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The Effects of Habituation on Separation-Distress Vocalizations in the Anxiety-Depression Continuum

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by
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Abstract

The chick model of the anxiety-depression continuum has been used to examine the disorder's pathology and treatment outcomes. However, little is known about how habituation to different stressors affects the behavior of the animal. In this study, different groups of socially-raised chicks received habituation to the experimenter via handling, acclimation to the apparatus with a social companion, or acclimation to the apparatus under an isolated condition for 4 days prior to a 60 min isolation test in which the animal's latency to vocalize and number of distress vocalizations were recorded. The latency to vocalize decreased and the number of distress vocalizations increased as a function of increasing habituation exposure. This information about the relationship between variables will be useful in future research using the anxiety-depression model in psychopharmacology research.

Introduction

Mental disorders are extremely serious diseases due to their early age of onset, chronicity, and high prevalence. Over their lifetime, about half of the American population will find themselves impaired by at least one mental disorder (Andrade *et al.*, 2000). Kessler *et al.* conducted a survey and found that of those who meet the criteria for a *DSM-IV* diagnosis of a mental disorder, 55% have just one disorder; 22% have two disorders; and 23% have three or more disorders. The lifetime prevalences of the various types of disorders are: social phobia, 12.1%; specific phobia, 12.5%; alcohol abuse, 13.2%; substance use disorders, 14.6%; major depressive disorder, 16.6%; mood disorders, 20.8%; impulse control disorders, 24.8%; and any disorder, 46.4%. The most prevalent and most chronic class of mental disorders is anxiety disorders with a prevalence of 28.8%. Usually mental disorders appear in childhood or adolescence: half of the disorders start by age 14, and three fourths starts by age 24 (Kessler *et al.*, 2005).

Not only burdensome, mental illnesses can also be very dangerous to the individual and others. A large percentage of mental disorders (40.4%) are classified as being mild. However, 37.3%, which is a significant figure, are classified as moderate. A moderate disorder is depicted by: moderate work limitations, substance addiction without significant role impairment, and suicidal ideation, scheme, or gesture. Even graver are the remaining 22.3% of mental disorders which are classified as serious. The characteristics of serious disorders are critical: work disability, untreatable psychosis, bipolar disorder, substance addiction with serious role impairment, and a 12-month suicide attempt with serious lethality intent. Therefore, having a mental disorder can contribute to an individual's suicidal state. Among the people who have suicidal ideation, 52.4% also have at least one anxiety disorder. Correspondingly, 64.1% of those who have attempted suicide have at least one anxiety disorder (Sareen *et al.,* 2005). In addition to suicide, mental illnesses are also a factor in some inappropriate or violent behavior, which results in severe consequences.

In the United States, one sixth of adult prisoners suffer from a mental disorder. Prisons contain three times more mentally ill people than mental health hospitals. Not only are the mentally ill prisoners locked behind bars for crimes which were likely beyond their control, but they are also regularly punished for their improper behavior: aggression, violence, and unruliness. Sadly, the most probable victims of beating, manipulation, extortion, and sexual abuse in prison are the mentally ill inmates (Davies 2003). Several untreated individuals with mental disorders suffer in other ways; one half of all homeless people are mentally ill. They endure very serious psychiatric disorders: major mood disorder, 19-30%; schizophrenia, 11-17%. They also suffer from drug abuse (20-30%) and alcohol abuse (57-63%) (McQuisiton *et al.,* 2003).

Mental disorders obviously cause much distress and agony to their victims. They diminish the quality of relationships, daily activities, and most importantly, life, for millions of Americans. The symptoms of these debilitating diseases could be reduced or eliminated by a rich understanding of each disorder that could lead to improved drugs. Therefore, research must continue to be conducted in order to broaden our knowledge about specific characteristics of mental disorders and to test the effects of various drugs: antipsychotic, anxiolytic, and antidepressant medications. Animal models have proven useful in understanding the etiology, pathophysiology, and treatment options for a variety of human clinical conditions. Using animal models should directly impact the quality of human life.

Animal models allow researchers virtually limitless exploration in unexplained disorders and their victims. The best animal models allow researchers to study all aspects of a disease, including its etiology and pathology. Some thorough investigations of a disorder cannot possibly be conducted using humans. For example, a researcher cannot study a baby's perinatal disease in its embryo stage; this stage can be studied in animals, though (Hafezparst *et al.,* 2002). Even though the research is not conducted on humans, the resulting information that is obtained is beneficial to them. This is due to the fact that animal models actually do model human disorders and symptoms. Research has shown that a cross-reference can be made between humans and other species. The mouse, for example, has nearly the same DNA sequence as people; it therefore, offers an excellent model for those studying genetic disorders (2002). Jesberger and Richardson found that antidepressants that are effective in people also produce behavioral and neurochemical effects in animals; thus, animal models contribute to drug research (Jesberger 1985). Sufka *et al.* (2006) modeled an anxiety-depression continuum in domestic fowl chicks which permitted observations of the disorder and the effects of various anxiolytics and antidepressants. Multiple animal models have provided the scientific community with advanced information about disorders. There are various ways in which animal models can be used, depending on what is being researched.

Willner has suggested that there are three types of behavioral models: behavioral bioassays, screening tests, and simulations. Neuroscientists utilize behavioral bioassays to understand brain function. This model permits one to analyze the operative state of a fundamental physiological system. The behavioral bioassays are used for purposes such as examining the structures which cause changes in brain functioning. Industrial pharmacologists utilize screening tests since they concern drug actions. Screening tests assist in accelerating the findings of novel drugs by uncovering agents that have anxiolytic, antidepressant, or certain other medical effects. The object of a simulation is to model a human behavior, particularly an abnormal behavior, in animals by inducing the symptom(s) of a disorder or a complete syndrome. Disorders are induced via various means: stressors, ageing, or brain damage. Once the animal has the desired symptom or disorder, its behavioral state can be observed so that psychologists can determine the etiology, physiological basis, and treatment of the disease. If the treatment is successful, the underlying physiological systems can also be studied. These observations of animal behavior allow psychologists to broaden their comprehension of human psychological processes (Willner 1991).

In this laboratory's recent work, we have tried to simulate an anxiety-depression continuum with young domestic fowl. The purpose of this experiment is to illustrate the close relationship and similarities between anxiety and depression. In our anxiety-depression model, chicks are socially raised, and then put in a 2-hour isolation stress test. During this test, their distress vocalizations are measured. In general, the rate of distress vocalizations start high; then after 20 min, they diminish to half of the initial rate. The initial high rate of distress vocalizations is characteristic of an anxiety phase, and the later reduced rate of distress vocalizations is characteristic of a depression phase, or learned helplessness. Anxiolytics diminish the number of distress vocalizations during the anxiety phase, and antidepressants increase the number of distress vocalizations during the depression phase. The reversal of the rates of distress vocalizations in each phase by these drugs demonstrates that the chicks are experiencing anxiety, then depression. Corticosterone, which indicates the chick's degree of stress, is also measured throughout the experiment. Plasma corticosterone levels are significantly higher at first, then decline; this further supports the validity of the anxiety-depression continuum. Therefore, this animal model is a simulation for an anxiety-depression continuum.

According to Willner (1991), a good simulation must meet certain criteria: same etiology, same symptoms, same pathology, and same treatment outcomes. One criticism of the chick anxiety-depression continuum model is that it may not effectively simulate the true etiology of depression. In humans, depression is the outcome of many factors accumulating in a gradual process: genetic factors, general psychological susceptibilities, and specific psychological susceptibilities. In some animal models of depression, this etiology is simulated by the application of chronic mild stressors. For example, Kenneth *et al.* (2006) used an animal model to simulate depression in domestic pigs by means of recurring stressors. This stressor treatment involved repeatedly placing a test pig with an unfamiliar, larger pig. Consequently, each test pig continually experienced aggressive encounters which resulted in hypercortisolaemia and lower weight gain. There were other environmental stressors as well: removal of bedding, wetting of bedding or unavoidable airflow. Therefore, this animal model, like many others, involves inducing depression by means of repeated measures. In contrast, our model, the chronic mild stressor paradigm, creates depression in chicks using one persistent stressor over a long period of time. Using an acute, but chronic stressor for the chick makes the model unlike the actual stressors that create depression in humans. The criticism is valid since a simulation requires similar etiology, and people do not get depressed from an acute stressor.

Pilot studies in this laboratory sought to simulate the etiology of anxiety-depression by incorporating a chronic mild stressor procedure into the chick separation stress paradigm. Social separation was used as the mild stressor. On days prior to the 1hr test session, socially raised chicks were put in isolation for 15 min either 4 times, 3 times, 2 times, 1 time, or not at all. Distress vocalizations were measured during the test session. We predicted that birds which had not received the chronic mild stressor would experience anxiety, and then depression. We predicted that birds which had been chronically mild stressed would never experience anxiety, but instead, immediately go into a phase of learned helplessness (depression). The experiment produced unexpected results: chicks that received a greater number of the mild stressor of social separation displayed a greater number of distress vocalizations during a subsequent isolation test session.

The current study is an attempt to explain the unanticipated results from the chronic mild stressor study. This experiment controls the extent of habituation each group of chicks receives: being handled by the experimenter, being handled and acclimated to the apparatus, or being handled and acclimated to the apparatus under an isolation condition. By varying the amount and type of habituation each group of chicks receives, we can study the relationship between that and distress vocalizations in the anxiety-depression model. Chicks distress vocalize in response to separation stress; the total number of distress vocalizations reflects the degree of stress they feel. However it is hypothesized that the relationship between stress and distress vocalizations is best represented by an inverted U-shape function with the greatest amount of distress vocalizations falling between a medium and a high stress level. Further increases in stress would lead to a slight decline in distress vocalizations. When a chick is maximally stressed, it does not produce a large number of distress vocalizations because it has a panic-like response that inhibits them. It is hypothesized that any manipulation that attenuates the stress the chicks experience should initially elevate distress vocalizations during the isolation test.

Method

Subjects

Cockerels (*Gallus gallus*, strain W-36; Cal-Maine Foods, Mendenhall, MS USA) were obtained 1-day posthatch and housed in stainless steel cages (44 x 61 x 40 cm) at a population density of 12 chicks per cage. Food (Purina Start and Grow, Purina Mills, St. Louis, MO, USA) and water were available ad libitum through 1-quart gravity-fed feeders (Model 4YQW0, Murray MacMurray, Webster City, IA, USA) and waterers (Model 4YQW0, Murray MacMurray, Webster City, IA, USA). Room temperature was maintained at 29 ± 1 °C and overhead florescent illumination was maintained on a 12-h light-dark cycle provided by fluorescent overhead lighting. Daily maintenance was conducted during the first quarter of the light cycle. Maintenance included refreshing water, replenishing food, and changing the newspaper under the cages.

Apparatus

The six-unit test apparatus contained Plexiglas viewing chambers (25 x 25 x 22 cm) situated in sound-attenuating enclosures. Each unit was illuminated by a 25-W light bulb and ventilated by an 8-cm-diameter rotary fan (Commonwealth Model FP-108AXS1, Rodale, Great River, NY, USA). Miniature video cameras (Model PC60XP, SuperCircuit, Liberty Hill, TX, USA) mounted at floor level in the corner of the enclosures and routed through a multiplexer (Model PC47MC, SuperCircuit, Liberty Hill, TX, USA) allowed for animal observation. Distress vocalizations were recorded by microphones [Model 3-675-001 (modified), Lafayette Instruments, Lafayette, IN, USA] mounted at the ceiling of the Plexiglas chamber and connected to digital sound-activating relays (Model 630400A, Lafayette Instruments, Lafayette, IN, USA; settings: 75% sensitivity and 0.10-s delay) that triggered electromechanical counters (Model 58004, Lafayette Instruments, Lafayette, IN, USA).

Procedure

The experiment was conducted across days 2-6 post-hatch. The experimental design consisted of 4 treatment conditions. These included 1) a group of chicks that was neither handled nor received any apparatus habituation before test (No HH), 2) a group of chicks that received handling by the experimenter for < 1 min daily but received no apparatus habituation (Hand), 3) a group of chicks that for 4 days received handling and apparatus habituation in which they were placed in the test chamber for 15 minutes with

1 social companion (Hab-Soc), and 4) a group of chicks that for 4 days received handling and apparatus habituation in which they were placed in the test chamber for 15 minutes under an isolated condition (Hab-Iso). Sample sizes were n=12. The habituation trials were conducted on Days 2-5 post-hatch. On day 6, all 4 groups were placed in isolation in the apparatus for a 60-min stress test session. Dependent measures collected during the 4 habituation trials and the isolation test session were (1) latency to first vocalize and (2) number of distress vocalizations. Distress vocalizations were counted in 5-min blocks. Animals were returned to their home cage following tests. These procedures were approved by the University of Mississippi IACUC (Protocol # 06-013) and were conducted in accordance with the principles of laboratory animal care as detailed in the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Data Analysis

Data were analyzed using one- and two-way repeated measures analysis of variance (ANOVA). Post hoc analyses were conducted using Fisher's LSD.

Results

The latency to vocalize and the total number of distress vocalizations during the four 15 minute habituation trials for the Hab-Soc and the Hab-Iso test groups are summarized in Fig 1A and B, respectively. In general, isolated groups had shorter latencies to vocalize and higher rates of distress vocalizations than social groups. A two-way ANOVA on the latency to vocalize data revealed a significant Isolation Treatment effect $[F(1, 45) = 6.65, p < .03]$. The Habituation Treatment and the Isolation x Habituation interaction terms were not significant. A two-way ANOVA on total vocalizations during the habituation test session revealed a significant Isolation Treatment effect $[F(1, 42) = 44.76, p < .0001]$ and a significant Isolation x Habituation interaction term $[F(3, 42) = 11.76, p < .0001]$. The Habituation Treatment effect was not significant. In general, isolated groups tended to increase distress vocalizations while social groups tended to decrease distress vocalizations over the 4 habituation trials.

The latency to distress vocalize during the 60 min isolation test session for all 4 groups (No HH, Hand, Hab-Soc, Hab-Iso) is summarized in Fig 2. The No HH group and the Hand group had longer latencies to vocalize than the Hab-Soc and the Hab-Iso groups. Consistent with these observations, a one-way ANOVA revealed a significant Treatment effect $[F(3, 40) = 2.79, p = .0528]$. Post hoc analyses demonstrated that the Hab-Soc and the Hab-Iso groups had significantly shorter latencies to vocalize than did the No HH group ($p < .05$).

A time-course analysis, in 5 min blocks, of distress vocalizations across the 60 min isolated test session for the 4 treatment groups is shown in Fig 3. The pattern of distress vocalizations illustrates two notable effects. First, the No HH control group had lower distress calls, whereas those animals that were handled or handled and habituated had higher distress vocalizations. Second, distress vocalizations were highest during the initial 5 min block, declined over the next 3 blocks to about 40% of the initial rate and remained stable thereafter regardless of treatment group. A two-way repeated measures

ANOVA conducted on these data revealed a significant main effect for Habituation Treatment [$F(3, 440) = 2.261$, $p = .09$], a significant main effect for Time Block [$F(11, 440) = 23.841$, $p < .0001$], and a significant Habituation Treatment x Time Block interaction [$F(33, 440) = 1.594$, $p < .05$]. Generally, the Hab-Iso group displayed more distress vocalizations than the other 3 groups initially, but declined to a level equaling that of these groups by the end of the test session. No further analyses of these data were performed.

Previous research from this laboratory has shown that this isolation stress procedure models both panic-like (0-5 min) and depression-like (30-60 min) behavior in chicks. Therefore, we converted distress vocalizations to a rate function for these two phases of the stress response and these are summarized in Fig 4. The habituation manipulation was effective in altering the rate of distress vocalizations during the panic-like phase (Panel A) but not the depression-like phase (Panel B) in that the rate of distress vocalizations increased with respect to greater habituation manipulation. A one-way ANOVA on rate of distress vocalizations during the panic phase revealed a significant Habituation Treatment effect [$F(3, 40) = 7.733$, $p < .005$]. Post hoc analyses revealed that the Hab-Soc group and the Hab-Iso group distress vocalized significantly more than the No HH group ($p < .005$). In addition, chicks in the Hab-Iso group distress vocalized significantly more than the Hand group ($p = .0101$). There was a marginally significant increase in distress vocalization rate of the Hand group compared to the No HH group ($p = .059$). Finally, a one-way ANOVA on rate of distress vocalizations during the depression phase failed to reveal a significant Habituation Treatment effect.

A summary of the relationship between latency to vocalize and rate of distress vocalizations during the panic phase is provided in Fig 5. Chicks that had higher rates of vocalizations tended to have the shortest latency to vocalize. The correlation between these two variables was r = -0.60

Discussion

Previous research from this laboratory yielded an unanticipated effect: chicks that were more chronically mild stressed by being socially separated produced a greater amount of distress vocalizations. We believe this occurred due to an inverted U-shape function and effects of habituation. Thus, in this study animals were subjected to various amounts and types of habituation prior to testing. During testing, each group of chicks was isolated in the testing apparatus for one hour. In general, there was a gradual decline in distress vocalizations over the 60-min test session, with the greatest number of distress vocalizations being made by the chicks that received the most types of habituation.

During habituation trials, two measures were collected: latency to first vocalize and total number of distress vocalizations. Isolated animals had a shorter latency to vocalize and higher rates of distress vocalizations over 4 habituation trials than the social birds. The observation that isolated animals distress vocalize more than social animals is consistent with other research. Sufka *et al.* (2006) found that chicks tested in an isolated condition distress vocalized significantly more than chicks tested in a social condition.

One interesting finding was that social animals tended to have a longer latency to vocalize and fewer distress vocalizations over time. There were observable changes in distress vocalizations that may have been due to both habituation and the age developmental process of animals. Fig 1B shows that the social birds decrease in their number of distress vocalizations because they are getting used to being in the apparatus with their companion. Therefore, they are acclimated to the apparatus and are not experiencing social separation anxiety. There may be a learning component involved in the animal's response. During each habituation trial, isolated chicks distress vocalize in order to reestablish social contact. After 15 minutes, the chicks are put back with their companions, and the distress vocalizations are positively reinforced. Therefore, the isolated chicks learn that if they distress vocalize, then social contact will soon be restored. This explains the isolated chick's increase in distress vocalizations over the 4 habituation trials.

We are not familiar with any studies that measure latency; we interpret it the following way. On test day, there were considerable differences across the 4 treatment groups. The No HH group exhibited the longest latency whereas the Hab-Iso group exhibited the shortest latency to vocalize. The No HH group might be experiencing the highest amount of stress or fear, which might be inhibiting their distress vocalizations. The Hab-Iso group shows that the more exposure to panic the animal has, the less stressed it becomes.

Among all treatment groups, the total number of distress vocalizations started out high, and declined over time. Sufka *et al.* (2006) have described this as an anxiety-depression continuum: the first 5 minutes represent an anxiety-like state and the last 30 minutes represent a depression-like state. The current data are consistent with their study. The level of variance in the anxiety phase is due to the amount and types of habituation each group received.

Fig 4A shows that distress vocalizations increased with the more habituation the animal received. The highest level of stress is in the No HH group because they were not habituated. The Hand group received 1 type of habituation, so they distress vocalized more than the No HH group. The Hab-Soc group received 2 types of habituation, so they distress vocalized more than the Hand group. Finally, The Hab-Iso group distress vocalized the most because they received the greatest amount of habituation. The distress vocalizations are a measure of the amount of stress the animal is experiencing and this relationship follows an inverted U-shaped function. As the stress levels increase, distress vocalizations increase to a point where they begin to decline under high levels of stress. Habituation to the experimenter, the apparatus, and an isolated experience has the effect of reducing the high levels of stress the animal experiences in the paradigm that yield higher levels of distress calls. Therefore, distress vocalizations indirectly measure the amount of habituation an animal has received. These findings are consistent with previous reports of habituation effects on other stress-related behaviors in chicks, specifically stress-induced analgesia (Feltenstein *et al.*, 2002).

Previous studies have shown that chronic mild stressors induce depression sooner, but do not make the animal more depressed. Porsolt *et al.* (1977) found that in the forced swim test, animals did not become more depressed. They did, however, stop swimming sooner; this means they entered learned helplessness, or depression, sooner. Unlike Porsolt's study, the animals in the current study did not enter the depression state earlier because our model, being an anxiety-depression continuum, is slightly different from other models. Consistent with previous research, the chicks did not become more depressed. In chicks, habituation doesn't affect the depression phase. Regardless of the initial level of anxiety the animals experience, they all eventually have the same level of depression

Collectively, these findings explain the relationship between stress and distress vocalizations and the kinds of manipulations that affect those stress calls. Changing the degree of stress by means of habituation seems to change the animal's behavior in significant ways. The habituation manipulations affect distress vocalizations in both latency and quantity (first 5 min). This occurs because distress vocalizations follow the inverted U-shape function relative to stress. These habituation techniques reduce stress, which causes the distress vocalizations to return to a higher amount due to the less panicked level.

Future studies exploring this complex relationship between stress and behavior offer several predictions. For example, rather than using exposure to multiple stressors, habituation to varying amounts of a single stressor would likely lead to a similar increase in distress calls in chicks. That is, varying the amount of handling or apparatus exposure or an isolation experience should yield similar effects as that of the combination of all three types of stressors. Future studies exploring this hypothesis are currently being planned in this laboratory.

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Figure 1

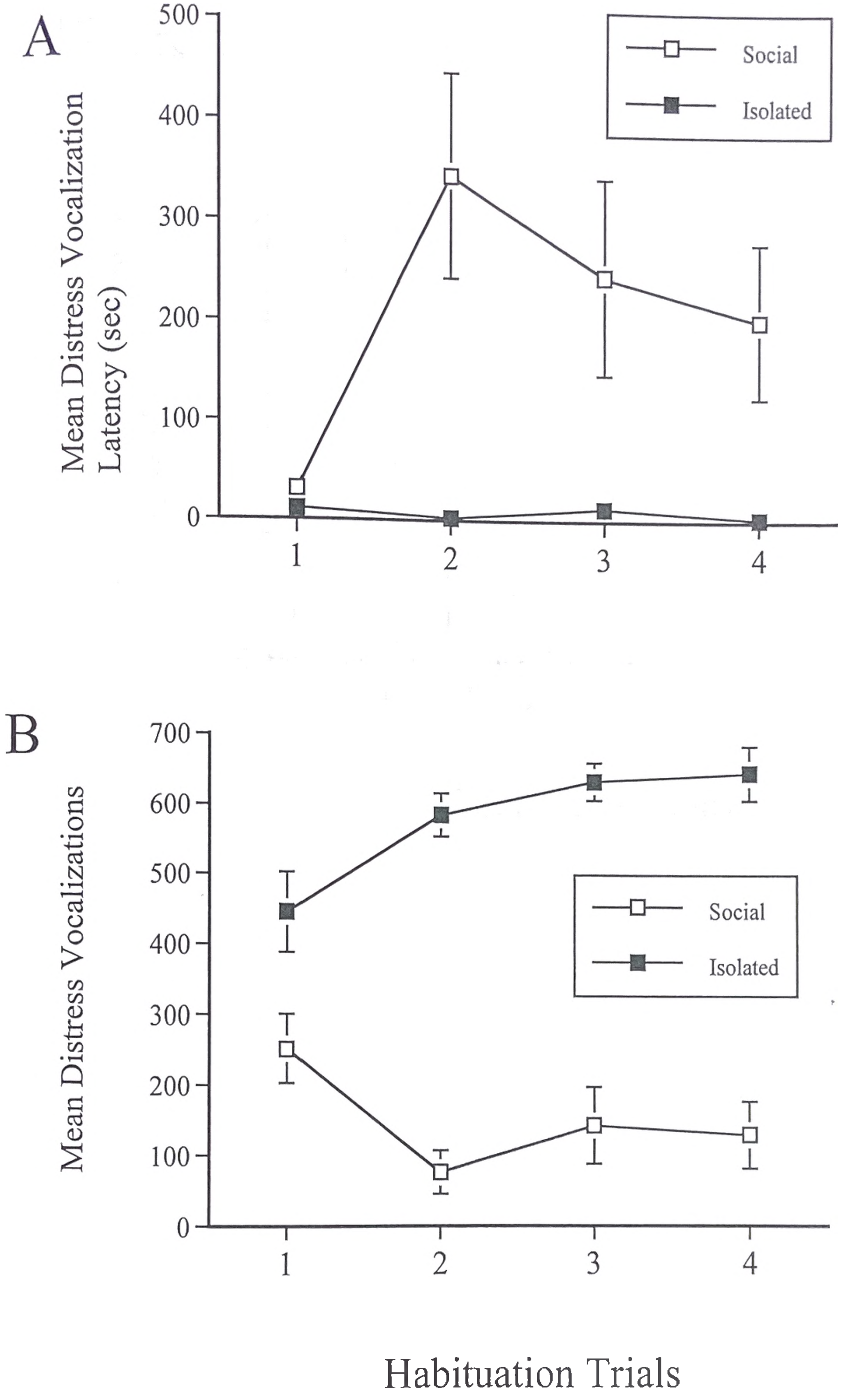
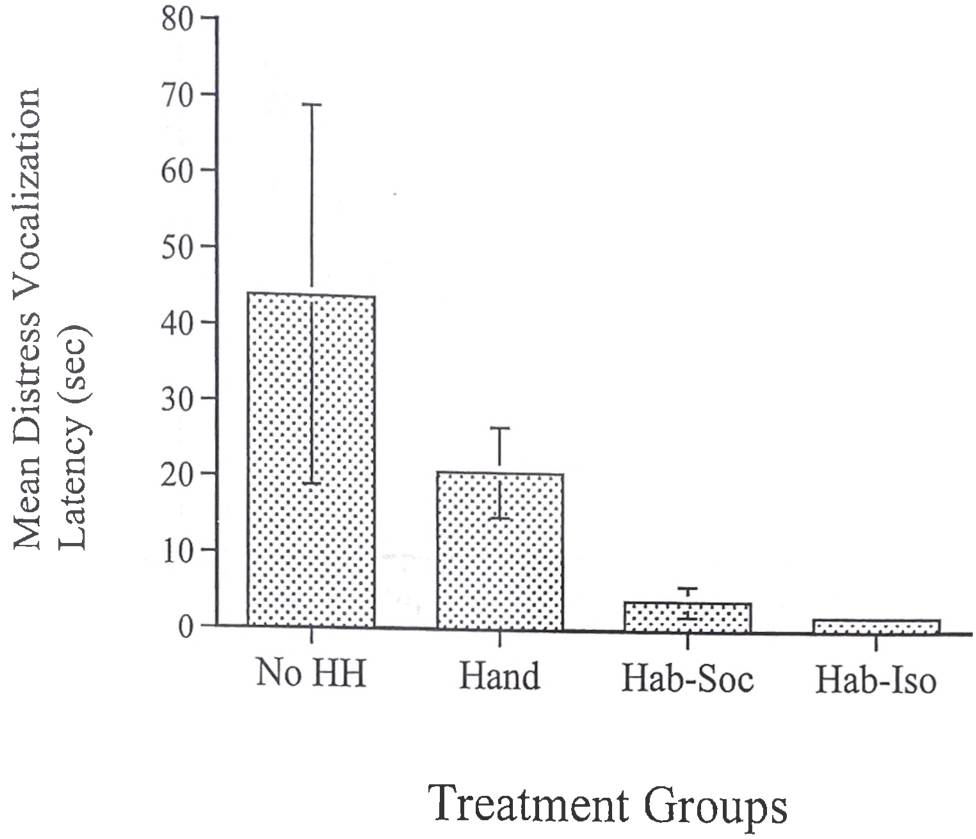

A
Mean Distress Vocalization Latency (sec)
500
400
300
200
100
0
1
2
3
4
Social
Isolated
B
Mean Distress Vocalizations
700
600
500
400
300
200
100
0
1
2
3
4
Social
Isolated
Habituation Trials

Figure 2



80
70
60
50
40
30
20
10
0
Mean Distress Vocalization
Latency (sec)
No HH
Hand
Hab-Soc
Hab-Iso
Treatment Groups

Figure 3

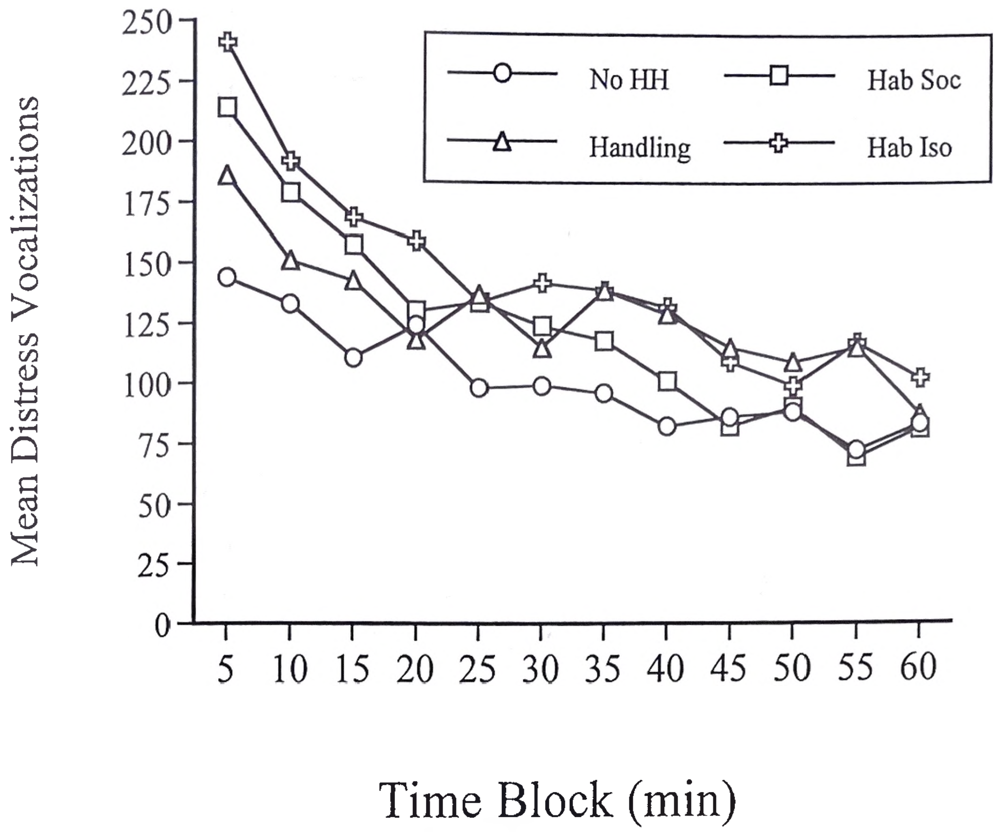

250
225
200
175
150
125
100
75
50
25
0
Mean Distress Vocalizations
No HH
Hab Soc
Handling
Hab Iso
5
10
15
20
25
30
35
40
45
50
55
60
Time Block (min)

Figure 4

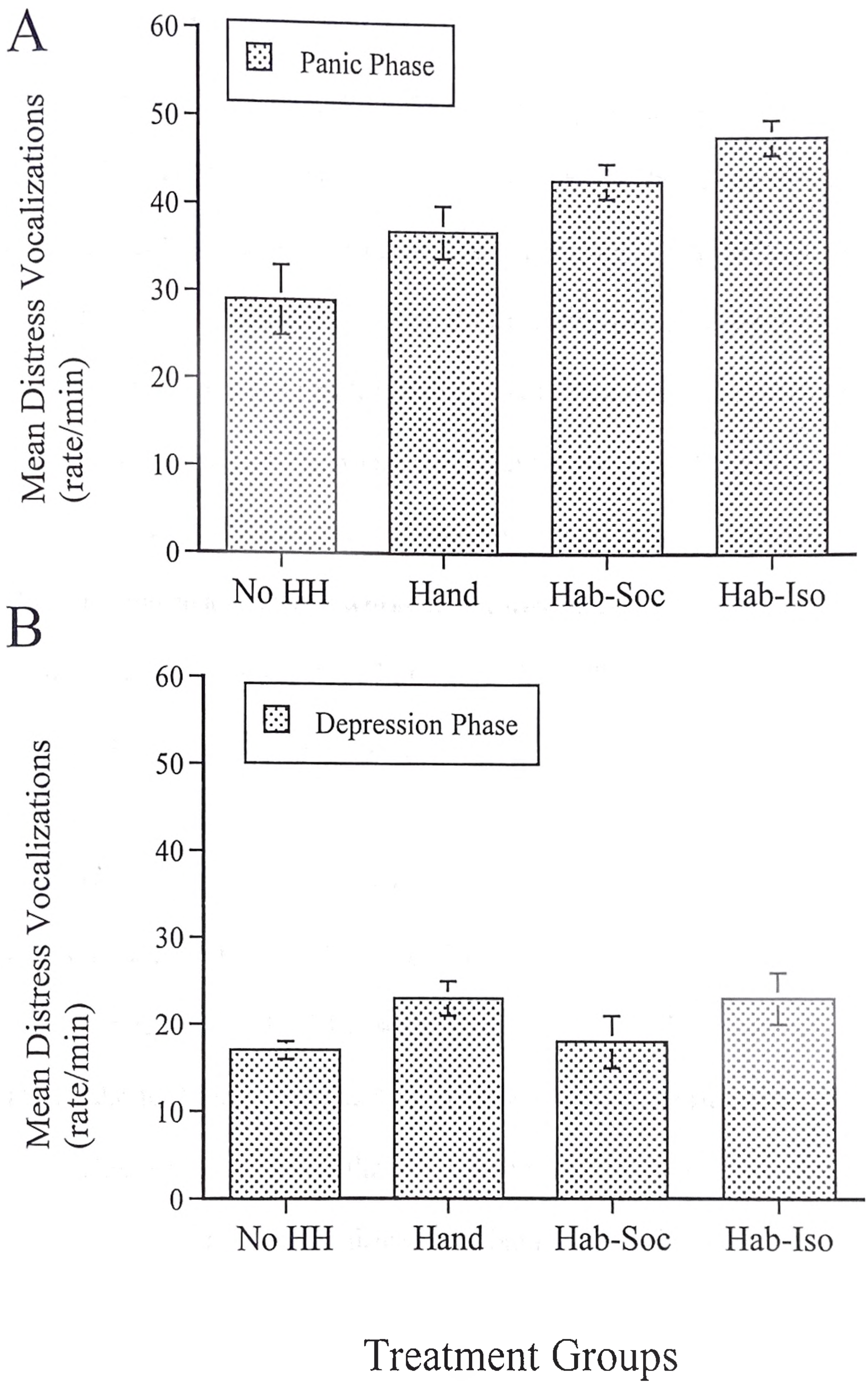

A
Panic Phase
Mean Distress Vocalizations (rate/min)
60
50
40
30
20
10
0
No HH
Hand
Hab-Soc
Hab-Iso
B
Depression Phase
Mean Distress Vocalizations (rate/min)
60
50
40
30
20
10
0
No HH
Hand
Hab-Soc
Hab-Iso
Treatment Groups

Figure Captions

Fig. 1 Panel A: Mean (SEM) latency to distress vocalize in the social (Hab-Soc) (open symbol) and isolated (Hab-Iso) (filled symbol) test conditions as a function of habituation trials (n = 12). Chicks tested in the social condition displayed an initial increase in latency to vocalize followed by a gradual decline over the 4 habituation trials. Isolated animals had a significantly shorter latency to vocalize than the socially tested chicks over the 4 habituation trials ($p < .03$). **Panel B:** Mean (SEM) distress vocalization in the social (Hab-Soc) (open symbol) and isolated (Hab-Iso) (filled symbol) test conditions as a function of habituation trials (n = 12). Chicks tested in the social condition displayed a decrease in distress vocalizations over the 4 habituation trials. Chicks tested in the isolated condition displayed an increase in distress vocalizations over the 4 habituation trials. In general, over the 4 habituation trials, isolated chicks distress vocalized significantly more than socially tested chicks ($p < .0001$).

Fig 2 Mean (SEM) latency to distress vocalize during the 60 min isolation test session for non-habituated (No HH), handled (Hand), socially tested (Hab-Soc), and isolated (Hab-Iso) groups (n = 12). No HH chicks had the longest latency to distress vocalize. Hand chicks had a shorter latency than Hand, but a longer latency then Hab-Soc. Hab-Iso chicks had the shortest latency to vocalize. Hab-Soc and the Hab-Iso groups had significantly shorter latencies to vocalize than did the No HH group ($p < .05$).

Fig 3 Behavioral characterization of the chick anxiety-depression continuum model. Data points represent means (n = 12). The No HH group (○) had lower distress vocalizations, whereas the other 3 groups that had received varying amounts of habituation had higher distress vocalizations. In general, distress vocalizations were highest during the first 5 min block marking the panic phase and significantly declined within 20-25 min to approximately 40% the initial response rate and remained relatively stable throughout the test session marking the depression phase. The isolated chicks displayed more distress vocalizations than the other 3 groups initially, but declined to a level equaling that of these groups by the end of the test session.

Fig 4 **Panel A:** Mean distress vocalizations during the panic phase for the non-habituated (No HH), handled (Hand), socially tested (Hab-Soc), and isolated (Hab-Iso) groups (n = 12). The Hab-Iso group displayed the most distress vocalizations during the first 5 min of the test session than any other group. The Hab-Soc group distress vocalized less than the Hab-Iso group but more than the Hand group. The No HH group displayed the least number of distress vocalizations. In general, the rate of distress vocalizations increased significantly with respect to greater habituation ($p < .005$). The Hab-Soc group and the Hab-Iso group distress vocalized significantly more than the No HH group ($p < .005$). Chicks in the Hab-Iso group distress vocalized significantly more than the Hand group ($p = .0101$). **Panel B:** Mean distress vocalizations during the depression phase for the non-habituated (No HH), handled (Hand), socially tested (Hab-Soc), and isolated (Hab-Iso) groups (n = 12). The habituation manipulation was not effective in altering rate of distress vocalizations during the depression-like phase.