

## Coping strategies in male and female rats exposed to multiple stressors

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### Abstract

Because of the pathogenic effects of chronic stress exposure, it is important to identify factors, such as effective coping strategies, that mitigate stress-induced pathology. Of interest in the present study was the consistency of behavioral responses across a diverse array of stressors. Sixteen male and 16 female Long–Evans rats were assigned to either a stress or control group. The stressed animals were subsequently exposed to a battery of ecologically relevant stressors (e.g., predator odor, novel stimuli, and immunological challenge) to determine trends in coping strategies. Blood was collected for corticosterone (CORT) assay and brains were harvested for assessment of *fos* immunoreactivity in the paraventricular hypothalamus (PVH) and central amygdala (CEA) following exposure to the final stressor of fox urine. A correlational analysis indicated that certain response strategies (e.g., latency to respond in different stress tests such as the open-field and novel item tests) persist across several behavioral tests, especially those tests involving exploratory components. A subsequent principal component factor analysis revealed the following four components: initiative to explore, low reactivity, variable reactivity, and high reactivity. Females exhibited higher recovery CORT levels than males; however, sex only affected one behavioral response measure (i.e., females demonstrated more attempts to climb the wall in the forced-swim test than their male counterparts). In conclusion, these results support the importance and prevalence of initiative to explore as a common factor in many stress tests; additionally, the principal component analysis indicated that physiological correlates of stress are more closely associated with more challenging environments and stimuli such as forced swimming, immunological challenges, and exposure to predator odors.

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### 1. Introduction

A growing literature suggests that the stress response, although vital for responding to challenges to one's homeostasis, can become pathogenic when engaged for extended durations. Chronic stress is associated with a greater risk of atherosclerosis, hypertension, atrophied hippocampal neurons, suppressed immune function, and cancer [1–8]. Stress also has been linked to the manifestation of several psychological disorders. Elevated concentrations of cortisol (CORT), a steroid hormone often used as an index of stress, are observed in individuals suffering from anxiety, depression, and eating disorders [9–12]. Further, schizophrenic patients often develop symptoms during stressful periods in their lives and subsequently exhibit impaired regulation of

CORT concentrations [13]. Higher relapse rates in addiction are also observed during times of stress [14].

With the mounting evidence that chronic stress poses a significant threat to the psychological and physical health of an individual, a better understanding of behavioral responses to stress would be extremely valuable. Toward this goal, more information concerning the influence of all relevant variables in the complex stress response is needed. Of specific interest in the current study was the animal's behavioral response to a stressor. The physiological response to stress (e.g., activation of the hypothalamic–pituitary–adrenal [HPA] axis) is well documented, but less is known about behavioral responses to stress. It has recently been suggested that animals develop consistent response strategies to a diverse array of stressors [1,15]. An animal's coping style can be categorized as proactive (characterized by a rigid response that is intrinsically driven, as observed in aggression) or reactive (characterized by a more flexible response that is more externally driven; this response would be adaptive in an unpredictable environment) [1,15]. Ani-

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mals that demonstrate a proactive style tend to have lower HPA axis activity but higher sympathetic reactivity whereas more passive, reactive animals have higher HPA axis activity and higher parasympathetic activity [1,15,16]. The active coping style is also associated with higher humoral immune response than passive coping strategies [17]. However, the categorization of coping styles is considered controversial by many researchers and has been applied under relatively restricted experimental conditions [1]. For example, most studies select animals exhibiting the coping extremes and do not assess the majority of animals that fall in the middle range of the coping categories. In addition, because the stress response upon exposure to acute and chronic stressors has been found to differ [18], it is important to assess coping strategies in response to durations of both stressors [15,19].

Prior research also indicates sex differences in behavioral and physiological responses to stress. For example, female rats are more active than males in the open-field test, which suggests that females exhibit lower stress responsivity than males [20]. In contrast, males generally mount smaller stress-induced CORT responses than females, which would suggest that males exhibit lower stress responsivity than females [21]. However, when male and female rats are exposed to repeated stressors, sex differences in behavioral responsivity diminish. Overall, females appear to be more vulnerable to the physiological effects of chronic stress [22].

The primary goal of this study was to determine the degree of generalization in an animal's response strategies to varying types of stressors. Commonly used behavioral tasks in stress research include the open-field test, the elevated plus maze, the defensive burying task, and the forced-swim test. Most studies select one of these tests to gauge the animal's stress responsivity. Although these tests often yield consistent, reliable responses, one question of interest in the current study is the robustness and appropriateness of a single test as an accurate representation of the animal's overall coping strategy. In the present study, we assessed consistencies in stress-response behavioral strategies. Following the exposure of male and female Long–Evans rats to a series of acute stressors, the behavioral measures were correlated to identify significant relationships in the battery of ecologically relevant stress tests (e.g., predator odors, novel stimuli, immunological challenge) as well as the more contrived tail-clip and forced-swim tests. Because the neurobiological response to stress is also an important component of the coping response, following the final stress exposure, CORT was assessed to provide an endocrinological index of the animal's stress response and two brain areas known for their involvement in stress and fear, i.e., the paraventricular hypothalamus (PVH) and central amygdala (CEA), respectively, were investigated by assessing *c-fos* immunoreactivity as an index of activation. Of additional interest was the question of the existence of various types or categories of coping strategies utilized upon exposure to

acute stressors. To determine if certain stress responses loaded onto components such as the aforementioned low-reactivity and proactivity (or high-reactivity) strategies, a factor analysis was conducted. Finally, the effect of sex was assessed in all dependent measures to provide further information concerning the conflicting evidence sometimes found between physiological and behavioral responses in males and females.

## 2. Materials and methods

### 2.1. Animals and housing

Thirty-two adult, Long–Evans hooded rats (16 males, 16 females; Harlan Sprague Dawley, IN) were housed (in  $53 \times 20 \times 15$  cm cages) in same sex pairs on a 12 h light/dark cycle. Rats were given water and food (Mazuri, MO) ad libitum during the experiment. The rats were divided randomly into an experimental group that was exposed to stressors ( $n = 16$ ; 8 males and 8 females) and a control group that was not exposed to stressful stimuli ( $n = 16$ ; 8 males and 8 females).

Following the 2-week habituation period, the experimental animals were exposed to a battery of stress tests. To avoid habituation, each test was conducted only once. The control rats were handled only during routine care of the animals and cages. The battery of stress tests consisted of the following tests in the order in which they were performed over a 2-week period.

### 2.2. Procedures

#### 2.2.1. Sawdust-digging escape task

The sawdust-digging escape task assesses the rat's response to a novel stimulus. A wood and Plexiglas box ( $96 \times 20 \times 30$  cm) containing two ramps at either end that sloped downward towards the center of the box was used [23]. A wooden divider was placed in the middle of the apparatus allowing 4 cm of open space between the bottom of the box and the divider. The base of the box was filled with sawdust until the 4-cm opening was covered and the animal could not visually detect an escape route from the starting compartment. A battery-operated, plastic toy lion ( $13 \times 5$  cm) that walked in a single direction was used as mobile stimulus.

During this test, an individual rat was placed at one end of the box and the simulated intruder was activated so that it was approaching the rat. The intruder was placed between the rat and the middle partition of the sawdust-digging apparatus. Rats were observed for 3 min, during which time latency to dig and duration of freeze responses were recorded. No animal escaped sooner than 3 min, so this was the most appropriate time to use for response duration. The apparatus was cleaned and the sawdust changed between animals.

### 2.2.2. Open-field test

The open-field task assessed exploratory behavior in a novel environment. Specifically, the open-field apparatus consisted of a wooden box (87.5 × 75 cm) consisting of a white floor with 42 evenly spaced squares (5.5 cm) outlined in black. The apparatus was cleaned with an unscented disinfectant solution (diluted one part disinfectant to two parts water; Purina, MO) between trials. Rats were placed in a corner of the open-field apparatus at the beginning of the 5-min test. Latency to explore (moving beyond the three corner squares), number of squares traversed (two feet inside the square), rearing frequency, and center squares traversed were quantified for each animal.

### 2.2.3. Novel item emergence test

The novel item emergence test assesses the rat's tendency to leave a familiar environment to investigate a novel stimulus. Black PVC pipe (15 cm long, 7.5 cm in diameter) was used to provide a familiar area in which the rats were able to hide. A green Lego piece (5 × 2.5 × 2.5 cm) was used as the novel item. The rats were allowed to habituate to the tubes overnight. The following day, the cagemates were separated from one another and individually tested. Once the experimental animal entered the tube, a Lego piece was placed in the front of the cage approximately 8 cm from the opening of the tube. Latency to emerge (head and body stretched out of the tube) and the latency to exit (all four feet outside the tube) were recorded following placement of the novel item.

### 2.2.4. Forced-swim test

The forced-swim test assesses behavioral responses to inescapable stress. A Plexiglas aquarium (60 × 30 × 50 cm) filled to three-fourths its capacity with room-temperature water was used to assess floating, diving, and attempted escapes. Rats were placed in the water for 5 min. The latency to immobility (defined as 3 s or more of immobility), the duration of immobility, dive frequency, and frequency of attempted escapes (defined as each movement toward the wall of the aquarium in which the animal displayed a climbing movement directed to the top of the aquarium) were recorded.

### 2.2.5. Tail-clip test

The tail-clip test assesses an animal's response to a chronic, nonpainful irritant. A small plastic clip (about 2 cm long) with five rounded teeth per side was placed on the rat's tail for 5 min. The latency for the animal's initial attempt to remove the clip was recorded.

### 2.2.6. Lipopolysaccharide (LPS) test

The LPS emergence test provided an opportunity to assess the animal's behavioral response to an immunological challenge. For 3 days before testing, the rats were allowed ad-libitum access to a sweetened milk solution

(Carnation; 25 mg sweetened condensed milk/75 ml water). The black tube, used in the novel item emergence test, was placed in the rat's cage the night before the LPS test. On the day of testing, the rats were injected intraperitoneally with 100 µg LPS [Sigma, MO; in isotonic saline (0.2 cc)]. The rats were left undisturbed for 3 h following the LPS injection [24]. Three hours after the LPS injection, the rats were given access to the sweet milk for 30 min. Latency to drink the milk and the total amount consumed were recorded. After the 30-min exposure to sweet milk in the LPS test, the milk was removed and the novel item emergence test was repeated.

### 2.2.7. Fox urine

This test assesses behavioral response to predator odor. A cotton swab soaked with fox urine (Buck Stop Lures, MS) was secured inside the test cage, 2.5 cm above the bedding, using duct tape. The rat was placed in the cage with the swab. During the 10-min test, latency to bury or bite the swab, as well as the duration of the action, was recorded. The rat was then placed back in its original cage. Bedding in the test cage was changed after each rat, and a new swab was prepared.

### 2.2.8. Blood sampling

Blood samples were collected from the retro-orbital sinus of anesthetized rats (Halothane, nasal route) 1 h following exposure to fox urine. The rats were then prepared for perfusion as described below. The blood samples were centrifuged at 3000 rpm for 10 min. The plasma was collected and frozen at –70 °C until assay. CORT concentrations were determined using a standard radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA).

### 2.2.9. Perfusion

Following the blood collection, the rats were given an overdose of sodium pentobarbital (0.3 cc ip). Rats were then perfused using phosphate-buffered solution (PBS) for 3 min and 4% paraformaldehyde (Paraform) for 10–12 min. Following removal from the skull, the brains were stored overnight in a sucrose–paraformaldehyde solution and subsequently sectioned (at 40-µm sections) using a Zeiss Cryostat. Using the Paxinos and Watson's stereotaxic atlas [25] as a guide and starting at plate 25, six sections were taken through the CEA and PVH. Every other section was subsequently exposed to *c-fos* primary antibody (at 1:6000 dilution; Immunostar, MI) followed by steps consistent with a standard immunohistochemistry protocol [26]. Following staining, the tissue was mounted on gelatin-coated microscope slides and coded for neuroimaging analysis.

### 2.2.10. Neuroimaging

All sections were analyzed using a Zeiss Axioscope (at 400 × magnification) and accompanying NeuroLucida neuroimaging software (MicroBrightfield, VT). The PVH was

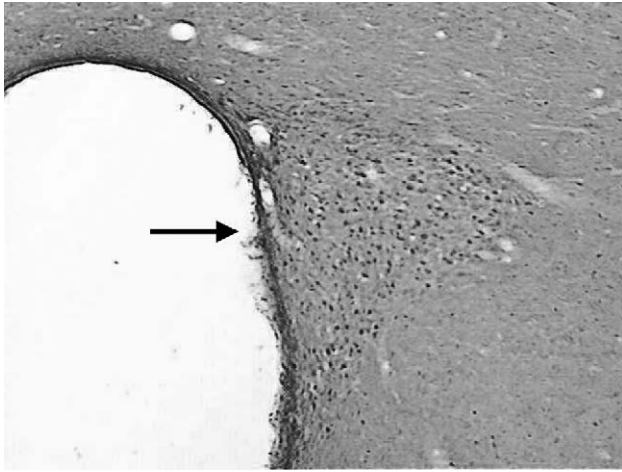


Fig. 1. Photomicrograph showing *fos* immunoreactivity in the PVH (at  $100\times$ ). Arrow indicates paraventricular area and serves as scale bar ( $200\ \mu\text{m}$ ).

identified by locating the dorsal tip of the third ventricle and moving  $200\ \mu\text{m}$  ventrally. The stage was then moved laterally until the brain tissue fully covered the  $140\times 160\ \mu\text{m}$  tracing box. All *fos*-immunoreactive cells were then counted in the PVH-designated area on each side of the ventricle by a single observer. See Fig. 1.

To locate the CEA, the ventralmost part of the cortex was used as a starting point. The reference point was then moved  $1400\ \mu\text{m}$  dorsally and  $400\ \mu\text{m}$  medially. Using NeuroLucida, a  $140\times 160\ \mu\text{m}$  box was selected and *fos* expression was quantified in that region. The quantification box was then moved  $200\ \mu\text{m}$  medially and *fos* expression was quantified in that region as well. Thus, two  $140\times 160\ \mu\text{m}$  screening areas were assessed in each hemisphere of each brain slice in the CEA region. For both PVH and CEA areas, the mean number of *fos*-immunoreactive cells for each animal in each hemisphere's designated brain area was determined for subsequent analysis.

### 3. Results

The results were analyzed using SPSS software. Specific analyses used were Pearson's correlations, principal component factor analysis, and analysis of variance (ANOVA). An alpha level of .05 was used for all appropriate statistics.

#### 3.1. Behavioral data

##### 3.1.1. Pearson's correlations

Using Pearson's correlations, the results of the behavioral tests were analyzed to identify behavioral consistencies over the multiple stress tests. Latency to dig in the sawdust-digging escape task was significantly correlated with duration of freezing behavior in the sawdust-digging

escape task [ $r=.623$ ;  $P=.01$ ], latency to move in the open field [ $r=.554$ ;  $P=.026$ ], latency to emerge in the novel item emergence test [ $r=.551$ ;  $P=.027$ ], and duration of digging behavior in the fox urine defensive burying test [ $r=-.511$ ;  $P=.043$ ]. (See Fig. 2 for appropriate scatterplots.) Number of squares crossed in the open field was significantly correlated with latency to move in the open field [ $r=-.629$ ;  $P=.009$ ], number of rears in the open field [ $r=.772$ ;  $P=.001$ ], latency to emerge from the tube in the novel item test [ $r=-.652$ ;  $P=.006$ ], latency to exit the tube in the novel item test [ $r=-.536$ ;  $P=.032$ ], latency to float in the forced-swim test [ $r=-.531$ ;  $P=.042$ ], and latency to drink milk in the LPS test [ $r=-.591$ ;  $P=.016$ ]. (See Fig. 3 for appropriate scatterplots.)

Whereas some behavioral measures in the aforementioned open-field and sawdust escape tests were correlated with several measures used in other behavioral tests (see Fig. 4), other behavioral measures seemed to be independent. For example, the latency to remove the tail clip was only correlated with the latency to exit in the novel emergence test [ $r=.767$ ;  $P=.001$ ]. The diving and attempted escape responses in the forced-swim test were not correlated with any other behavioral measures, including other forced-swim measures.

##### 3.1.2. Factor analysis

Although caution should be taken when conducting factor analyses with small sample sizes typical of most behavioral neuroscience work, this analysis was conducted to determine if certain variables loaded on emerging factors in a theoretically meaningful way. The acceptance criteria for emerging components was conservative; following a principal component analysis, only factors with eigenvalues larger than 1.0 were accepted; accordingly, factors with high-loading scores ( $>.50$ ) were considered as relevant contributing factors [27,28].

Although eight components, or factors, emerged in the principal component analysis, the first four, accounting for 70% of the variance, were chosen for theoretical consideration. Factor 1 (eigenvalue=6.4) accounted for 31% of the variance and consisted of the following high-loading variables: decreased squares crossed in the open-field test; decreased rears in the open-field test; increased latency to explore in the open-field test; increased latency to emerge in the novel item test; increased latency to exit the tube in the novel item test; increased latency to drink in the LPS test; increased latency to emerge from the tube in the LPS test; increased latency to exit from the tube in the LPS test; increased latency to attempt to remove the tail clip; increased latency to dig in the sawdust-digging escape test; increased latency to dig in the fox urine test; increased latency to float in the forced-swim test; and decreased CEA *fos*. Because these variables seem to be primarily involved with increased latency to approach novel stimuli, this factor was designated the initiative to explore factor.

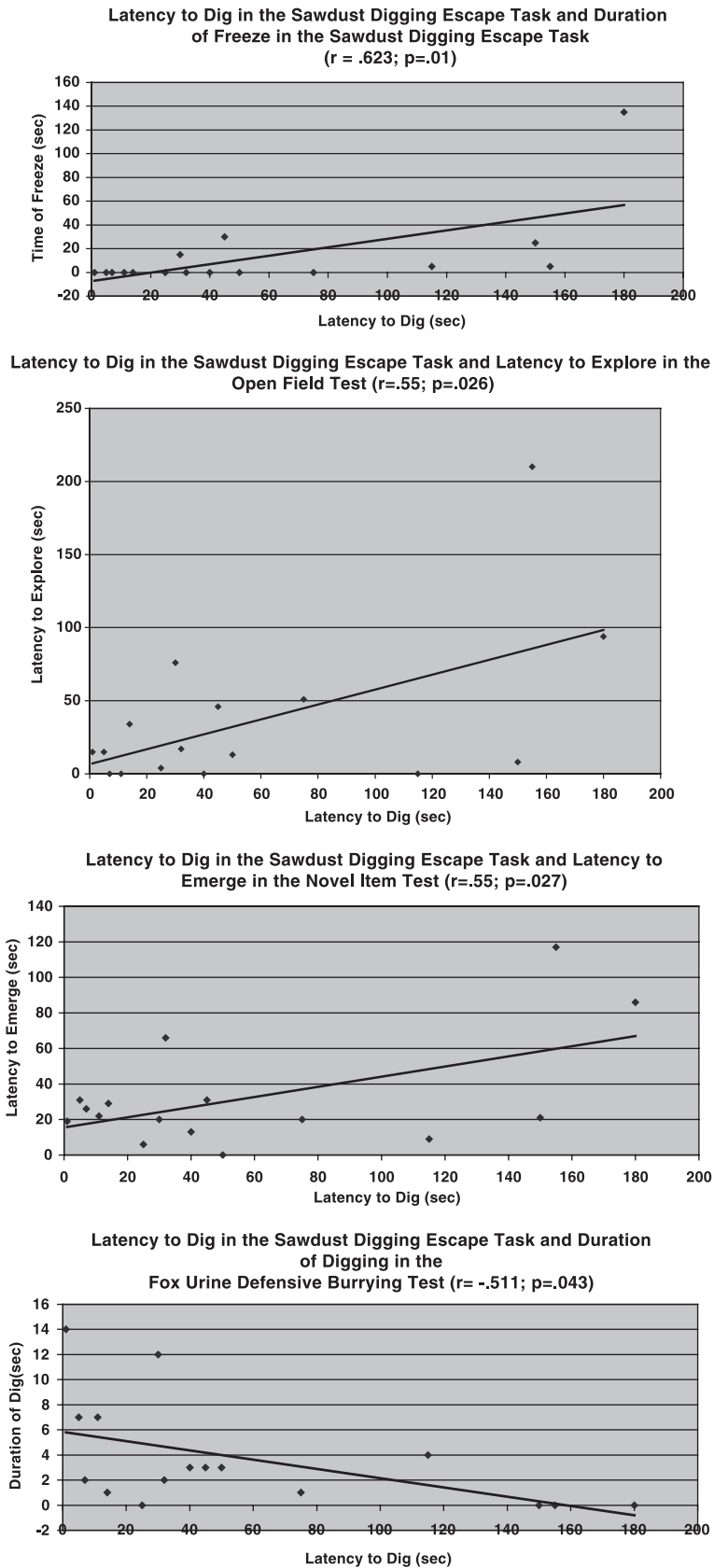


Fig. 2. Collection of scatterplots depicting the variables that were significantly correlated with the latency to dig in the sawdust escape task.

Factor 2 (eigenvalue=3.3) accounted for 16% of the variance and consisted of the following high-loading variables: higher recovery levels of CORT; increased latency to dig in the sawdust-digging escape test; increased duration of freeze response in the sawdust-digging escape test, and

decreased tendencies to dive in the forced-swim test. Accordingly, these responses were characterized as the low-reactive factor.

Factor 3 (eigenvalue=2.80) accounted for 13% of the variance and consisted of the following high-loading var-

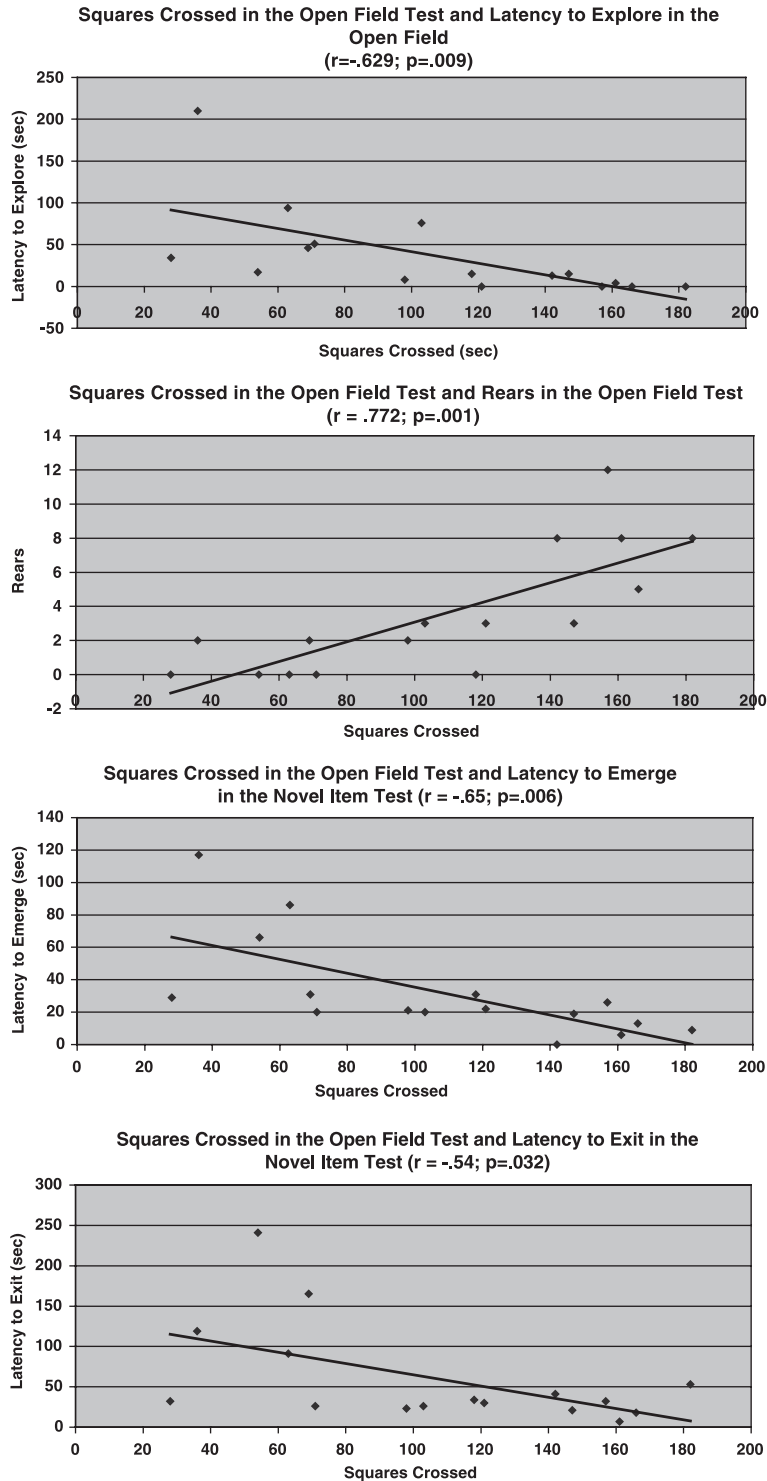


Fig. 3. Collection of scatterplots depicting the variables that were significantly correlated with the number of squares crossed in the open-field test.

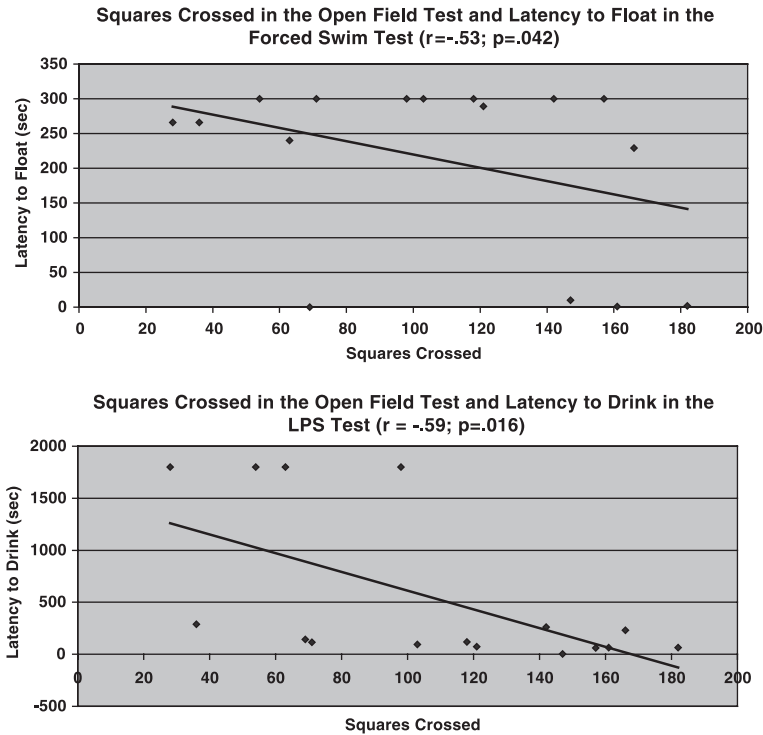


Fig. 3 (continued).

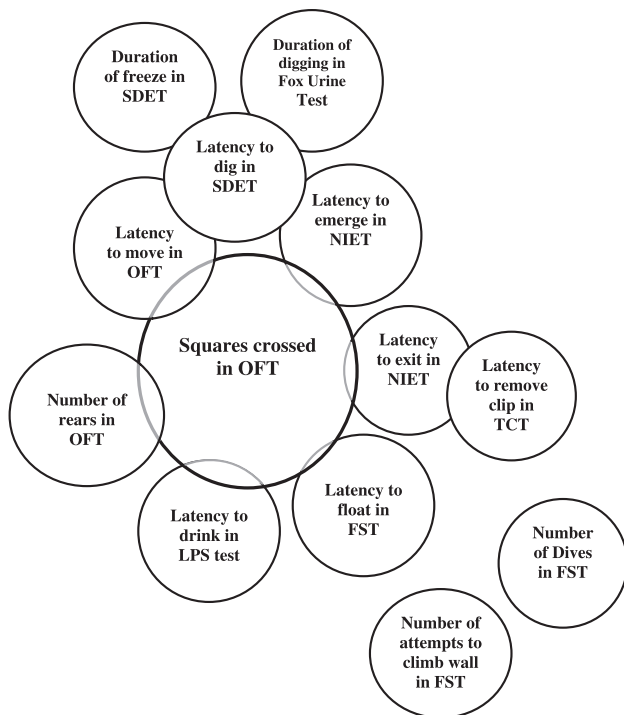


Fig. 4. Diagram showing the correlational relationship among behavioral measures recorded in the open-field test (OFT), novel item emergence test (NIET), forced-swim test (FST), sawdust-digging escape test (SDET), tail-clip test (TCT), LPS test, and fox urine test. Overlapping circles indicate that the two measures were significantly correlated.

ables: increased latency to dig in the fox urine test; decreased latency to emerge in the LPS test; decreased latency to exit in LPS test; and increased CEA and PVH *fos* immunoreactivity. Because of the variability observed in the behavioral responses (i.e., increased latency to dig in the fox urine test and decreased latency to emerge in the LPS test), they were characterized as the variable-reactive factor.

Finally, Factor 4 (eigenvalue = 2.2) accounted for 10% of the variance and consisted of the following high-loading variables: increased squares crossed in the open-field test; decreased duration of immobility; and increased latency to immobility. Due to the high persistence in responding to both novel environments, these responses were designated as the high-reactive factor. See Table 1 for a list of high-loading factors for each component.

### 3.1.3. Analysis of variance

One-way ANOVAs were used to determine the effect of gender on all of the dependent measures assessed in this study. In the forced-swim test, a significant main effect of sex on the number of times an escape attempt was made up the wall of the aquarium was observed [ $F(1,13)=21.871$ ;  $P=.0001$ ], where females tried to escape more times than males [means  $\pm$  S.E.M. =  $114 \pm 4.6$  and  $81 \pm 4.4$ , for females and males, respectively (see Fig. 5)]. There were no sex differences observed in the remaining dependent measures assessed in this study.

Table 1  
Emergent components in principal component analysis and the accompanying high-loading factors

Component	High-loading factors
Initiative to explore	< squares crossed in open field < rears in open-field test > latency to emerge in novel item test > latency to exit in novel item test > latency to drink in LPS test > latency to emerge in LPS test > latency to exit in LPS test > latency to remove tail clip > latency to dig in fox urine test > latency to float in forced-swim test < immunoreactive amygdala <i>fos</i> in response to fox urine test
Low reactivity	> CORT recovery values > latency to dig in sawdust-digging test > duration of freezing in sawdust-digging test < number of dives in forced-swim test
Variable reactivity	> latency to dig in fox urine test < latency to emerge in LPS test < latency to exit in LPS test > CEA and PVH immunoreactive <i>fos</i>
High reactivity	> squares crossed in center of open field < duration of floating > latency to float in forced-swim test

### 3.2. Immunohistochemistry data

A  $2 \times 2$  ANOVA revealed no effect of experimental condition (stress vs. control) or sex on the amount of *fos* immunoreactivity in the CEA or PVH.

### 3.3. Corticosteroid level

A  $2 \times 2$  ANOVA revealed that there was a significant effect of sex on the CORT concentration [ $F(1,25) = 24.589$ ;  $P = .001$ ]. Females had higher CORT concentrations [mean  $\pm$  S.E.M. =  $147 \pm 12.5$  ng/ml] than males ( $73 \pm 8.1$  ng/ml). (See Fig. 6.) There was no difference in CORT concentrations between stress and control animals of either

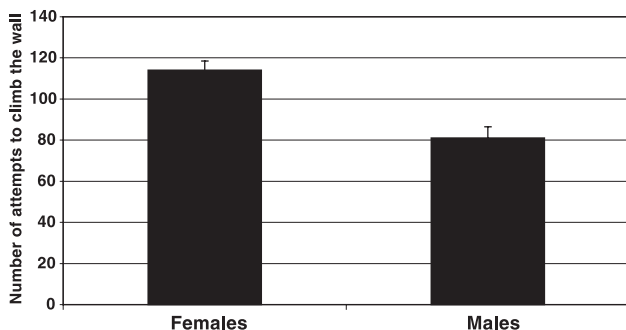


Fig. 5. Average number of attempts for males and females to climb the wall in the forced-swim task.

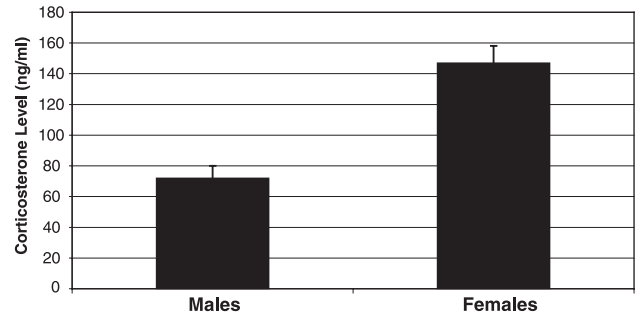


Fig. 6. Mean CORT levels in male and female rats approximately 1 h following the predator odor stressor.

sex. Since blood samples were not collected until 1 h after exposure to fox urine, it is possible that HPA axis activity had already returned to baseline.

## 4. Discussion

### 4.1. Behavioral consistencies in the correlational analyses

The relationship among the animals' latency to actively respond in some tasks (i.e., latency to dig in the sawdust escape task, latency to explore in the open field, and latency to emerge in the novel item test) were all significantly correlated. Further, general activity in the open-field test (i.e., number of squares crossed) was significantly correlated with more behavioral measures than any other behavioral index used in the current study. Number of squares crossed was correlated with six measures including latency to move in the open-field test, rearing frequency in the open-field test, latency to float in the forced-swim test, latency to emerge from the familiar tube in the novel object test, latency to exit the familiar tube in the novel object, and latency to consume milk in the LPS test. These data suggest that behavior in the open-field test may be predictive of responses in several other behavioral tests routinely used in stress studies. Combined with the aforementioned latency data, the consistencies observed in the latency correlational data in the current study support a previous study suggesting that measures of initiative are the most discriminative of tasks to assess coping strategies [1]. These consistencies, however, did not appear over all behavioral tasks. For example, none of the open-field measures were significantly correlated with the tail-clip measures.

The forced-swim test also appears to have two types of potential responses—a more passive response of floating and the more active escape responses of swimming, climbing the walls of the swim tank, and diving. Interestingly, a high level of general activity in the open field was associated with a long latency to float. Thus, animals that exhibited an active response to novelty (increased exploratory behavior) in the open-field test were less likely to adopt an inactive response to the forced-swim test. Ulti-

mately, whether an animal exhibits an “active” or “passive” coping style, or a reactive or proactive coping style, in a battery of behavioral tests may be influenced by aspects of initiative or inclinations toward general exploratory behavior [1].

#### 4.2. Principal component analysis

In accordance with the significant correlations observed with several latency scores, the most prominent component in the factor analysis was also related to latency to explore novel stimuli, or avoidance of novelty. All of the latency scores across the many stress tests (in addition to decreased exploratory behavior in the open-field test and decreased CEA *fos* immunoreactivity following exposure to fox urine) emerged as significant factors in this *initiative to explore* component. Interestingly, similar components have emerged in factor analyses of coping strategies in a variety of animals including an avoidance/disinterest factor [29] and activity and avoidance factors [30] in octopuses, curiosity and activity factors in the male stickleback fish [31], and both approach and avoidance components in children [32]. Physiological indices of HPA axis reactivity in response to the fox urine test, however, did not load onto this initiative variable, which may suggest that an animal’s initiative to explore a novel environment or stimulus may not be closely tied to HPA axis responsivity.

Factor 2, *low behavioral reactivity*, seemed to be the most closely related to HPA axis reactivity because the recovered level of CORT was the highest loading variable in this component. Because prior research suggests that more passive, or immobile, responses are associated with higher CORT responses [1], it is interesting that other high-loading variables, including latency to dig, duration of freeze in response to the animated intruder in the sawdust escape test, and decreased dives in the forced-swim test (all suggesting a rather immobile response) loaded onto this component along with the higher CORT recovery response.

Factor 3, *variable reactivity*, incorporated more flexible responses including increased latency to dig in response to the predator cue, fox urine, decreased latency to emerge and exit from the tunnel in the LPS novelty test, and higher activation in the PVH and CEA. The observation that acute stress sometimes facilitates immunoresponsivity [18,33] may explain why animals that seemed to have the highest neuroresponse to the stimulus recovered from the LPS novelty test by demonstrating decreased latencies in emerging and exiting the tubes following the LPS injection. Although most of the stress coping research has included animals selected for the extremes of coping strategies—that is, high active or low active—it is difficult to make direct comparisons of this theoretical category with past research. However, pigs with a more passive response strategy experienced enhanced humoral immunoresponsiveness, as observed in the recovery following the LPS test in the current study [17].

Factor 4, *high reactivity*, appears to be related to more active, persistent coping responses. These animals were more likely to explore the center activity squares in the open-field test and were more active in the forced-swim test, evidenced by a decreased latency to float and a decreased duration of floating. Although prior research suggests that these more active animals have lower levels of CORT, no endocrinological effects were observed in this study.

#### 4.3. Sex differences

As previously described in the literature [22], sex differences were not observed to be consistent across the behavioral and neurobiological measures in the present study but CORT concentrations were higher in females than males [21]. Male values were 50% less than female values. This difference in CORT levels, however, was not accompanied by differences in the behavioral response (i.e., digging) in the test preceding histological processing. Because of the high within-subjects variability in CORT responses, accompanied by the interesting varying sex effects, additional research exploring the efficiency of CORT recovery to baseline values following exposure to ecologically relevant stressors in male and female animals is warranted.

### 5. Conclusion

Although animal models are playing an increasingly important role in medical and psychiatric research [34], a plethora of questions exist concerning interpretation of the behavioral responses assessed in these models. Of interest in the present study was the identification of consistent patterns and categories of responses in male and female rats exposed to various stressors. Although the stress responses and/or coping strategies assessed in the present study were quite diverse, latency to explore novel stimuli was consistently correlated with behavior in open-field test. The strongest correlations existed among the behavioral tests that required exploration or movement within the test environment. Similarly, the first factor in the principal component analysis, the initiative to explore component, also was related to exploration of novel stimuli. Hence, an animal’s initiative (or hesitancy) to explore novel stimuli or environments seems to be an important aspect of “coping” that is assessed in several types of ecologically relevant stress tests. Additionally, more passive responses such as freezing in the sawdust-digging test and decreased dive responses in the forced-swim test loaded significantly onto the low-reactivity component. Brain *fos* immunoreactivity, as observed in the CEA and PVH, and decreased latency in the LPS novelty test were observed in the variable-reactive factor. Finally, the more proactive responses were observed in the high-reactive factor. Taken together, these results suggest that stress-induced coping responses to a cluster of tests may be related to an initiative to explore novel stimuli component. However,

propensity to explore is not necessarily a predictive factor for coping strategies employed when animals are exposed to more direct physical threats (e.g., threats that may require the animal to escape rather than explore) such as an approaching intruder. Finally, although CORT concentrations were higher in females than males, sex differences were observed in only one behavioral response test (i.e., climbing the wall in the forced-swim test). No sex differences were observed in *fos* immunoreactivity. The lack of consistency in physiological and behavioral responses to stress in male and female animals provides additional support for the increasingly evident sex-related dichotomies in physiological and behavioral stress responses.

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