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The Role of Prior Stressor Controllability and the Dorsal Raphé Nucleus in Sucrose Preference and Social Exploration

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Abstract

Research investigating how control over stressors affects behavior often utilizes freezing and shuttle escape learning as the behavioral endpoints. These endpoints have been argued to reflect anxious or depressed states, but these descriptions are problematic. The present study sought to determine the impact of stressor controllability and the dorsal raphé nucleus (DRN) on sucrose preference and juvenile social exploration, putative measures of anhedonia and anxiety that are commonly used in studies of stress per se. In Experiment 1 rats were exposed to escapable (ES) or yoked-inescapable (IS) tailshocks. In Experiment 2 ES or IS was given 7 days before all rats received IS. In Experiment 3 the DRN was inactivated during IS by microinjection of 8-OH-DPAT. Sucrose preference and social exploration were tested for several days after stress. A fourth experiment confirmed that juvenile social exploration is sensitive to traditional β -carboline and benzodiazepine manipulations. Both ES and IS reduced sucrose preference, but only IS reduced social exploration. Prior treatment with ES prevented the effect of IS on social exploration but did not prevent the effect of IS on sucrose preference and inactivation of the DRN prevented the effect of IS on social exploration but did not change sucrose preference. The present results indicate that social exploration but not sucrose preference is sensitive to prior stressor controllability, and that DRN activation mediates the effect of IS on social exploration. We argue that DRN-5-HT activation mediates a state of generalized anxiety produced by uncontrollable stress and that juvenile social exploration is a useful behavioral endpoint in stressor controllability studies.

Keywords

learned helplessness; anxiety; depression; serotonin; rat; social interaction

1. Introduction

The degree of behavioral control that an organism has over a stressor often determines the sequelae of that stressor [26] and may play a critical role in the development of pathological behaviors after a traumatic event [46]. Unfortunately, cognitive factors such as coping processes are difficult to study in animals. The stressor controllability paradigm, however,

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permits the manipulation of behavioral control, a component of coping. Here, rats are assigned to one of two treatments in yoked pairs: escapable stress (ES) in which the rat can escape an electric tailshock by turning a wheel or yoked-inescapable stress (IS) in which each shock is terminated only when the ES subject responds. For the IS subject turning the wheel has no effect. Exposure to IS produces behavioral sequelae that do not follow ES [5,26,43,44]. Consequences of stress that are unique to IS have been extensively reviewed [15,29,35,43] and called “learned helplessness effects” [26].

Although a number of behavioral and physiological endpoints have been used in stressor controllability experiments (see the reviews listed above), the experiments exploring the neural mechanisms underlying the dimension of control have typically utilized freezing in response to footshock and shuttle escape performance measured 24 hr or more after the stressor experience (e.g. [1,24]). IS in one environment later increases freezing immediately after footshock in a different environment and interferes with shuttlebox escape, but ES has no effect [22]. These outcomes reflect the stress history of the subject and have been argued to correlate with anxious [27] or depressed states [51]; for this reason variations of these procedures are commonly used as preclinical screens for novel anxiolytic or antidepressant compounds. Poor performance in the shuttlebox has been argued to reflect associative deficits [25], motor impairment [50], fatigue [30] and reduced pain sensitivity [18], as well as anxiety [27]. Thus, the meaning of poor shuttlebox escape is unclear. Furthermore, the shuttlebox procedure is complex, and different laboratories have used procedures that are quite varied, thereby generating a number of discrepancies (e.g. [31,48]).

To address these weaknesses the current experiments explored whether control over a stressor would modulate the impact of the stressor on later juvenile social exploration and sucrose preference. Social exploration has been argued to reflect approach/avoidance [12] and sucrose preference to reflect the hedonic status of the animal [52]. These procedures have the advantages of being simpler than the shuttlebox and easier to standardize. Thus, they might be viable alternative dependent variables for investigators interested in the investigation of stressor controllability.

The effects of stress exposure on sucrose preference have been the focus of much research (for reviews see [32,52]). However, the results have been equivocal. Sucrose preference is typically reduced following chronic stress exposure [52] but acute stress has been reported to increase sucrose consumption [10]. These outcomes have led to divergent interpretations. When sucrose preference is reduced, the behavior is described as anhedonia (e.g. [52]), but when consumption increases it is argued to reflect a response to metabolic demand [10]. Nevertheless, it is unknown whether or not alterations in sucrose preference are the result of stress *per se* or the result of uncontrollable stress because the parameter of control has not been manipulated in the reported research. In the present set of experiment sucrose preference is observed during the first 4 hours of the dark phase, a time when feeding behavior is greatest [4]. Since sucrose is only available to rats during the preference test, as opposed to continuous access as used by Dess (1992), any metabolic changes resulting from stress could be met by consuming food. Therefore, the sucrose preference test employed here may better reflect the rat’s hedonic state. In any case, it is presently unknown whether the controllability of the stressor is an important modulator of any effects of the stressor on sucrose preference.

The *adult* social interaction method of File and her colleagues (see [12]) has been used in our laboratory. IS, but not ES reduced time spent in social interaction for a period of days after stress exposure [44]. Social interaction is a widely used and pharmacologically valid assay of anxiety in the rat [12,28]. However, no experiments designed to explore the neural mechanisms underlying the effects of stressor controllability on social interaction have been reported. Thus,

whether the effects of IS on social interaction are mediated in a manner similar to those on escape behavior (e.g., critical involvement of the serotonin system [26]) is unknown.

A modified *juvenile* social exploration procedure adapted by Pollack et al. [37-39] from the widely cited Bluthé et al. procedure [7] is used here. In the juvenile test, a naïve juvenile (28-32 days old) stimulus rat is added to the cage of an experimentally treated adult and investigation of the juvenile by the adult is quantified by an observer. This method has three critical advantages over the adult social interaction procedure. First, the juvenile test is conducted in a familiar plastic cage rather than a test field and changes in social exploration can therefore not be attributed to novelty. Second, the adult experimental subject is less likely to engage in aggressive behaviors with a juvenile [6] which may confound interpretation of the behavior as relating to anxiety. Instead the adult primarily interacts with the juvenile by anogenital sniffing, allogrooming, chasing and pinning, behaviors categorized as investigative [12]. Third, in the adult method the dependent measure is the total interaction time initiated by a pair of rats with the same experimental treatment, thus requiring twice the number of rats and treatments than the statistical N reflects. Only social exploration initiated by the adult experimental subject is quantified in the juvenile test and the juvenile stimulus rats can be reused for multiple tests. Therefore, the quantities of rats are significantly reduced in the juvenile social exploration test.

Experiment 1 intended to replicate the findings of Short and Maier [44] using the juvenile social exploration procedure and to examine the impact of stressor controllability on sucrose consumption in a two-bottle sucrose preference test. These endpoints provide a basis for ascertaining the effects of stressor controllability on putative measures of anxiety and anhedonia. To further characterize the sensitivity of the social exploration and sucrose preference measures to the control dimension, Experiment 2 utilized a “behavioral immunization” procedure. The term behavioral immunization refers to the finding that an initial session of ES blocks the effects of a subsequent session of IS on freezing, shuttle escape failure and serotonergic activation [3], even if the treatments occur in different environments. Immunization has only been examined with shuttle escape and freezing to footshock as measures, and so the generality of this effect is unknown. The extent to which ES could block or reduce later IS effects on social exploration and sucrose preference is unknown. With regard to neural mediation, IS-induced changes in post-shock freezing and shuttle escape depend upon dorsal raphe nucleus (DRN) activation at the time of IS [24], this activation being prevented by behavioral control [1] (for review see [26]). The role of the DRN has only been examined with escape and freezing to shock as measures. And so the generality of these results and whether they would extend to social exploration and sucrose preference is unknown. Experiment 3 tested whether stress-induced changes in social exploration and sucrose preference are dependent upon activation of the DRN. To our knowledge juvenile social exploration has not been tested for sensitivity to classic anxiogenic or anxiolytic pharmacological manipulations. In Experiment 4, the anxiogenic β -carboline-3-carboxylic acid ethyl ester (β -CCE) or the anxiolytic benzodiazepine, diazepam, were administered to naïve rats 1 h before a 3 minute juvenile social exploration test. Sensitivity to these compounds would add support to the argument that time spent exploring a juvenile is an index of an anxious state.

2. Materials and Methods

2.1 Animals

Adult (60-70 days old and 275-360gm at time of testing) and juvenile (28-32 days old and 90-100gm at the time of testing) male Sprague-Dawley rats (Harlan; Indianapolis, IN) were housed in pairs. For Experiments 1-3 the vivarium maintained a reverse light/dark cycle (12 h light: 12 h dark, lights OFF at 9:00AM) with all stress treatments occurring during the first 5 h of the light cycle and behavioral testing occurring in the first 5 h of the dark cycle. For

Experiment 4 behavioral testing occurred in the first 5 h of the light cycle. Rats were allowed at least 7 days of acclimation. All procedures were approved by the University of Colorado Animal Care and Use Committee.

2.2 Escape/Yoked and Inescapable Tailshock Procedure

Tail shock was administered in $14 \times 11 \times 17$ cm acrylic wheel turn boxes enclosed in sound-attenuating chambers described previously [22]. Electric shock was delivered through copper electrodes augmented by electrode paste by a Precision Regulated Animal Shocker (Coulbourn Instruments, Allentown, PA). In the ES condition each rat was given 100 tailshocks presented on a variable interval-60 s schedule (VI-60). Turning the wheel at the front of the chamber terminated each tailshock according to a protocol previously described [1]. Shock intensity was 1.0 mA for the first 33 trials, 1.3 mA for the following 33 trials and 1.6 mA for the remaining 34 trials. These parameters were used to maintain escape behavior. Each tailshock for the IS subject terminated whenever its ES partner responded. A restrained control group was simply placed in the apparatus for a period equal to the ES and IS treatment while rats in a naïve homecage control group remained in their cages on the stress day. In Experiments 2 and 3 inescapable tailshocks were administered in acrylic restraint tubes (23.4 cm L. X 7 cm DIA).

2.3 Social Exploration and Sucrose Preference Tests

Each experimental subject was allocated a separate plastic tub cage with shaved wood bedding, food and a wire lid. Experiments 1-3 were conducted in 4 phases: Day 1 habituation, Days 2-4 sucrose preference baseline, Day 5 stress and Days 6-12 post-stress testing. On Day 1 tap water in the homecage was replaced with 2% sucrose in tap water to habituate rats to the novel solution. On Days 2-4, each rat was transferred to a single cage for the first 4 h of the dark cycle. Sucrose preference was determined with a two-bottle choice test using standard bottles, one filled with tap water and one with 2% sucrose in tap water. The locations of water and sucrose (left/right) were counterbalanced across days and treatments. Rats had free access to food during the two-bottle test. Sucrose preference was computed each day as the volume of sucrose consumed divided by the total fluid volume consumed multiplied by 100. Rats that did not establish reliable preference for sucrose (greater than 65%) were excluded.

On post-stress days the sucrose preference test was followed by a 3 minute juvenile social exploration test. Water bottles were removed and each rat, still in the test cage, was transferred to a dimly lit room (40 lux). After 15 min a 28-32 day old juvenile was introduced to the cage for 3 min and exploratory behaviors (sniffing, pinning, and allogrooming) were timed by an observer blind to treatment. After the test the juvenile was removed and the experimental adult rat was returned to the homecage. Although juvenile stimulus rats were reused for multiple tests, the adult was never tested with the same juvenile.

2.4 Cannula Placement and Microinjection Procedures

In Experiment 3 rats were surgically implanted with guide cannula to the DRN. Rats were anesthetized with isoflurane and a single guide cannula (26g, 15.5 mm; Plastics One, Roanoke, VA) was lowered to the DRN (AP=-8.1, LM=0, DV=-5.1) and fixed to the skull. Coordinates were taken from bregma and dura according to Paxinos and Watson [36]. A stylet was placed in the cannula and rats were allowed at least 7 days to recover. Microinjections of 0.5 μ l of 2 mg/ml 8-OH-DPAT (Sigma) in 0.9% saline or saline alone were administered 20 min before stress. Rats were gently restrained in a towel and a microinjector was inserted that extended 1mm beyond the cannula tip (33 g; Plastics One, Roanoke, VA). 8-OH-DPAT or saline was injected at a rate of 1 μ l/min; injectors remained in place for 2 min for diffusion. Cannula location was verified from 40 μ m sections stained with cresyl violet. Subjects were only included if the cannula tips were at least 50% within the target nucleus.

2.5 Experiment 1: The role of stressor controllability on sucrose preference and social exploration

After acclimation rats were given access to sucrose and 3 days of sucrose preference testing (Day 1: Habituation and Days 2-4: Baseline) to establish sucrose preference. On Day 5 rats were randomly assigned to either ES, yoked-IS, Restraint (R) or Homecage control (HC) groups ($n = 8/\text{group}$) and treated accordingly in the light phase of the light/dark cycle. At the beginning of the next dark cycle, 12 h after stress, post-stress sucrose preference and social exploration were measured. Sucrose preference was tested on 3 consecutive days. Social exploration was tested 12 & 36 h after stress. Based on the report of Short and Maier [44], it was expected that IS but not ES would reduce social exploration time. Although sucrose preference is reduced following several stressor regimens [52], the effects of stressor controllability on sucrose preference have not been studied.

2.6 Experiment 2: The effect of prior ES on IS-induced changes in sucrose preference and social exploration

To determine whether an initial ES experience would block, or immunize against, the effects of IS on social exploration and sucrose preference rats were first exposed to either ES, IS or HC treatment in wheel-turn boxes ($n = 8/\text{group}$). Restraint was not included because it had no effect on either behavioral endpoint in Experiment 1. HC remained in their cages. After the initial stress treatment rats remained undisturbed in their homecages for 3 days. On the next day, sucrose preference habituation and baseline began with the same schedule as in Experiment 1. On the seventh day after immunization treatment, all rats received 100, 5 s inescapable tailshocks (1.6 mA) on a VI-60 sec schedule in a restraining tube. Shock was administered in the restraining tube to reduce context similarities between the first and second stress exposures. Unstressed controls were not included because the critical comparison was between ES and IS pretreatment. Sucrose preference was tested 12, 36 & 60 h after stress and social exploration was tested 12, 36, 60 and 168 h after stress to explore the time course of effects.

2.7 Experiment 3: The role of DRN activation in the effects of IS on sucrose preference and social exploration

Rats were assigned to one of 4 groups in a 2 (IS or HC) by 2 (8-OH-DPAT or Vehicle) design, $n_s = 10/\text{group}$. Rats were then treated as in Experiment 1 with habituation, baseline, stress and post-stress testing. IS consisted of 100, 5 s 1.6mA tailshocks on a VI-60 sec schedule. This procedure exposed rats to approximately the same amount of shock as in yoked-IS subjects. An ES versus IS comparison was not made as the purpose of the experiment was to determine whether inhibition of the serotonergic dorsal raphe nucleus before IS would prevent the behavioral effects of IS. In the DRN the serotonin 1A receptor (5-HT_{1A}) behaves as an inhibitory somato-dendritic autoreceptor. 8-OH-DPAT is a 5-HT_{1A} agonist and can be used to silence serotonergic neuronal activity [19]. Since ES does not alter social exploration (Experiment 1 and [44]) or sensitize the DRN [2], there would be no effect of ES to block. The current procedure results in a significant reduction in animal usage. HC animals remained in their homecages. 20 min prior to IS, stressed and HC rats received a microinjection of 8-OH-DPAT or saline. This method has been used frequently to inhibit DRN 5-HT activity [19,24, 47]. After stress rats were tested for sucrose preference and social exploration as in Experiment 2.

2.8 Experiments 4A and 4B: Sensitivity of the juvenile social exploration test to anxiogenic and anxiolytic compounds

In Experiment 4A, rats were randomly assigned to either 0 or 1 mg β -carboline-3-carboxylic acid ethyl ester (β -CCE, Sigma) per kg body weight sonicated in 0.9% saline with a drop of

hydrochloric acid per 5ml ($n = 10/\text{group}$). In Experiment 4B, rats were randomly assigned to either 0 or 3 mg diazepam (Sigma) per kg body weight sonicated in 0.9% saline and 1% Tween 20 ($n = 10/\text{group}$). Injection volumes were 1 ml/kg body weight. In both experiments, naïve rats were weighed, injected according to group and placed in a standard plastic tub cage with bedding and a wire lid. After 1 h a juvenile was added for 3 min and exploratory behaviors were observed.

2.9 Data Analysis

Sucrose preference was computed as percent of baseline by dividing the preference of the test day by the percent preference of the third baseline day multiplied by 100. Data were converted in this way to normalize each rat's post-stress sucrose preference as percent of baseline preference and to account for individual differences in basal sucrose preference. Social exploration was analyzed as total exploration time in seconds. Sucrose preference and social exploration data were analyzed with separate analyses of variance (ANOVAs) or *t*-tests with Stress and Drug as between-subjects factors and Time as a within-subjects factor. Main effects and interactions were deemed significant if $p < 0.05$ and further explored with Fisher's Protected Least Significant Difference (PLSD) comparisons.

3 Results

3.1 Experiment 1: The role of stressor controllability in sucrose preference and social exploration

Mean sucrose preference is depicted in Figure 1 Panel A. One cohort of rats (ES, IS, R & HC) was excluded from analysis because the ES subject did not learn to escape tailshock. The resulting groups had $n_s = 7$. Although both ES and IS treatments reduced sucrose preference 12 and 36 h after stress, whereas HC and R treatments had no effect, a 4 (Stress) X 3 (Time) ANOVA revealed no significant effects for Stress, Time or the Stress X Time interaction. Still, post hoc pair-wise contrasts showed that ES and IS reduced sucrose preference compared to R and HC at 12 h, $ps < 0.05$, indicating that stress, regardless of controllability, produced a transient reduction in sucrose preference. Mean time spent in social exploration is depicted in Figure 1 Panel B. IS suppressed social exploration compared to HC controls which did not differ from ES and R treated rats. A 4 (Stress) X 2 (Time) ANOVA revealed a main effect for Stress $F(3,24) = 4.287$, $p < 0.05$ and no significant effects for Time or Stress X Time interaction. Post hoc comparisons indicated that time spent in exploration was significantly reduced in IS treated animals compared to all other groups 12 h after stress, but only differed significantly from R and HC 36 h after stress, $ps < 0.05$. ES did not significantly differ from HC or R at either time point. These data indicate that social exploration is reduced by stress and that this is dependent on the controllability of stress. However, sucrose preference was reduced by stress *per se*. Restraint alone had no effect on either measure indicating that no part of the effect of IS can be attributed to the handling or restraint inherent in IS treatment.

3.2 Experiment 2: The effect of prior ES on IS-induced changes in sucrose preference and social exploration

The goal of Experiment 2 was to determine if the reduction in social exploration and sucrose preference observed after IS could be prevented by prior ES. Sucrose preference is depicted in Figure 2 Panel A. As in Experiment 1, sucrose preference was reduced in all groups that received IS. A 3 (Stress) X 3 (Time) ANOVA detected a significant main effect for Time, $F(2, 42) = 19.541$, $p < 0.001$, but no other significant effects. The effect of stress is evident across days as each rat's baseline preference provided its own control. Sucrose preference returned to baseline levels in all rats by 60h. Post hoc comparisons between days indicated that sucrose preference increased significantly at each timepoint, $ps < 0.01$. That is, at 12 h sucrose preference was significantly lower than baseline levels that returned at 60 h but the

controllability of prior stress had no proactive effect on sucrose preference. Mean time spent in social exploration is depicted in Figure 2 Panel B. Here, prior ES prevented the reduction in social exploration by later IS. A 3 (Stress) X 4 (Time) ANOVA identified a significant main effect for Stress, $F(3, 21) = 4.075, p < 0.05$, but no other significant effects. Post hoc comparisons found that social exploration was significantly greater in the ES group compared to both IS and HC at 60 & 168 h, $ps < 0.05$. Control during the initial stress experience appeared to proactively interfere with the development of social exploration deficits caused by IS. To confirm that the effects observed on social exploration were not related to motor deficiency or avoidance by the juvenile the number of social exploration encounters initiated by the adult was tallied for each pair of rats at the 168 h timepoint, the time at which group differences in social exploration time were greatest. The group means (S.E.M. in parentheses) were ES = 42.4 (4.0), IS = 39.9 (4.3) and HC = 40.8 (2.8). A one-way ANOVA found no significant effect of prior stress treatment indicating that the adults initiated the same amount of exploratory behavior regardless of the adult's treatment. However, taken with the group differences in exploration time, these data provide compelling evidence that IS-treated adults have equal opportunity to engage with the juvenile but actually disengage from interaction with the juvenile. This active avoidance accounts for the difference in time spent in exploration after IS, compared to ES.

3.3 Experiment 3: The role of DRN activation in the effects of IS on sucrose preference and social exploration

Experiment 3 determined whether DRN activation by IS is necessary for IS-induced reductions in social exploration and sucrose preference. Subjects with misplaced cannulae or symptoms of infection prior to stress were excluded; cannula placements are illustrated in Figure 3 Panel C. The size of each treatment group was: IS-VEH, $n = 10$; IS-DPAT, $n = 8$; HC-VEH, $n = 9$; and HC-DPAT, $n = 6$. Sucrose preference is depicted in Figure 3 Panel A. Again, IS reduced sucrose preference but inhibition of the DRN by 8-OH-DPAT appeared to have no interaction with that effect. A 2 (Stress) X 2 (Drug) X 3 (Time) ANOVA revealed a significant main effect for Time, $F(2, 58) = 15.45, p < 0.001$, a significant main effect for Stress, $F(1, 29) = 5.79, p < 0.05$ and a significant Stress X Time interaction, $F(2, 58) = 4.50, p < 0.01$. No other effects approached significance. To explore the Stress X Time interaction, the mean sucrose preference of IS was compared to the mean of HC (pooling between drug conditions) at each timepoint. IS significantly reduced sucrose preference at the 12 h point, $p < 0.01$. Therefore, IS reduced sucrose preference but the effect was not prevented by 8-OH-DPAT. Mean time spent in social exploration is depicted in Figure 3 Panel B. Here, IS appeared to reduce social exploration but only in the IS-VEH group. A 2 (Stress) X 2 (Drug) X 4 (Time) ANOVA revealed a significant main effect of Stress, $F(2, 29) = 5.24, p < 0.05$ and a significant Stress X Drug interaction, $F(1, 29) = 10.50, p < 0.01$. No other effects reached significance. To explore the interaction, the simple effect of Drug was tested at each level of Stress. 8-OH-DPAT significantly increased social exploration only in the IS group, $F(1, 29) = 7.36, p < 0.05$. Thus, IS reduced social exploration for 7 days, and this effect of IS was blocked by intra-DRN 8-OH-DPAT microinjection. Thus, sucrose preference appears to be modulated by neither stressor controllability or by the DRN but both modulate social exploration.

3.4 Experiments 4A and 4B: Sensitivity of the juvenile social exploration test to anxiogenic and anxiolytic compounds

Figure 4 depicts the mean time spent in social exploration after β -CCE or diazepam treatment. For Experiment 4A, a t -test identified a -CCE, $t(18) = 3.037, p < 0.01$. For Experiment 4b, a t -test identified a significant increase in social exploration produced by 3mg/kg diazepam, $t(18) = 2.193, p < 0.05$.

4 Discussion

The present experiments sought to determine whether the controllability of a stressor modulates the impact of the stressor on a putative measure of anhedonia, sucrose preference, and a putative measure of anxiety, juvenile social exploration. Although the control dimension has been accorded a prominent role in discussions of how stressors participate in the induction of various psychopathologies (e.g., [46]), actual research has been almost entirely restricted to an examination of shuttlebox escape and post-shock freezing. The rationale for using these endpoints is largely historical, rather than because the behaviors model aspects of clinically relevant conditions. The study of stressor controllability stemmed from a report that exposure to classical conditioning in which a tone was paired with shock in one environment, a Pavlov harness, produced failure to learn to escape and avoid in a different environment, a shuttlebox [33]. The subjects did not learn to escape or avoid after the initial classical conditioning, an unpredicted finding. Of course, the shock during classical conditioning was inescapable, and Seligman and Maier [40] attempted to determine whether this aspect of the initial classical conditioning was responsible for producing the later failure to learn, and it was. This 1967 study, along with a report that control reduced the impact of the stressor on ulcer formation [49] began the study of stressor controllability. Quite naturally, failure to learn to escape became a behavioral endpoint in studies designed to examine the neurobiological mechanism(s) by which control exerts its actions and post-shock freezing was added because it could be easily measured during the escape session.

Thus, shuttlebox escape and post-shock freezing were studied as the behavioral endpoints in stressor controllability studies only because Seligman and Maier [40] were attempting to discover why classical conditioning had interfered with escape and avoidance learning, not because the behavior was thought to reflect some underlying state of interest. Although the shuttle escape deficit is sensitive to some anxiolytic and antidepressant treatments (e.g., [11, 42,55]), escape learning or post-shock freezing have not been extensively validated as animal models of any particular condition. Other behaviors have been used as endpoints in uncontrollable stress experiments, such as reduced duration of swimming when placed in water (e.g., [45]), but to our knowledge there are no published studies demonstrating that reduced swimming was a result of the uncontrollability/controllability of the stressor. Only paradigms demonstrated to be sensitive to control are relevant for determining the mechanisms by which control operates, and not all sequelae of stressors are sensitive to control. For example, exposure to tailshocks identical to those used here reduced later activity in a running wheel, but this effect was not sensitive to control [54]. That is, ES produces a reduction in activity equal to that produced by IS. Thus, studies using this endpoint would not be relevant to understanding mechanisms by which control or lack of control operates. It may be noted that other behaviors have been shown to be sensitive to stressor controllability (e.g., shock-elicited aggression [23] and eyelid conditioning [21]), but these have not been used in experiments designed to determine underlying mechanisms of controllability.

The results of the present experiments were clear. The stressor-induced reduction in social exploration with a juvenile was controllability dependent, with IS but not ES reducing social exploration. In Experiment 2 of the present study we observed that the juveniles engage equally with the adults regardless of the adult's stress treatment, therefore a reduction in social exploration is likely due to an active avoidance of social exploration. Finally, Experiment 4 demonstrated that the juvenile social exploration procedure is sensitive to classic anxiogenic and anxiolytic pharmacological treatments, further supporting the conclusion that reduced social exploration by IS reflects a state of anxiety.

Stressor controllability does not modulate the effects of stressors on all assessments of anxiety. For example, both ES and IS reduced open arm exploration in the elevated plus maze to the

same degree [14]. The fact that two very different social interaction paradigms have now been shown to be sensitive to stressor controllability suggests that social behavior might be selectively responsive to control. File and colleagues (e.g., [8]) suggested that social interaction reflects generalized anxiety while the elevated plus maze reflects the escape aspects of panic. If this is so, then the present results indicate that control is important in regulating generalized anxiety. It has been argued that the activation of DRN 5-HT neurons is uniquely related to generalized anxiety [13,41], and so the dissociation between the effects of control on social exploration and the lack of effect on the elevated plus maze is consistent with a critical role of DRN 5-HT in mediating the effects of control on anxiety.

The present experiments add two new findings with regard to stressor control and social interaction, and these strengthen the parallel to shuttle escape as an outcome. First, as with shuttle escape, a prior experience with ES blocked the effects of later IS on juvenile social exploration. This immunizing effect has not been previously demonstrated with any behavioral outcome other than shuttle escape. As with effects on shuttle escape, DRN 5-HT inhibition with 8-OH-DPAT during IS blocked the reduction in social exploration. It can be noted that social interaction seems especially sensitive to DRN 5-HT manipulations (e.g., [9,34]) and to peripheral 5-HT treatments after different inescapable stress procedures [16,17]. Furthermore, reduced binding of the 5-HT_{1A} autoreceptor, a biomarker of 5-HT sensitization, within the midbrain raphe has been correlated with social anxiety disorder [20]. These findings parallel the current data in that activation or sensitization of serotonergic cells, as would be the result of low 5-HT_{1A} binding and IS, enhances anxious behavior in a social setting.

Interestingly, the stress-induced decrease in sucrose preference was not prevented by a) behavioral control, b) the prior experience of control, or c), intra-DRN 8-OH-DPAT during the stressor. A search of the literature on sucrose preference and the DRN yielded only one paper. Wirtshafter and Asin [53] found that lesion of the DRN prevented the catalepsy produced by haloperidol, but not the reduction in sucrose preference. Thus, if the critical mediator of stressor controllability effects involves regulation of DRN activity, then it is sensible that control did not modulate the impact of the stressor on sucrose preference. The fact that controllability and intra-DRN 8-OH-DPAT failed to prevent the effect of stress on sucrose preference while both effectively prevented the effects on social exploration supports the contention that stressor controllability modulates behavior by regulating DRN activity.

The absence of a modulating effect of control or of DRN 5-HT inhibition on the stressor-induced reduction in sucrose preference underscores the need to manipulate stressor controllability in studies that are concluded to be relevant to stressor controllability/learned helplessness. There are numerous instances in which an uncontrollable stressor such as IS is shown to have an effect on some measure, relative to non-stressed controls. Some biological variable is then shown to mediate the effect, and it is concluded that it is relevant to the uncontrollable nature of the stressor. Such studies can be misleading as not all outcomes of exposure to stressors are related to stressor controllability—some are simply produced by stress *per se*. The data here, for example, demonstrate that changes in sucrose preference are not related to the presence or absence of behavioral control.

In sum, the present data provide strong support for the use of juvenile social exploration as a behavioral endpoint in neurobiological studies of stressor controllability. It has the advantages that it requires little or no instrumentation, is easy to standardize, and has been validated as relating to anxiety. Indeed, Maier & Watkins [27] have argued that the full pattern of behavioral sequelae of uncontrollable stress can be captured by anxiety.

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Figure 1. Mean ($\pm$ S.E.M.) sucrose preference (Panel A) and social exploration (Panel B) after ES, IS, R, or HC treatment. *ES and IS significantly reduced sucrose preference 12 h after stress, p s < 0.05 . Sucrose preference returned to unstressed levels by 60 h post-stress. **IS significantly reduced social exploration 12 h after stress but neither ES nor R had an effect.

Figure 2.

Mean ($\pm$ S.E.M.) sucrose preference (Panel A) and social exploration (Panel B) after a session of 100 inescapable tailshocks. Group designations indicate stress treatment 7 days prior to testing. Sucrose preference was significantly lower than baseline levels 12 and 36 h after stress, $p < 0.05$ but prior stress treatment had no detectable effects. Prior escapable stress treatment appeared to interfere with the reduction of social exploration after IS. *ES pretreated rats spent significantly greater time in social exploration than IS or HC pretreated rats 60 and 168 h post-stress, $p < 0.05$.

Figure 3.

Mean ($\pm$ S. E. M.) sucrose preference (Panel A) and social exploration (Panel B) after IS and microinjection of 8-OH-DPAT (DPAT) within the DRN. Locations of microinjector tips are illustrated in Panel C. IS significantly reduced sucrose preference 12 h post-stress regardless of drug condition, $p < 0.05$. Preference returned to unstressed levels by 60 h post-stress. Social exploration was reduced only in IS-Saline treated subjects, $p < 0.05$, whereas 8-OH-DPAT prevented this effect on all test days.

Figure 4.

Mean (+ S. E. M.) social exploration 1 h after systemic β -CCE. (Panel A) or diazepam (Panel B). * β -CCE significantly reduced social exploration compared to vehicle controls, $p < 0.01$.

**Diazepam significantly increased exploration time compared to vehicle controls, $p < 0.05$.