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A Pretest Procedure Reliably Predicts Performance in Two Animal Models of Inescapable Stress

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DRUGAN, R. C., P. SKOLNICK, S. M. PAUL AND J. N. CRAWLEY. *A pretest procedure reliably predicts performance in two animal models of inescapable stress.* PHARMACOL BIOCHEM BEHAV **33**(3) 649–654, 1989.—Rats exposed to inescapable tailshock fail to learn a shuttle-escape task 24 hours later, an effect referred to as "learned helplessness." However, within most rat strains only 10–50% of the animals tested develop this syndrome. In the present study a significant correlation was found between rats that displayed learned helplessness on the first test and those that displayed learned helplessness on a second test performed either 2 weeks ($r = .80$, $p<0.001$) or 4 weeks ($r = .74$, $p<0.001$) later. An analysis of the mean session latency of the shuttlebox task in these two tests suggested a bimodal distribution of animals that failed and learned. A significant correlation was found between individual rats that learned this task on the first test and those which learned this task 2 or 4 weeks later. Similarly, in the "behavioral despair" test, a significant correlation was observed for floating time for individual rats on the first test and on the second test either 2 ($r = .72$, $p<0.001$) or 4 weeks ($r = .63$, $p<0.001$) later. However, for the forced-swim test, a unimodal and rather graded response was observed across individual subjects. Thus, performance on the first round predicted performance on the second round in both models. When rats experienced the learned helplessness paradigm on round 1 and the behavioral despair paradigm in round 2, there was no correlation between rats that displayed helplessness following inescapable tailshock and the rats that demonstrated "behavioral despair" on a later test. While both the "learned helplessness" and the "behavioral despair" models may assess the ability of individual animals to "cope" with stressors, the lack of cross-predictability strongly suggests that the two models may be mediated by different neurochemical mechanisms.

Learned helplessness Forced-swim test Stress Depression Anxiety

INESCAPABLE shock has been reported to produce a variety of effects including failure to learn a simple escape task 24 hours later (17, 18, 25), subsequent inactivity in the presence of shock (2, 6, 7, 10), analgesia (12,13), reduced aggressiveness or subordinate behavior (15, 19, 22, 23), enhanced susceptibility to growth of implanted tumors (27,28), development of gastric ulcers (29) and immunosuppression (13). Some authors have suggested that "learned helplessness" paradigms employing inescapable shock represent animal models of depression (26,30). This view is supported by the pharmacologic profile of drugs that block this syndrome (26).

The use of learned helplessness as an animal model for studying the neurochemistry of stress-induced psychopathology has been confounded by the problem that not all rats exposed to inescapable shock develop the syndrome. In fact, the percentage of rats developing learned helplessness varies considerably, depending upon the strain (32), the difficulty of the escape response (24), and the type of escape task employed (1,14). At present, there is no reliable method to distinguish animals that will develop these syndromes. Neurochemical analyses are often performed immediately or within several hours after inescapable shock. Using animals after the learning task (24 hours later) for biochemical analysis introduces yet another source of variance, since the animals that subsequently fail to learn the shuttlebox task have experienced greater footshock exposure than the animals which learned the task. The development of a behavioral pretest procedure that reliably identifies rats that will develop the learned helplessness syndrome while permitting sufficient time after the shuttlebox escape task for any changes in neurochemical parameters to return to baseline, could allow assay of more homogeneous groups.

Forced-swimming-induced "behavioral despair" has been reported to be another animal model of behavioral depression

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(20,21). Twenty-four hours following a 15-minute forced-swim in a cylinder from which they cannot escape, rats will quickly maintain a characteristic immobile posture. The duration of this immobility was reduced following administration of clinically effective antidepressant drugs at doses which otherwise decreased spontaneous motor activity in an open field (20). The duration of immobility was also reduced by electroconvulsive shock, rapid eye movement (REM) sleep deprivation, and "enrichment" of the environment (20,21).

In the present paper we investigated the reproducibility of both the learned helplessness and the forced-swim-induced behavioral despair models in individual rats. If the models have predictive value for individual rats, then a pretest procedure may have heuristic value for selecting homogeneous populations of rats for subsequent behavioral and biochemical analyses. In addition, cross predictability between the two animal models would suggest that similar or identical neurochemical mechanisms are involved in the development of the learned helplessness and the behavioral despair syndromes.

METHOD

Subjects

Male Sprague-Dawley rats were purchased from Taconic Farms (Germantown, NY), weighing 175–200 g at the start of experimentation. All rats were maintained on a 12-hour light/dark cycle in a temperature- and humidity-controlled vivarium, with free access to food and water.

Apparatus

Inescapable shock pretreatment was given to rats restrained in Plexiglas escape-yoke wheel-turn boxes ($15.5 \times 12 \times 17$ cm) modeled after those used by Weiss *et al.* (31), Maier *et al.* (16), and Drugan *et al.* (4). A grooved Plexiglas wheel extended 1.7 cm from the floor of the box. The wheel was locked prior to experimentation so that it could not be turned. The rat's tail was extended through a slot in the rear wall of the chamber and was taped to a Plexiglas rod parallel to the floor of the chamber. Shock generators (Lafayette Instruments Model No. 82400) were used to apply eighty 5-second inescapable shocks (incremented from 1–2 mA) through electrodes attached to the rat tail. All rats were tested for shuttle escape performance in a two-way shuttlebox (BRS/LVE Model RSC-044). The gridshock to the shuttlebox floor was produced by a BRS/LVE (Model No. SGS-004) shock generator/scrambler.

For both forced-swimming pretreatment and subsequent immobility testing, a vertical Plexiglas cylinder (height: 36.4 cm, diameter: 19.5 cm) containing 28 cm of water (25 ± 1°C) was used.

Procedure

Experiment 1. The purpose of Experiment 1 was to determine the "stability" of performance of rats which were tested for shuttle-escape performance 2 or 4 weeks following an initial exposure to inescapable shock and subsequent shuttlebox-escape testing.

Rats were given eighty 5-second inescapable tailshocks (incremented from 1–2 mA) on an average of one per minute. Twenty-four hours later, all rats were tested for escape performance in a two-way shuttlebox as previously described (5, 8, 9, 17). In brief, each trial began with a warning tone (80 dB, 2.8 kHz) followed by a 1.0 mA gridshock 5 seconds later. The first five trials required a single crossing of the shuttlebox in order to terminate shock. These trials (FR-1) are unaffected by prior exposure to shock and serve as a control for nonspecific effects such as sedation. The subsequent 25 trials require two crossings to terminate shock (FR-2). If the required escape response did not occur within 30 seconds of shock onset, the trial was automatically terminated.

Escape latencies were measured on both the first and second rounds of testing. An arbitrary criterion was established such that rats with a mean FR-2 session latency >20 seconds were termed "fail" (helpless rats), while those rats with mean session latencies ≤20 seconds were termed "learn" (nonhelpless) rats. Comparison between the number of rats which learned and failed on the two rounds of testing was performed by a chi-square test. Comparison of individual session latency scores on both rounds of testing was performed by Pearson Product Moment Correlation Analysis.

Immediately following the shuttlebox testing, all rats were returned to their home cages and were given free access to food and water. Two or four weeks later, rats were removed from their home cages and administered the two-day procedure described above, i.e., a session of inescapable tailshock followed twenty-four hours later by the shuttlebox-escape testing.

Experiment 2. Experiment 2 was designed to assess individual variation in "immobility-time" for rats placed in ambient water for a 5-minute immobility test, 24 hours following a 15-minute forced-swim pretreatment.

Naive rats were individually placed in ambient temperature (25°C) water in the vertical Plexiglas cylinders described above. Following 15 minutes in the water, they were removed and placed in a Plexiglas cage warmed by incandescent heat lamps for 30 minutes before being returned to their home cages. Twenty-four hours later, each rat was placed back in the water-filled cylinders and the duration of immobility was measured during a 5-minute test. Immobility was assessed by a lack of vigorous struggling such that the forepaws did not break the surface of the water. Thus, total test time consisted of the sum of floating (immobility) or struggling (active swimming with front paws breaking the surface of the water). This procedure is similar to the methods described by Porsolt *et al.* (20,21) except that the depth of the water was greater in the present study (28 cm versus 15 cm).

Experiment 3. In light of the individual variability observed for "immobility time" in Experiment 2, Experiment 3 investigated the reproducibility of this behavioral performance in the behavioral despair test 2 or 4 weeks following initial testing.

In a separate group of rats, two or four weeks after the first round of forced-swimming and immobility testing, all rats were placed back in the water-filled cylinders for a second 15-minute forced-swim pretreatment. Twenty-four hours later the swim test was again administered in which the duration of immobility was recorded as previously described.

Experiment 4. Experiment 4 evaluated the cross-predictive value of performance in a shuttlebox-escape task following inescapable shock and a subsequent test of forced-swim immobility 2 weeks later. The learned helplessness procedure was chosen as a pretest predictor for behavioral despair, because of the low variability around the apparently bimodal distribution of escape performance (learn vs. fail) which emerged from the learned helplessness procedure (see Fig. 1). If the behavioral despair test had been used as a predictor for learned helplessness, the variability may have confounded an interpretation of cross-predictability, due to the graded continuum of immobility that was observed with the forced-swim procedure (see Fig. 3).

Rats were either untreated (naive) or given a session of 80 5-second inescapable tailshocks as described in Experiment 1. Twenty-four hours later the previously shocked rats were tested for shuttlebox-escape performance and categorized as either fail (helpless) or learn (nonhelpless) according to the mean session latency criterion described in Experiment 1. Immediately following the shuttlebox testing all rats were placed back in their home cages.

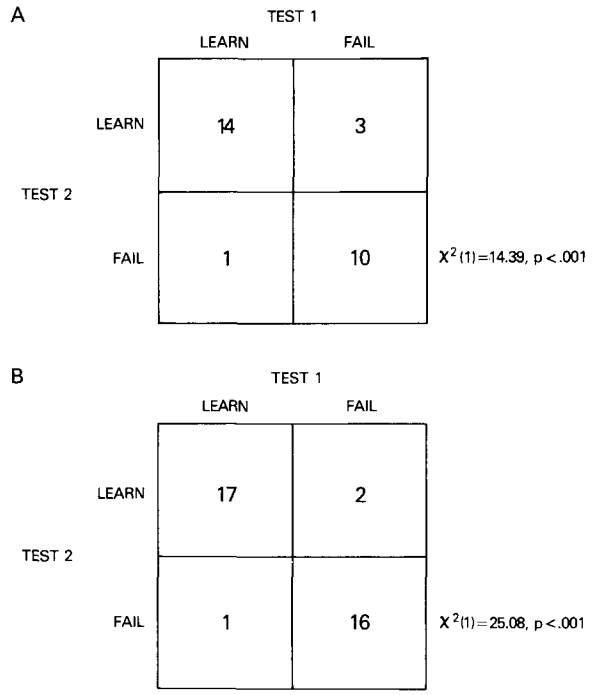

FIG. 1. Shuttle escape performance of rats that were exposed to 80 inescapable shocks and tested for shuttlebox escape learning 24 hours later (Test 1), then exposed to a second session of 80 inescapable shocks and tested for shuttlebox escape learning 24 hours later (Test 2), either two weeks later (Panel A) or four weeks later (Panel B).

Two weeks later the three groups of rats (naive, learn and fail) were placed in the water-filled cylinders for the 15-minute forced-swim pretreatment. Twenty-four hours later all rats were placed back in the cylinders and the duration of immobility was recorded during a 5-minute test session.

RESULTS

Figure 1 compares the escape performance of rats in the first and second test sessions in the learned helplessness paradigm. Panel A represents the results of the two sessions of shuttle-escape performance when the testing was separated by a two-week period for twenty-eight rats. Fourteen out of fifteen of the rats that initially learned the escape task, also learned when tested two weeks later, 24 hours after the second session of inescapable tailshock. Conversely, of the thirteen rats that initially failed on the escape task, ten also failed two weeks later. As compared to the null hypothesis of equal distribution of 28 rats into $N=7$ for each of the four χ^2 cells, $\chi^2=14.39$, $p<0.001$. Panel B illustrates the two sessions of shuttle-escape performance when the testing was separated by a four-week period. Seventeen out of eighteen rats that initially learned the shuttlebox-escape task, also learned when tested four weeks later, 24 hours following a second session of inescapable tailshock. Sixteen of the eighteen rats that failed on the initial session also failed to learn the shuttlebox-escape task when tested four weeks later. As compared to the null hypothesis of equal distribution of 36 rats into $N=9$ for each of the four χ^2 cells, $\chi^2=25.08$, $p<0.001$.

Figure 2 illustrates the mean FR-2 session latencies for both the first and second rounds of shuttlebox-escape testing. This scatterplot supports the data of Fig. 1 which employed a 20-second

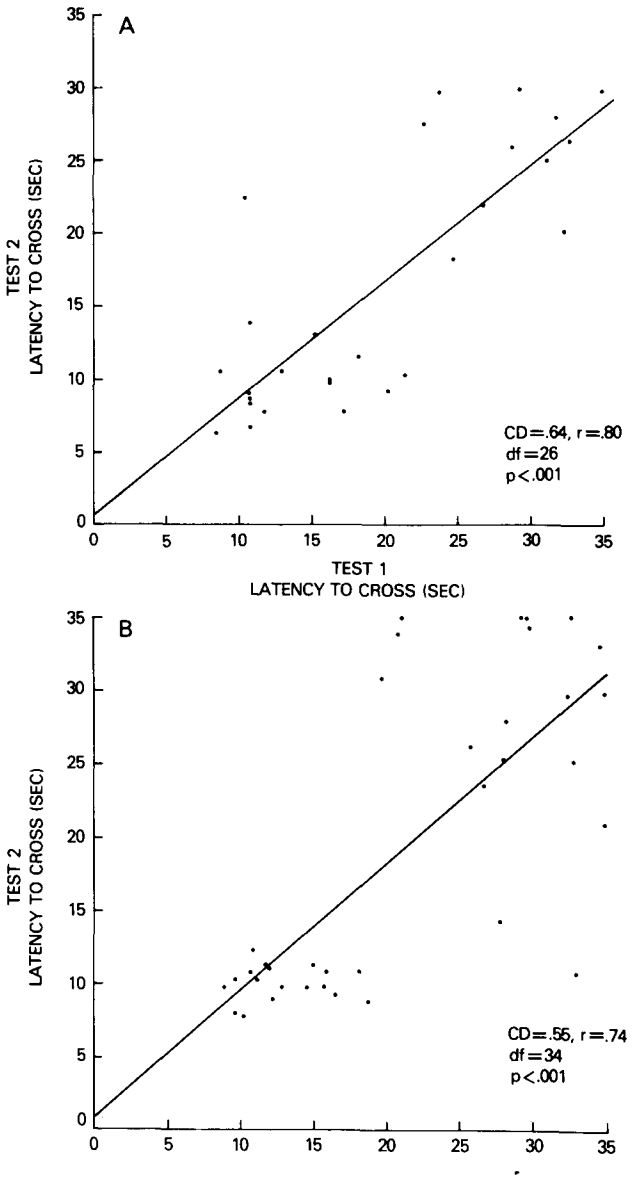

FIG. 2. Mean FR-2 session latency for individual rats exposed to 80 inescapable shocks and tested for shuttle escape performance 24 hours later (Test 1), then exposed to a second round of 80 inescapable shocks and tested for shuttlebox escape learning after 24 hours (Test 2), either two weeks (Panel A) or four weeks (Panel B) later.

time-based criterion for defining learn versus fail rats. Figure 2A represents the scatterplot of the mean FR-2 session latency of the first and second shuttlebox-escape tests when the testing was separated by a two-week period. A significant correlation between the performance on the first and second test was found by Pearson Product Moment Correlation Analysis, $r(26)=.80$, $p<0.001$. Furthermore, the distribution of latencies strongly suggests a bimodal distribution, since two clusters of points comprise the scatterplot. Figure 2B shows a scatterplot of the mean FR-2 session latency of the first and second sessions of shuttlebox-escape performance when the testing was separated by a four-week period. A significant correlation between the escape performance on the first and second test was found by a Pearson Product Moment Correlation Analysis, $r(34)=.74$, $p<0.001$, with an apparent bimodal distribution of points.

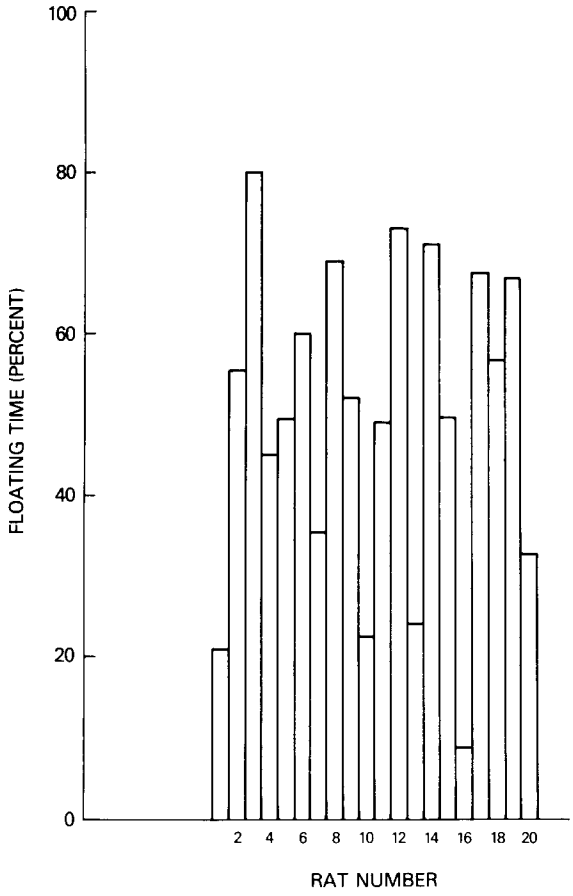

FIG. 3. Mean percent floating time for individual rats during a 5-minute immobility test 24 hours following a 15-minute forced swim pretreatment.

Figure 3 illustrates the time spent floating during the 5-minute test session for individual rats in a modified Porsolt test. As can be seen, there is considerable variability in the percent of time spent floating by rats that were equally exposed to a 15-minute forced-swim pretreatment 24 hours earlier.

Figure 4 shows a scatterplot of the time spent floating during the initial test and the second test two or four weeks later. Panel A represents the time spent floating on Test 1 and 2 when the testing was separated by a 2-week period. Pearson Product Moment Correlation Analysis, $r(38) = .72$, $p<0.001$, confirmed that the behavior of the rat on Test 1 is significantly correlated with the behavior on Test 2. Panel B demonstrates that time spent floating on Test 1 is correlated with the time spent floating when the subsequent testing is 4 weeks later [Pearson Product Moment Correlation Analysis, $r(38) = .63$, $p<0.001$].

Figure 5 shows the time spent floating by rats previously exposed to inescapable tailshock and shuttlebox-escape testing. While a slight increase in floating behavior was observed in rats that were exposed to inescapable tailshock, analysis of variance revealed no significant difference in floating behavior between naive, learn and fail groups, $F(2,40) = 1.31$, N.S.

DISCUSSION

The present paper demonstrates that the behavioral response of an individual rat to inescapable tailshock is similar on two rounds of testing separated by up to four weeks. This finding suggests

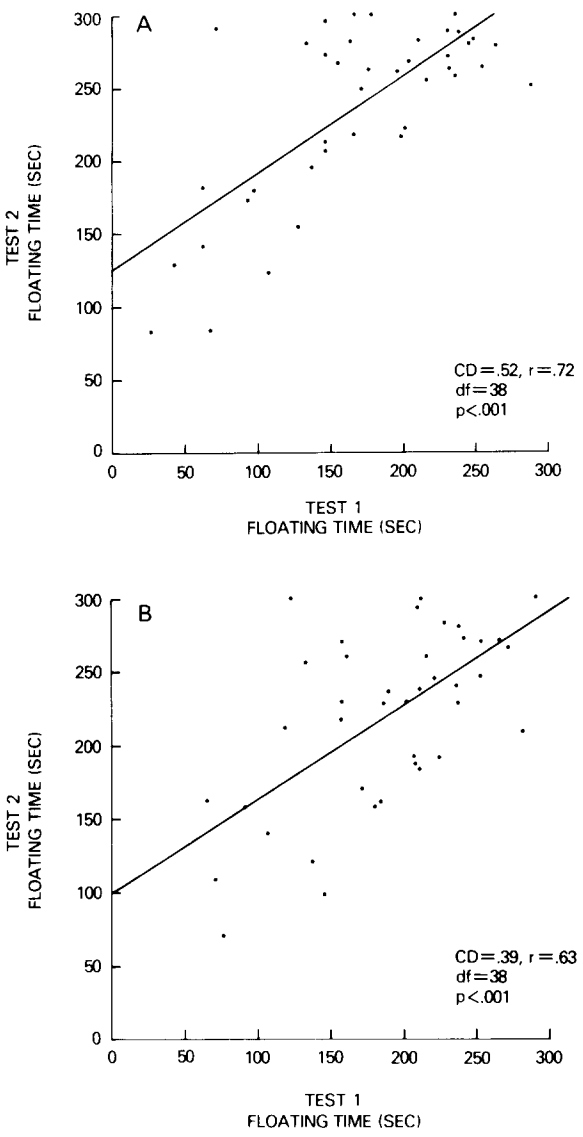

FIG. 4. Floating time for individual rats exposed to a 15-minute forced swim pretreatment and tested for immobility after 24 hours (Test 1), then were exposed to another round of 15-minute forced swim pretreatment and tested for immobility after 24 hours (Test 2), either two weeks (Panel A) or four weeks (Panel B) later.

that, in rats, the propensity to learn or fail to learn a simple shuttlebox-escape task 24 hours following a session of inescapable shock is a reproducible and stable characteristic. The bimodal distribution of approximately 50% learn, 50% fail seen under these conditions might be conceptualized as stress-sensitive (fail) versus stress-resilient (learn) or "coping" populations.

Despite the marked variability in the duration of immobility in the forced-swim-induced behavioral despair test between individual rats, immobility also appeared to be a reproducible characteristic for individual animals. The rats which initially floated for only a small percentage of the test session time also showed similar floating times 2 or 4 weeks later. In contrast to the bimodal distribution observed in the learned helplessness model, the behavioral despair model produced a unimodal, graded continuum of float-time scores. Similar to the learned helplessness model,

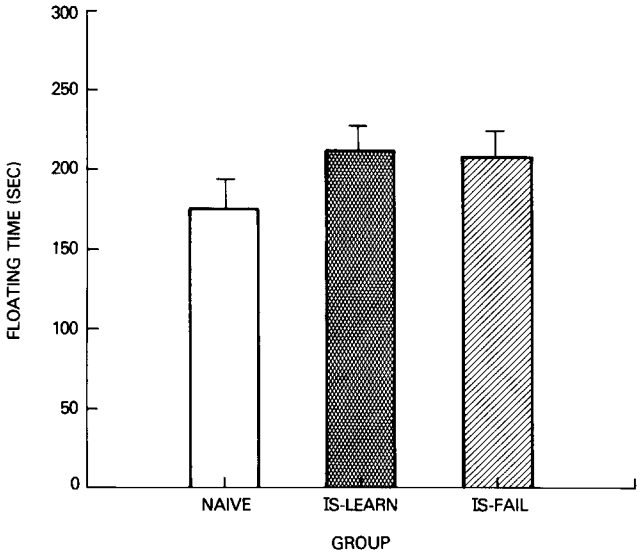

FIG. 5. Mean floating time of rats 24 hours following a 15-minute forced swim test in rats that were previously exposed to 80 inescapable shocks and tested for shuttlebox escape learning 2 weeks prior. Rats were helpless (IS-fail) nonhelpless (IS-learn) or not given any prior treatment (Naive). Vertical bars represent standard error of the mean.

the behavioral despair model may reflect a behavioral "trait" or predisposition in individual rats which is predictive of subsequent behavioral responsiveness to this type of stress.

Our results are consistent with other studies which suggest the presence of a genetic predisposition to the development of learned helplessness [Weiland *et al.*, (32)]. Alternatively, the mechanism responsible for the stability of the behavioral performance might be the result of transfer of training of a memory effect. Some stressors have measurable effects on behavioral paradigms which persist for 2 weeks but dissipate by 4 weeks (3). The 4-week study was designed to extend the time between the first and second rounds to twice the interval of the 2-week study. The correlation coefficient in the 4-week experiment was as robust as the correlation coefficient in the 2-week experiment, suggesting that it is less probable that the correlation between the two rounds of testing was due to a memory effect. However, the 4-week experiments cannot rule out a transfer of training or memory effect as responsible for the behavioral stability. Task-related memory can be stable for months or years in some paradigms.

In addition, there was no correlation between rats that failed on the shuttlebox task and the animals that subsequently demonstrated immobility on the forced-swim test. Since these two models, which employ qualitatively and quantitatively different stressors, did not prove to be predictive of one another, it is possible that 1) the observed correlations within models represent a memory effect, not seen between models or 2) different neurochemical mechanisms may be responsible for the stress-induced behavioral deficits in these two models.

Tests of both learned helplessness and behavioral despair appear to have predictive validity for behavioral responsivity within that specific model. This predictability could be useful for genetic breeding (to create substrains analogous to the Maudsley reactive and nonreactive, Roman high and low avoidance, or Flinders sensitive and nonsensitive rats). It is interesting to note that within a single strain (i.e., Sprague-Dawley, Taconic Farms) individual animals exhibit divergent behavioral responses when challenged by inescapable stress. By using a pretest procedure to create more homogeneous groups, it may be possible to detect neurochemical correlates or predisposing factors responsible for individual differences in stress responsivity.

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