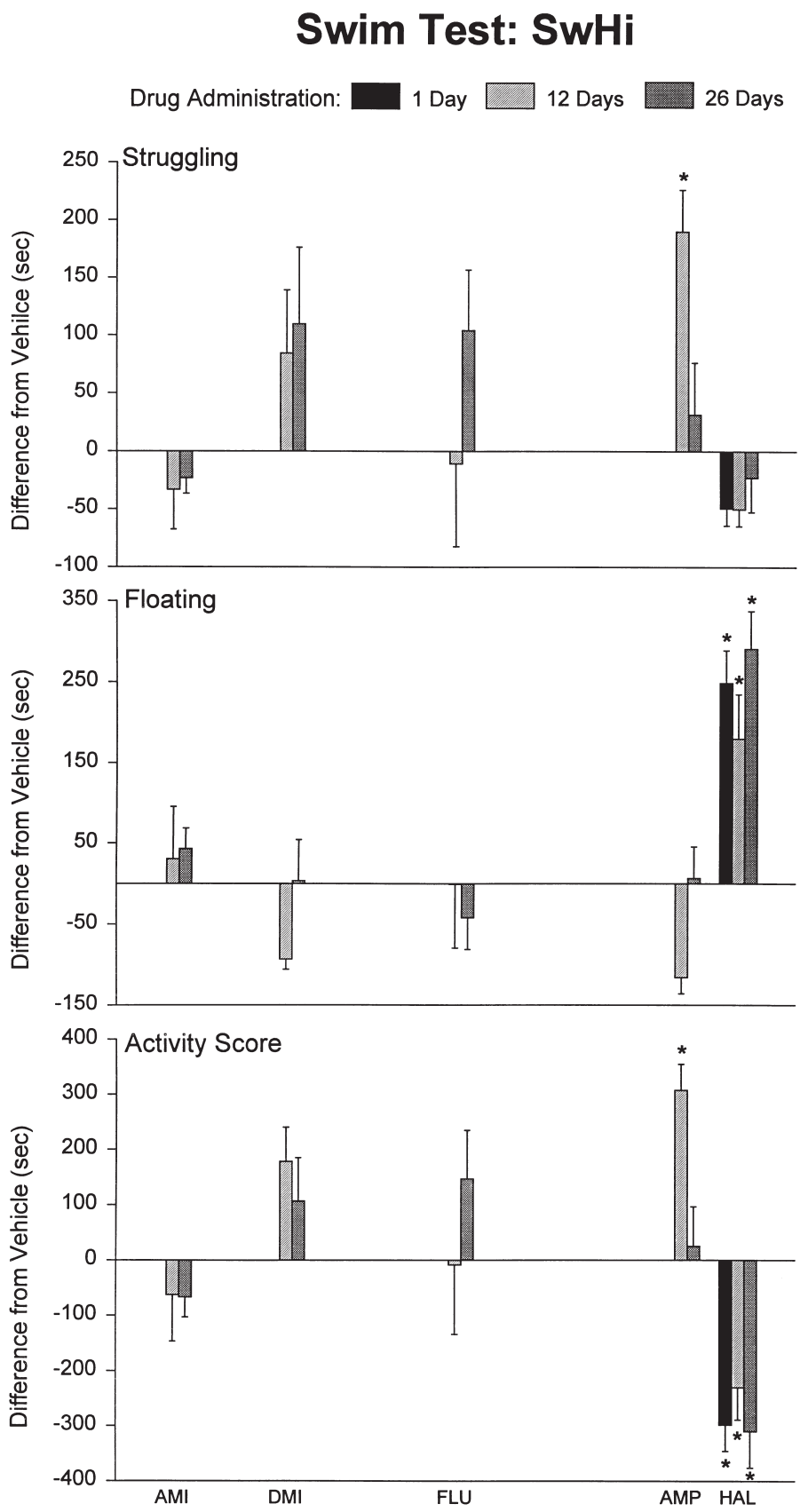
FIG. 4. Swim-test activity of male SwHi rats that received different antidepressant and nonantidepressant drugs. Drugs were administered via subcutaneous minipump and testing took place 1, 12, or 26 days after onset of drug administration. Animals received either vehicle (VEH), or the antidepressant drug amitriptyline (AMI; 10 mg/kg/day), desipramine (DMI; 10 mg/kg/day), or fluoxetine (FLU; 10 mg/kg/day). Nonantidepressant drugs given were amphetamine (AMP; 2 mg/kg/day) or haloperidol (HAL; 0.5 mg/kg/day). At top is shown the mean (and standard error) time spent struggling (seconds) and at bottom is shown the mean (and standard error) time spent floating (seconds) in a 15-min swim test. *Significantly different (at least $p < 0.05$) from vehicle-treated animals tested on the same day after drug administration commenced.

tion. Therefore, the results from all animals that received vehicle for a particular duration of administration were combined to simplify presentation and analysis of results.

Swim test. Figures 4 and 5 show the results obtained from testing SwHi rats in the swim test. Vehicle-treated SwHi rats showed, as expected, large amounts of struggling behavior (mean = 165.0 s) and lesser amounts of floating behavior (mean = 130.7 s). As was the case for SwLo rats, different durations of vehicle administration (i.e., 12 or 26 days) did not affect performance of vehicle-treated animals.

Effects on struggling behavior. The only group of SwHi rats that differed significantly from vehicle-treated animals was the group that had received AMP for 12 days. Although DMI given for 12 and 26 days and FLU given for 26 days somewhat elevated struggling behavior, differences between these groups and vehicle-treated animals did not reach significance.

Effects on floating behavior. HAL markedly increased floating of SwHi rats at all times of administration (HAL was also tested after being given for 1 day; the mean for these animals differed from that of 12 and 26 day vehicle-treated animals by *t*-test, $p < 0.001$). Although DMI and AMP tended to decrease floating when given to these animals for 12 days, none of the antidepressants tested produced significant differences from vehicle-treated SwHi animals.


Swim Test: SwHi
Drug Administration: 1 Day 12 Days 26 Days
Struggling
Difference from Vehilce (sec)
250
200
150
100
50
0
-50
-100
Floating
Difference from Vehicle (sec)
350
250
150
50
-50
-150
Activity Score
Difference from Vehicle (sec)
400
300
200
100
0
-100
-200
-300
-400
AMI
DMI
FLU
AMP
HAL

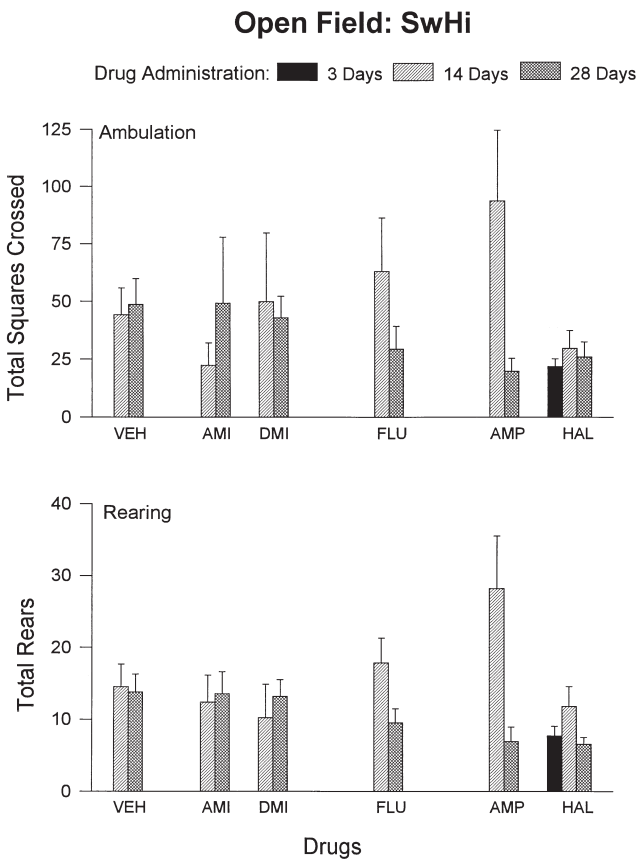


FIG. 6. Open-field ambulatory activity and rearing behavior of SwHi rats that received different antidepressant and nonantidepressant drugs. All details are given in legend to Fig. 1. Animals were tested 2 days after the swim test.

Effects on activity score. Changes in activity score (see Fig. 5) were commensurate with the effects described in the two categories directly above—activity score was significantly increased in SwHi animals that received AMP for 12 days (but was not elevated after treatment with this drug for 26 days) and was significantly decreased in all HAL-treated groups (due to large elevations in floating behavior).

Effects on diving. SwHi rats normally show some diving in the swim test; 34.7% of vehicle-treated SwHi rats were observed to dive during the test. None of the antidepressant drugs caused any notable change in the percentage of SwHi rats that dove. The only statistically significant effect for this measure was that HAL decreased diving; none of the animals given HAL showed any diving. Also, although AMP did not change the percentage of SwHi rats that engaged in diving, a few of the animals receiving this drug that dove showed very large amounts of this behavior.

Open field. Figure 6 shows the findings for the major categories of behavior quantified in the open-field test (total squares entered and total number of rearing responses). For both measures, the ANOVAs failed to yield a significant overall effect of group for each duration of drug administration (14 or 28 days); thus, for SwHi rats no significant effects of the drugs were found on the behaviors measured in the open field. Defecation was increased in SwHi rats given AMI and DMI, which defecated significantly more [mean = 8.2 and 6.8 boluses, respectively (both durations of drug administration combined)] than did vehicle-treated SwHi rats (mean = 3.5 boluses). No differences were observed in the percentage of animals that urinated in the open field.

DISCUSSION

Responses of SwLo Rats to Antidepressants—Selective Detection by Assessing Struggling Behavior But Complications Caused by Sedation

The present study examined the influence of several antidepressant as well as nonantidepressant drugs on swim test and open-field activity of SwLo rats. The focus of this investigation was on the swim test, and consistent effects were seen here. When SwLo rats were given antidepressant drugs (via subcutaneous minipump) for at least 12 days (i.e., 12 or 26 days), several of the drugs increased their struggling behavior. In comparison to the normally minimal amount of struggling that SwLo rats show in the swim test (see vehicle-treated SwLo animals), the following drugs significantly elevated struggling after prolonged administration: IMI, DMI, VEN, PHE, and BUP. With respect to floating behavior of SwLo rats, which is the dominant response of these rats, PHE and BUP also markedly and significantly reduced floating when administered for either 12 or 26 days. Thus, the results showed that behavior of the SwLo rats in the swim test responds to the long-term administration of several antidepressants, as was reported in the preceding study (27).

With respect to this preceding study, comment should be made about the finding that PHE elevated struggling in the present study but did not do so in the earlier test with this drug (27). There are three possible explanations for this difference. First, the later generations of SwLo rats used in the study reported here may have been more responsive to PHE. Second, minipumps lasting 2 weeks were used in the present study vs. pumps that lasted 1 week in the previous study, thereby reducing replacement surgeries and resultant stress in the present study relative to the earlier one. Third, and perhaps most likely, a lower dose of PHE was employed in the present study (5 mg/kg/day) to attempt to reduce the marked emotionality and reactivity that is observed in animals that receive this drug, and this may have facilitated the emergence of struggling behavior by reducing adverse reactions of the animals to being handled when they were introduced into the swim test.

Two issues are of relevance in evaluating the response of SwLo rats to antidepressants: 1) specificity (or selectivity) of response to antidepressants, and 2) capability for detecting different antidepressants. Regarding specificity, this was particularly good with respect to effects on struggling behavior.

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FIG. 5. Swim-test activity of SwHi rats that received various antidepressant and nonantidepressant drugs, shown as the difference (in seconds) from vehicle-treated animals that were tested on the same day after drug administration commenced. Differences from vehicle-treated animals are shown for struggling (at top), floating (at middle), and activity score (at bottom). All groups and drug doses are given in legend for Fig. 4. Means (and standard errors) are shown. *Differs significantly (at least $p < 0.05$) from vehicle-treated animals tested on the same day after drug administration commenced.

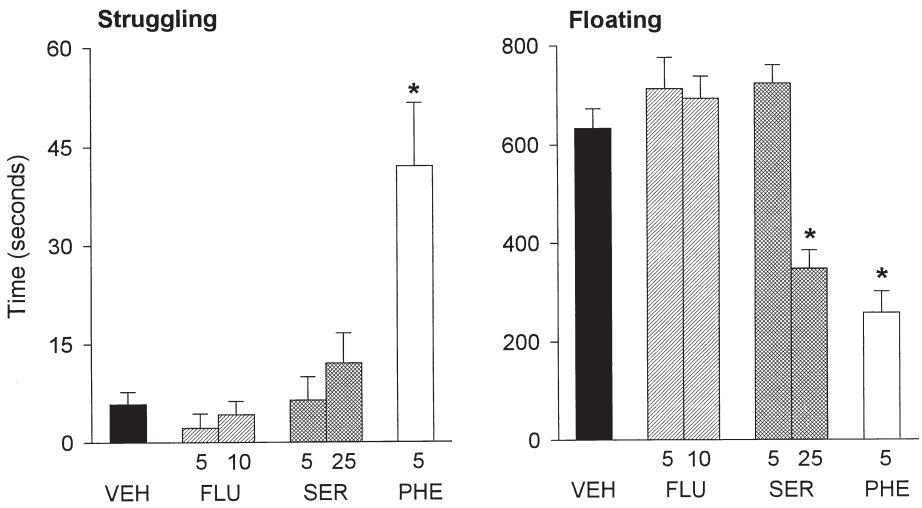

FIG. 7. Effect of additional testing of SSRIs on activity of SwLo rats in the swim test. In addition to FLU at 10 mg/kg/day (results in main study), a lower dose of FLU and also sertraline (SER) was tested (5 mg/kg/day; $n = 5$ per group) as well as a higher dose of SER (25 mg/kg/day; $n = 7$); numbers shown below columns indicate dose (mg/kg/day). Results for all groups are from swim test after 12-day drug administration via subcutaneous minipump; SER (25 mg/kg/day) required implantation of two pumps to deliver this dose. In addition to SSRIs, results of 12-day administration of vehicle (VEH) and phenelzine (PHE) from the main experiment are reproduced for comparison. Means and standard errors are shown. * = Differs significantly (at least $p < 0.05$ by t-test) from vehicle-treated animals. Note that SER (25 mg/kg/day) only affects floating behavior whereas PHE has similar effect on floating but also increases struggling.

As indicated above, several antidepressants increased struggling activity of the SwLo rats. In contrast, none of the nonantidepressant drugs given (CAF, AMP, HAL) significantly increased struggling of these rats at any duration of administration. Floating of SwLo rats was also significantly affected (i.e., reduced) by two of the antidepressant drugs—the atypical antidepressant BUP and the monoamine oxidase inhibitor PHE. However, floating behavior was also decreased markedly by AMP at all times of its administration, so that a decrease in floating was not found to be produced specifically by antidepressants. Finally, when drugs were given to SwHi rats, none of the three antidepressants tested (DMI, AMI, FLU) significantly altered either struggling or floating, thereby indicating that the SwHi rats do not show the same responses to antidepressants as are seen in SwLo rats.

Regarding detection of different antidepressants, although SwLo rats responded to several antidepressant drugs as detailed above, not all antidepressant drugs successfully altered their swim-test behavior. Most notably, FLU and AMI failed to show effects. FLU in particular was a focus of interest—one motivation for development of the SwLo rat was the possibility that use of these subjects in the swim test might overcome a weakness of the Porsolt immobility test in that SwLo rats might prove to be responsive to SSRIs. A significant shortcoming of the Porsolt swim test is its poor detection of SSRIs [see (18); for review, see (2)]. To more thoroughly determine the response of SwLo rats to SSRIs, both lower and higher doses of different SSRIs were also assessed in limited studies. The results are shown in Fig. 7. A lower dose of both FLU and a second SSRI, sertraline (SER), similarly failed to affect behavior as did FLU in the main experiment. However, SER at a higher dose (25 mg/kg/day) was effective in reducing floating behavior. These results are similar to various reports that immobility in the swim test can be reduced by SSRIs but that high doses of these drugs are required (4,7,17,20).

Why did AMI fail in this test and an SSRI have a positive effect only at a high dose? One possible answer is the sedative action of these compounds. Inactivity in the swim test, particularly floating behavior as well as immobility in the Porsolt test, is increased by sedation. In the preceding study, for example, the anxiolytic buspirone both decreased struggling and increased floating (27). As a particularly dramatic example of this influence, Weiss et al. (25) microinfused small quantities of clonidine into the region of the nucleus tractus solitarius/area postrema, which mediates the profound sedative action of clonidine, and dramatic increases in floating behavior were produced by this manipulation. Regarding the antidepressants tested in this study, AMI is the most sedating of the three tricyclics tested, with a high percentage of patients reporting drowsiness and sleepiness as a side effect of using this antidepressant (6). Regarding the SSRIs, results in the open field seem informative—as shown in Fig. 3, FLU, at the dose used, tended to decrase ambulation and significantly decreased rearing, which would be indicative of a sedative effect. Others have reported findings consistent with SSRIs having sedative effects (11). It can be hypothesized that a sedative effect of SSRIs occurs in rats at doses below the very high range, and therefore, antidepressant action is not easily detected in the swim test when low or moderate doses of these drugs are used.

To summarize, long-term administration of various antidepressants increased struggling activity of SwLo rats in the swim test; this response was specific to antidepressants as none of the nonantidepressant drugs tested increased struggling. Some of the antidepressants also decreased floating of SwLo rats; sertraline, an SSRI, had this effect at a high dose, similar to what has been seen by other investigators. However,

a decrease in floating was also produced by the nonantidepressant drug AMP, so that decreased floating, unlike increased struggling, is not a specific marker for antidepressants. Finally, AMI was ineffective in altering either struggling or floating, although this might have been due to sedative effects that appear able to block increases in activity in the swim test that would otherwise occur.

Different Catecholamines Affect Struggling vs. Floating, Which Can Explain "False Positive" Detection of SSRIs in the Swim Test

An interesting aspect of the present findings is that antidepressants that have different biochemical actions and/or primarily affect different monoaminergic systems appear to influence different aspects of swim-test behavior. To be specific, the extent to which struggling behavior of SwLo rats was increased by different antidepressants was directly related to the potency of the drugs in potentiating action of norepinephrine (NE) via reuptake blockade. Thus, DMI was clearly the most effective drug in increasing struggling behavior, followed by IMI. Based on the data of Richelson and Pfenning (19), the ability of antidepressants to increase struggling behavior of SwLo rats is positively correlated with their potency in blocking NE reuptake. On the other hand, floating behavior appears to be influenced by action on dopaminergic systems. Thus, BUP, the most potent of the clinically available antidepressants for blocking reuptake of dopamine (DA), markedly decreased floating. Of the nonantidepressant drugs, AMP, an extremely potent dopamimmetic, decreased floating at all times of administration whereas HAL, which blocks DA receptors, tended to increase floating, an effect that was particularly marked in the SwHi rats. Finally, the MAO inhibitor PHE, which will increase dopaminergic activity via blockade of DA catabolism (3), likewise decreased floating. Thus, assessing the effect of different drugs in the swim test indicates that struggling behavior is responsive to agents that potentiate NE in the brain, whereas floating behavior is responsive to agents that affect DA. This means that it may be possible to determine mechanisms by which antidepressants (or other drugs) affect behavior by assessing which aspects of swim-test behavior are altered by the drugs.

One example of a use of the preceding formulation is to explain effects of SSRIs in the swim test. From their microdialysis studies of FLU, Clark et al. (5) argue that very high doses of SSRIs cause elevations of extracellular DA in the nucleus accumbens. Based on the formulation described above, this accounts for why various studies, including the present one, find decreases in floating (or decreased immobility) in the swim test when very high doses of SSRIs are administered; i.e., these doses will increase DA release in the forebrain and thus decrease floating. Clark et al. argue that because the stimulation of DA by SSRIs occurs only at doses higher than are needed for antidepressant action, stimulation of forebrain DA does not appear to be relevant to the antidepressant action of SSRIs, which, by extension, means that the detection of SSRIs by decreased floating in the swim test also is not related to antidepressant activity. Thus, although high doses of SSRIs may decrease floating in the swim test, this effect is actually a "false positive" with respect to antidepressant action. Interestingly, Clark et al. found that 10 mg/kg FLU given intraperitoneally actually reduced extracellular DA in nucleus accumbens, which would be consistent with the sedative effect of SSRIs described above and would also tend to block detection of antidepressant action in the swim test.

SwLo Rats as a Model for Study of Depression

The final section of this discussion comments on SwLo rats as a model of depression. As noted in the introduction of Weiss et al. (27), an important impetus for development of the SwLo rats was the possibility that these animals would be of value for studying depression. In addition to reasons given in the introduction to that article for why SwLo should represent a model of depression, findings in the present study are relevant to this issue. In the previous study (27), two antidepressants (DMI and PHE) were found to be effective in altering swim-test behavior of SwLo rats when administered chronically but not when given acutely (i.e., for 1 day). The present study found this for all of the antidepressants that were effective in SwLo rats. The sole exception to this was that DMI decreased floating when administered for 1 day, but floating was not found to be a specific indicator of antidepressant action. The extent to which antidepressant drugs are ineffective in altering the swim-test behavior of SwLo rats when administered acutely supports the possibility that these rats are a homologous model of depression, as antidepressant medications are similarly ineffective in relieving depression in humans until the drugs have been administered for at least several days [e.g., (12,13,22)]. Concerning use of the term "homologous model" to describe SwLo rats, Weiss and Kilts (26), in surveying animal models of depression and schizophrenia, suggested that animal models can be viewed as falling in one of two categories—animals assays or homologous models. Animal assays measure responses of the animal (either behavioral or physiological responses) that are not necessarily similar to anything seen in the human disorder that the model is related to; the response observed is simply useful as a indication of some process of interest. Animal assays are most commonly used in drug screening to determine whether a drug has a particular desired (or undesired) action (i.e., will block DA receptors, will block NE reuptake, etc.). Homologous models, in contrast, attempt to reproduce the human disorder; thus, the behavior seen in these models is meant to be similar to, or derives from, the same pathological processes that are present in the human disorder. Homologous models also can be used in drug screening, and, in addition, are potentially more valuable than animal assays because, in so far as they validly reproduce the human disorder, they can be used to attempt to discover the underlying pathophysiology of the human disorder. In that swim-test activity, and specifically struggling activity, of SwLo rats responds positively to chronic administration of antidepressant drugs but not to acute administration, which also characterizes the antidepressant response of humans, this finding is consistent with the hypothesis that SwLo rats possess characteristics that are homologous to human depression.

However, not all antidepressants tested affected behavior of SwLo rats. Even if one permits nonspecific changes to be considered—such as decreases in floating—AMI was not effective in the SwLo rat. This would indicate that the SwLo rat is not a comprehensive model of depression. On the other hand, it is possible that the deficiency does not lie with the SwLo rat but with the screening test used (the swim test) in that sedative influences appear to block detection of antidepressant action in this test; in this event, the SwLo rats might be a better model than can be judged solely by response in the swim test in so far as this test may fail to detect sedative antidepressants. But this having been noted, it is clear that the SwLo rat responds to certain antidepressants in the swim test and, therefore, might be best characterized as a model of a

subtype of depression that responds to these particular drugs. What might that subtype be?

One possibility that can be suggested is that the SwLo rats seem to possess characteristics found in atypical depression. That the SwLo rats responded unambiguously to certain tricyclics (e.g., DMI, IMI) that have been described as activating (24) as well as the MAO inhibitor in the panel (PHE) corresponds to the effective treatments for this disorder. Atypical depression is most predominantly seen in late adolescents–young adults, and is marked by lethargy (psychomotor retardation), hypersomnia, and often weight gain (1). There are limitations to the suggested parallel; for examples, SwLo rats did not show indications of hyperphagia and excessive weight gain when food intake and body weight were measured under normal, home cage conditions [see (27)]. However, the lack of motor activity, at least in the swim test, of SwLo rats is consistent with psychomotor retardation (lethargy), which is an important symptom of atypical depression. Turning again to the response to antidepressants, although atypical depression will respond positively to tricyclic antidepressants (23), one group of investigators argues that the most effective treatment for this subtype is an MAO inhibitor (15). It is, therefore, interesting that PHE markedly decreased floating behavior of SwLo rats, an observation we also reported in the preceding article (27). Based on what was pointed out in the previous section of this Discussion, this decrase in floating indicates that PHE potently stimulates DA. AMP had a similar effect on floating. This is noteworthy as atypical depressives often abuse drugs, and activating drugs such as AMP in particular; also, abusers of AMP and closely related substances can be treated successfully with the same "activating" antidepressants that were effective in SwLo rats (9,10,14,29). The hypothesis can be advanced that the reduced swim-test activity of SwLo rats might derive, at least in part, from reduced function of central dopaminergic systems, which AMP will directly counteract, and, in so far as SwLo rats represent atypical depression, such individuals are "hypodopaminergic" and are liable to self-medicate with AMP to compensate for this dysfunction. Further studies, presently underway, are exploring whether the SwLo rat might be useful as a model for studying depressed individuals who are prone to abuse drugs.

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