Does Tactile Stimulation Attenuate The Adverse Effects of Maternal Separations in Rats?

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DOES TACTILE STIMULATION ATTENUATE THE ADVERSE EFFECTS OF MATERNAL SEPARATION IN RATS?

A Thesis

by

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ABSTRACT

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Handling has been used as a stressor and a stress-protector in research on development in rats. Tactile stimulation (TS), on the other hand, has consistently been shown to favorably affect the performance and exploratory behaviors of stressed rats in novel environments and this effect is usually interpreted as evidence of attenuating the developmentally-related emotional consequences of stress. In the present study, the effects of TS during maternal separation (MS) were explored during a critical period of development in rats. An open-field task to measure exploratory behavior was used as a dependent measure of anxiety at a pre-pubertal period. It was hypothesized that early handling would attenuate the emotional effects of MS with TS rats revealing more exploratory behavior.

DEDICATION

I dedicate the completion of my master's thesis to my daughter, Jacky. She is my inspiration, my joy and my strength. She has taught me so much. Her capacity to empathize is extraordinary and her ability to provide support is beyond her years. I am tremendously fortunate for having her in my life.

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Comparative studies involving animals have contributed significantly to the understanding of human behavior. The rat has been used in medical and psychological research and has been considered to be a very useful model of many behaviors in humans. Because early life experiences play a role in developmental processes, manipulations of rearing conditions have been made to study such effects later on in life. Periods of development were identified as early as 1898, when Wesley Mills made comparative observations of critical developmental stages. These critical periods are points in time in which the brain has the best capacity of mediating the establishment of particular abilities, given that the organism has had the proper set of environmental contingencies (National Institute of Health [NIH], 2010). The effects of both positive (Daskalakis, Kaperoni, Koros, Kloet, & Kitraki, 2009; Zanettini et al., 2009) and negative (Beane, Cole, Spencer, & Rudy, 2002; Bogoch, Biala, & Weinstock, 2007; Farkas et al., 2009; Panagiotataropoulos et al., 2004; Pohl, Olmstead, Wynne-Edwards, Harkness, & Menard, 2007; Stone & Quatermain, 1997) experiences on development have been examined at various stages of animal development. Such animal models provide important clues to understanding human development and the consequences of physical maltreatment at varying pivotal developmental stages in human infants, children, and young adults as well. Research using animals at various developmental periods allow us to more effectively study the problems that arise throughout the lifespan (Smith & Morrell, 2007).

Stress and Early Development

Problems early in life can arise from different types of stressors that individuals experience. As with research based on animal models, stressors associated with maltreatment are known to have long-term negative consequences in humans (Gross & Keller, 1992). Conversely, stress can also help individuals cope with dangers in the environment by enabling a complex mechanism of behavioral, metabolic, and neuroendocrine responses to be acquired as part of a coping repertoire (Abraham & Kovacs, 2000). This complexity, as well as the lack of consensus in defining stress (Woodmansee, Silbert, & Maier, 1992) have sparked much research and created an enormous body of literature.

The effect of stress on rodent behavior varies with age (Stone & Quartermain, 1997; Smith & Morrell, 2007); most likely a result of extensive brain development after birth (Bogoch, Biala, Linial, & Weinstock, 2007). This enables scientists to explore the influence of plasticity of the nervous system and behavior (Bogoch, et al., 2007).
For instance, Stone & Quartermain (1997) found that social stress reduced eating and open-maze-arm entries in young, but not in adult rats. Infant rats have demonstrated a greater tendency to be active in anxiety-provoking situations (Smith & Morrell, 2007). When comparing behavioral effects in young versus aged rats, age-related deficits were ameliorated by stress habituation measures and habituation procedures appeared to only benefit aged rats (Mabry, McCarty, Gold, & Foster, 1996). In addition, effects of stress on neurobehavioral development did not induce drastic changes in motor coordination

(Farkas et al., 2009). The differences in outcomes can often be attributed to differences in methodology and specific species' development. Furthermore, discrepancies in the performance at different stages of development clearly show that age impacts both the acquisition of a deficit, as well as getting habituated to a stressor.

Effects of stress on behavior have resulted in reduced learning and motivation (Mintz, Rüedi-Bettschen, Feldon, & Pryce, 2004; Šida, Koupilová, Hynie, & Klenerová, 2003). Specifically, Mintz et al. found that severe thermal stress results in reduced social motivation. Restraint stress also inhibits the retrieval mechanism in memory, resulting in learning impairments (Šida et al., 2003).

In another study, metabolic effects of stress in rats with inherited stress-induced hypertension were tested under environmental stress during puberty at developmental periods. Acute stress resulted in an increase of basal blood pressure and exaggerated blood pressure response in both young and adult rats (Maslova, Bulygina, & Markel, 2000). These results indicated by Maslova et al. (2000) illustrate the interaction between genetic (inherited hypertension) and environmental influences (prepubertal stress), given that both are known to affect hypertension in humans (Maslova et al., 2000).

Neuroendocrine effects due to stressors are commonly assessed as well. A number of studies have specifically looked at the effects of stress on the hypothalamic-pituitary-adrenal axis (HPA axis) (Beane et al., 2002; Parfitt, Walton, Corriveau, & Helmreich, 2007; Rüedi-Bettschen et al., 2006). Early environmental factors such as handling, early depravation, and maternal separation all resulted in influences in the HPA axis in all three studies. Handling was demonstrated to reduce corticosterone levels (Bean et al., 2002; Rüedi-Bettschen et al., 2006) and maternally separated mice revealed increased plasma corticosterone secretion (Parfitt, Levin et al., 2004). In fact, when both mice and rats have been tested on multiple types of maze procedures, diverse stressors such as restraint, exposure to novel environments, and noise have resulted in an increase of corticosterone levels (Meerlo, Horvath, Nagy, Bohus, & Koolhaas, 1999; Parfitt, Walton et al., 2007; Slotten, Kalinichev, Hagan, Marsden, & Frone, 2006).

Many different types of stressors are used to assess effects on behavior. Repeated restraint stress has shown to cause impairments on performance on spatial memory and active avoidance tasks (Luine, Villegas, Martinez, & McEwen, 1994; Sida, Hynie, & Klenerova, 2003; Stillman, Shukitt-Hale, Levy, & Lieberman, 1998). Social stressors were used by Rodgers and Cole (1993) to assess the effects on performance and they found a decrease in exploratory behavior in stressed rats when compared to controls. Karandrea, Kittas, and Kitraki (2002) also used swimming stress and restraint paradigms, in which the rats are confined in ventilated glass jars. These rats were repeatedly restrained and were exposed to swimming stress and as a result they had reduced sensitivity detected in both the hippocampus and the hypothalamus (Karandrea, 2002).

Perhaps the most long-standing and common method that has been previously used as a stressor is electric shock. Shock has shown to produce avoidance latencies to enter dark and arm compartments in mazes (Klenerova, Kaminsky, Sida, Krejei, & Hynie, 2002; Sida, Koupilova, & Klenerova, 2003). Other forms of stress included environmental stimuli and gestational stress (Bogoch et al., 2007; Lemaire, Lamarque, Moal, Piazza, & Abrous, 2005; Pohl et al., 2007). In recent years though, maternal influences, such as maternal separation and maternal isolation, have been the subject of extensive research with rodents (Aisa, Tordera, Lasheras, Del Rio, & Ramirez, 2008;

Farkas et al., 2009; Ladd, Thrivikraman, Huot, & Plotsky, 2005; Parfitt, Levin et al., 2004; Parfitt, Walton et al., 2007; Slotten et al., 2006).

Breed and Gender

Breed

Sprague Dawley rats have been shown to be excellent subjects to study behavioral, metabolic, and neuroendocrine responses (Conrad, Jackson, Wieczorek, Baran, Harman, Wright, & Korol, 2004; Luine et al., 1993; Stamatakis et al., 2002). Additionally, a substantial amount of experimental work has been done in this species and breed with regards to emotionality (Imanaka et al., 2008; Klenerová et al., 2002; Smith & Morell, 2007; Woodmansee et al., 1992). The rat's physiological design and functioning bares similarities among species and so this is a very useful model for this type of research.

By using specific strain of rats, variables can be better compared without the interference of small genetic differences between strains. Studies that have looked at the differences among various strains of rats have demonstrated this. For example, Klenerova, Kaminsky, Sida, Krejci, Hlinak and Hynie (2002) looked at the effects of restraint in combination with cold water stress on Sprague-Dawley and Lewis rats to test their learning and memory. The rats were restrained by immobilizing their front and hind legs, and then they were immersed in water at 22 degrees Celsius. The comparison of these two strains resulted in significant differences in behavior during the first adaptation period. Lewis rats exhibited longer exploratory latencies during first exposure to the novel environment compared to Sprague-Dawley rats while Lewis rats displayed habituation to their surroundings (Klenerova et al, 2002). Strain differences have also looked at various types of variables ranging from nociceptive (pain) behavior to acute and chronic stress (Paré, Blair, Kluczynski, & Shanaz Tejani-Butt, 1999; Vendruscolo, Pamplona, & Takahashi, 2004).

Gender

Sex differences are very important factors in understanding the effects of stress. Gender-specific outcomes have been found in studies dealing with development, behavior, stress and hormones (Karandrea, 2001; Paré et al, 1999; Weinstock, 2007). For example, the estrous cycle has been known to have an effect on acute stress (Conrad et al., 2004). Two month old Srague-Dawley rats were stressed by restraint procedures and later tested their performance on a Y-maze. It was found that acute stress effects were gender-dependant with acute restraint differentially impairing spatial memory in males and improving performance in females (Conrad et al., 2004). Also, according to Boissy and Bouissou (1994), male rodents have also been known to exhibit more fearful behavior than females. The use of only one gender is therefore useful since it eliminates possible sex differences in physiological and especially endocrine regulations (Boissy & Bouissou, 1994).

The general consensus in regards to gender and stress are that females are more prone to the deleterious effects of stress (Paré et al., 1999; Weinstock, 2007). These differences though are hypothesized to be stressor-specific (Karandrea, 2001). In the present study, both genders will be tested to determine if MS and TS effects interact with gender.

Open-field Tests as a Measure of Activity and Anxiety

Open-field tests are usually used to test performance of stressed rats. These tests are designed to measure behavioral responses such as locomotor activity, hyperactivity, and exploration behaviors. One of the most essential behaviors an organism displays is the exploration of its environment (Smith & Morrell, 2007). A highly anxious rat will exhibit less exploratory behaviors. However, by nature, they tend to avoid open spaces and are more inclined to travel along walls to avoid aviary predators. Therefore, open-field tests are used to operationally define and measure anxiety. The novelty of the open field environment acts as an anxiety-eliciting stimulus which permits measurement anxiety-induced inhibition of locomotor activity and exploratory behaviors. This measure is appropriate since rats tend to avoid brightly illuminated, novel, open spaces in the natural environment when stressed. Several studies have used open field test for similar purposes (Parfitt, Walton et al., 2007; Meerlo et al., 1999; Costela, Tejedor-Leal, Mico, & Gibert-Rahola, 1995; Smith & Morrell, 2007; Zanettini et al., 2009).

In the present study, the effects of TS during maternal separation (MS) were explored during the neonatal/infancy critical periods of development. Noenatal and infancy periods in rats are from postnatal day (PND) 0 through 21 (Ojeda, Urbanski, & Ahmed, 1986). An open-field task to measure exploratory behavior was used as a dependent measure of anxiety in adolescence in adulthood in rats. Maternal interaction data were also collected to control for maternal care influence on vulnerability to stress, since variation in maternal care has also been known to be a mediating factor in these types of interventions (Hancock, Menard, & Olmstead, 2005; Macrí et al., 2004).
Two primary hypotheses were tested in the present study:

1) Maternal separation during critical periods of development is a stressful experience resulting in adverse emotional consequences revealed in higher anxiety in new and potentially threatening environments.

2) Tactile stimulation during maternal separation attenuates the adverse emotional effects of maternal separation.

Maternal Separation

Maternal separation (MS) is a stressor that has been extensively studied. MS is a more natural occurring stressor compared to shock or isolation, and therefore, strengthens external validity of any findings. It also serves as an excellent model for human maternal separation and negligence. Neonatal MS has been shown to be one of only a few stressors capable of overcoming the effects of the hyporesponsive period (SHRP). This response occurs in early development in rats and minimizes pituitary-adrenal responses to stress (as cited in Parfitt, Levin et al, 2004). MS can therefore be used to assess how stress affects young rats while accounting for hormonal influences at that stage of development.

The maternal separation paradigm has been used to analyze effects on behavioral responses and brain function, e.g., the hippocampus and endocrine system. For instance, dampened motivation for acquiring a sucrose reward was found in a study by Matthews and Robbins (2003). MS was also found to reduce hippocampal mossy fiber density in Long Evan rats (Huot, Plotsky, Lenox, & McNamara, 2002). Furthermore, changes in hypothalamic-pitutiary-adrenal axis were reported as outcomes from MS (Aisa et al., 2008; Ladd et al., 2005; Macrí, Mason, & Würbel, 2004; Parfitt, Walton et al., 2007; Rüedi-Bettschen et al., 2006; Slotten et al., 2006). Given that adverse effects of MS stress are well established and that there is a comparative model in humans are the reasons MS was chosen as the independent variable for this investigation.

The Impact of Handling

A discussion of the research literature on handling is warranted when examining the effects of rearing conditions on emotional development because of its expected impact in development. In other words, handling, as a procedure that generally involves separating the dam from her pups for 15 minutes daily, appears to be very problematic, both because of the inconsistency in results and procedures and because it has been used as ameliorating the effects of stress, as well as, as a stressor itself (Maslova et al., 2000; Rüedi-Berrschen et al., 2006).

Positive effects of handling have been demonstrated in studies involving a variety of dependant variables including, maternal separation, corticosterone levels, reactivity of stress-related neuronal circuitries, emotionality, learned helplessness, anxiety, prenatal stress, HPA function, and gene expression (Ábrahám & Kovács, 2000; Beane et al., 2002; Bogoch et al., 2007; Costela et al., 2005; Fernández-Teruel, Escorihuela, Castellano, González, & Tobeña, 1997; Ferré, Núñez, García, Tobeña, Escorihuela, & Fernández-Teruel, 1995; Parfitt, Levin et al., 2004). The ameliorating effects of handling have been found on emotionality (fearfulness), reaction to stressors, and on exploratory behavior (Fernandez-Teruel et al., 1997). In terms of temporal effectiveness, handling appears to have anxiolytic effects in both short and long-term emotionality in mice and different strains of rats (Fernandez-Teruel et al., 1997; Maslova et al., 2000; Sternberg & Ridgway, 2003).

Handling has also revealed mixed results. For instance, effects of handling appear to be sensitive to other variables such as gender, breed, type of measure/conditions and coping strategies (Ladd, et al., 2005; Meerlo et al., 1999; Panagiotaropoulos et al., 2004; Papaionnou, Dafni, Alikaridis, Bolaris, Stylianopoulou; 2002; Parfitt, Walton et al., 2007; Stamatakis et al, 2008). In a study by Rüedi-Bettschen et al. (2006), early handled and non-handled rats resulted in having similar phenotypes to chronic stress revealing no effects of handling. Effects of handling on performance have also revealed conflicting results when studied under different conditions. For instance, it was found that handling had a beneficial effect on both genders since both spent more time in target quadrants in a Morris water maze than non-handled animals (Stamatakis et al., 2008). However, they emphasized that early experience interacts with gender and acute stress exposure in adults. In addition, it has been demonstrated that early handling only had a marginal reduction in anxiety-like behavior in mice (Parfitt, Walton et al., 2007).

In one study, opposite effects from those hypothesized were found when handling was used as a stressor (Maslova et al., 2000; Sternberg & Ridgway, 2003). It is difficult to understand why these investigators chose their hypothesis given that handling is most commonly used as a buffer to stress.

One major problem with handling procedure is that it is a form of maternal separation. In fact, claims that MS has prevented damage caused by neonatal cerebral hypoxia-ischemia (insufficient blood flow to cerebral cells and structures) have been published (Rodrigues et al., 2004). What is disconcerting is that MS was identified as 10 minutes of separation from dam by individually placing the pups in plastic box containers. In addition to separating pups from their dams, handling procedure also involved setting them in beakers or other holding containers for 15 minutes. Perhaps, if MS had been used as separating the dam for a substantial period of time, results would have been different.

The lack of consistency and clarity in the operational definitions for both handling and MS has resulted in discrepant and often conflicting results. In light of this, the procedures that can consistently produce ameliorating effects are necessary for this type of research. One procedure that has shown some promise in the literature is tactile stimulation.

Tactile Stimulation

Tactile stimulation (TS) has consistently been shown to favorably affect the performance and exploratory behaviors of stressed rats in novel environments and this effect is usually interpreted as evidence of attenuating the emotional consequences of stress. Only a few studies have focused on tactile stimulation. Anxiolytic effects of TS have been found in various animal models. For instance, TS has been shown to reverse the effects of neonatal isolation and anxiety-like behavior in Sprague Dawley (S-D) rats (Imanaka et al., 2007), behavioral responses to reward as an expression of anhedonia in Lister-hooded rats (Matthews & Robbins), and alterations in feeding behavior (Silveira et al., 2004). According to Silveira et al., TS increased consumption of sweet and savory snacks, and this was assumed to be due to CNS changes caused by the intervention. TS also appears to strengthen memory in a study using classical conditioning (Domínguez, López, & Molina, 1999) and neuronal plasticity (Zhang & Cai, 2007).

Only a few studies have focused on the interaction of tactile stimulation and maternal separation (Imanaka et al., 2008; Rodrigues et al., 2004). Imanaka et al., demonstrated that TS reverses adverse effects of MS, whereas, Rodrigues and colleagues revealed ameliorating effects of both TS and MS. The latter study used handling procedures for MS, hence showing positive outcomes of the separation from the dam. Again, a lack of consensus regarding the labeling of procedures has created problems in achieving a coherent understanding of equivocal findings in this area of research.

CHAPTER II

METHODS

Subjects

Sprague-Dawley rats of both genders were purchased from Ace Animals, Inc. and Charles River Laboratories. Animals were housed and bred in the laboratory facilities at the Biology Annex, room 116, at the University of Texas Pan American campus. Three female and two males were used for breeding (1:1 breeding ratio; 1 male-1 female housed at a time). Conception was confirmed by the presence of a vaginal plug beneath the wire-floor home cage. Once the rats were pregnant, they were housed individually and separately from the males through gestation and lactation. A total of 31 pups were utilized in this experiment (15 females, 16 males). Testing was carried out at constant room temperature (22±1°C) and at a 12:12 light-dark cycle (light on at 08:00 hrs). Animals were housed in standard polypropylene rat cages (10.5” x 19” x 8”). Cleaning of the cages occurred twice weekly with minimal and equal handling of all animals. The pups were weaned at 21 days and housed 2 or 3 per cage until behavioral assessments on PND 25 when the pups were juveniles (Ojeda et al., 1986). All experimental procedures were carried out using lightly powdered latex gloves. Food (Harlan-Teklad Global Rodent Diets) and deionized water were available ad libitum. Experimental procedures were approved by the University of Texas Pan American Institutional Animal Care and Use Committee (IACUC).

Design and Procedures

The hypotheses were tested using a 3 X 3 mixed factorial design with repeated measures on the second factor. That is, three between subjects comparisons were performed as each group [Maternal Separation with Tactile Stimulation (TS), Maternal Separation with No Tactile Stimulation (NTS), and Controls (C) who were neither separated nor stimulated] were tested in an Open Field apparatus on three successive days. Treatment group litters from each dam were randomly assigned. Procedures took place from PND 3 to PND 16 (2 weeks) throughout the neonatal and infantile periods (Ojeda et al., 1986). After postnatal day 16, offspring were left alone until weaning. On day PND 21, animals were weaned, weighed and housed, 2-3 per cage until behavioral assessment procedures (open-field task). Offspring in all groups were weighed on PND 25 to monitor appropriate growth and development.

Maternal Interaction

Maternal interaction data was collected to control for maternal care quality on vulnerability to stress. Dam observations were recorded on a behavioral datasheet from PND 1 to PND 19. Licking/grooming (LG), arched back nursing (ARN), "blanket" (B, passive nursing mother on side), carrying pups (CP), dam off (DO), and other (O) behaviors were observed pre- and post-maternal separation. Maternal behavior was obtained by recording what behavior dam was observed engaged in prior to MS and for 10 minutes after MS. Generally, LG and ARB are considered high quality care, whereas, DO is considered low maternal care. Data sheet is provided in Appendix A.

Maternal Separation (MS)

Procedures in the MS-NTS (no-tactile stimulation) and MS+TS groups were administered between 08:00-11:00 hrs. At PND 3, dams were removed from their pups for 180 minutes. The dams were placed in a cage in a separate room to isolate and prevent communication with their offspring. At the conclusion of the separation period, dams were returned to their offspring and remained there until the following day. Pups in the MS-NTS group did not receive TS. The control group was kept undisturbed under normal conditions with their dams.

Tactile Stimulation (TS)

At PND 3, same procedures of the MS groups were used. In addition, pups in the MS+TS group were stroked with a soft brush (100% hypoallergenic goat hair), while in their cage without holding/picking up the pups to prevent confounding effects of handling. Pups were gently stroked with the brush on the dorsal surface, in the rostral caudal direction for 10 minutes (location as described by Rodrigues et al., 2004), every hour for the 3hrs of MS. Each pup was stroked an average of 5 times per minute, for a total of 50 strokes per session. Consequently, each pup in this experimental group was tactilely stimulated for 30 minutes every day, from PND 3 to PND 17. The pups habituated to undisturbed, no-stress conditions for 1 week until PND 25. Figure 1 illustrates the general research design.

Open-Field Test

Anxiety was assessed in an open-field apparatus purchased from Med. Associates Inc., St. Albans, VT. This instrument includes a test environment (ENV-515) with interior dimensions of 43.2 cm x 43.2 cm and three 16 beam I/R arrays that enable the recording of exploratory behavior. Each rat was placed individually in a corner of the field and its behavior was recorded on three days for five minutes. All of the rats' activity was evaluated on how well they perform in novel environmental stimuli. All subject data was automatically entered into the open-field database where the total distance, average speed and time spent in various parts of the field (e.g. the border areas versus open-center area) was measured and analyzed.

Statistical Analysis

Statistical analysis was conducted using Statistica software (Statsoft, Inc., Tulsa, OK). Open-field activity measures were analyzed by two-way analysis of variance (ANOVA) with 3 between-group levels: TS, NTS, C and 3 repeated measures on the second factor (3 sessions of open-field activity). F-test was used for multiple comparisons. Statistical significance was set at p-value .05.

General Experimental Design

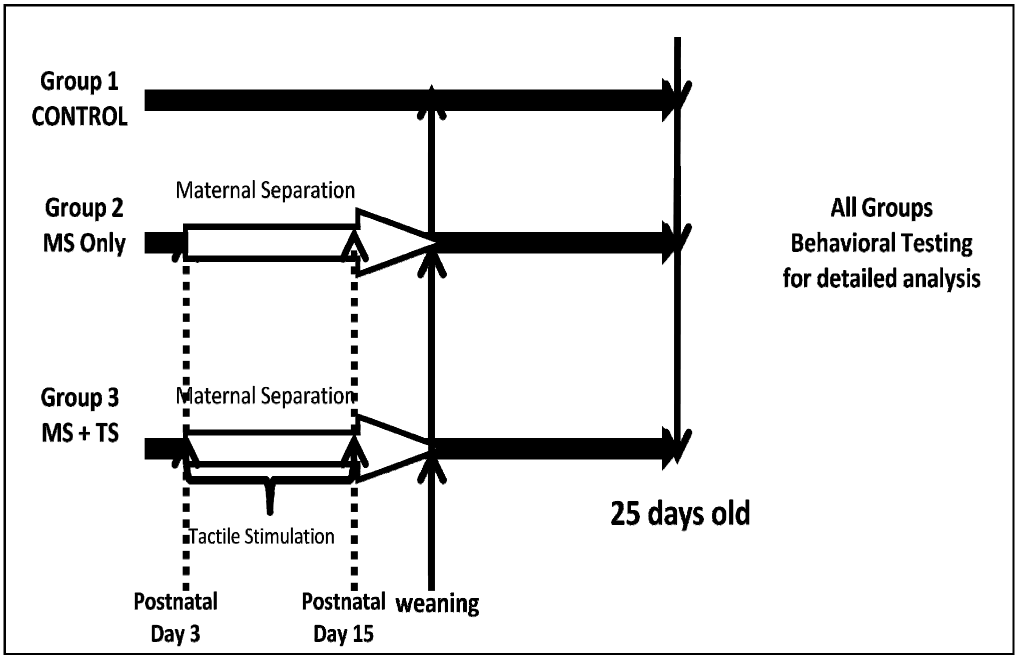

Figure 1. Three groups across time until postnatal day 25.

CHAPTER III

RESULTS

There were no differences in the quality of care of the pups between treatment groups as determined by the instances of LG, ARN, B, CP, DO, or O behaviors each dam exhibited prior and after maternal separation. Each dam spend equal amount of time engaged in each of the behaviors. LG occurred the majority of the observed time (98%), after MS, followed by ARB prior MS (98%). Very few instances of the other behaviors were observed

Both hypotheses of this study were strongly confirmed as revealed in the ANOVA results described in Table 1. The ANOVA results reveal that the NTS group traveled significantly less distance in the open field than the TS and control subjects.

Fisher LSD *post hoc* comparisons reveal that NTS subjects traveled less distance than both other groups on all three days and control subjects traveled significantly greater distance than TS subjects only on Day 1 (PND 25). The finding that NTS subjects also traveled less distance than TS subjects on Day 1 confirms hypothesis #2. However, TS subjects in this analysis reveal less distance traveled than control subjects on Day 1 as well. The results of the Fisher LSD post hoc comparisons are presented in Table 2

Table 1 also reveals a significant Groups X Day interaction effect reflecting the fact that all three groups converged by Day 3 of the 3-day repeated measures open field test. This finding and the group differences on Day 1 are clearly illustrated in Figure 2.

Table 3 reveals the results of the 3 X 3 ANOVA using average velocity/sec in the open field apparatus. Group comparisons yield a marginally significant difference in the speed with which the open field was explored when all three days are combined. Speed increases significantly across the three day test for all groups (repeated measures significant F-ratio) and a significant interaction effect reveals that the three groups began at different velocities on Day 1 but converged by Day 3 much like what was revealed in the data for distance traveled. These effects are illustrated in Figure 3.

Fisher LSD post hoc comparisons (Table 4) reveal that NTS subjects were moving slower on all three days than subjects in the TS and C groups. TS subjects moved faster than NTS subjects on Day 1 and 2 but not on Day 3. Each of these findings is clearly illustrated in Figure 3.

A more conservative statistical analysis (Bonferroni *post hoc*) also reveals statistically significant differences in the NTS group on day 1 compared to TS and C groups for distance. These findings are illustrated in Table 5. Table 6 illustrates statistically significant outcomes for average velocity. Even on this statistical conservative measure, the control moved faster than the TS, and the TS moved faster than the NTS groups.

It was also found that freezing during the entire 5-min open field test was revealed in 3 of the 9 NTS subjects (33%) while only one of twelve TS subjects froze during the first day of testing (8%) and none of the twelve control subjects froze (0%).

All analyses of gender were not statistically significant $p > .05$. Group X Condition X Gender ANOVA yielded no statistically significant differences.

Table 1. Summary of ANOVA Results for Group X Day Analysis of Distance Traveled

Effect	Sum of Squares	df	Mean Squares	F	p	eta^2	Power
Group	711,567	2	355,784	4.70	0.02	0.24	0.74
Error	75,701	30	75,701				
Time	39,544	2	19,772	0.57	0.57	0.02	0.14
Group X Time	677,771	4	169,443	4.85	0.001	0.24	0.94
Error	2,097,081	60	34,951				

Table 2. Summary of Fisher LSD *post hoc* Between-Group Comparisons for Distance (Least Conservative Analysis; Means Rounded to Integers)

Group/Day	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Means	311	508	555	791	681	540	515	574	571
(1) NTS Day 1		*	*	*	*	*	*	*	*
(2) NTS Day 2	*		†	*	*	†	†	†	†
(3) NTS Day 3	*	†		*	†	†	†	†	†
(4) C Day 1	*	*	*		†	*	*	*	*
(5) C Day 2	*	†	†	†		†	†	†	†
(6) C Day 3	*	†	†	*	†		†	†	†
(7) TS Day 1	*	†	†	*	†	†		†	†
(8) TS Day 2	*	†	†	*	†	†	†		†
(9) TS Day 3	*	†	†	*	†	†	†	†	

*$p<0.05$ †$p>0.05$

Table 3. Summary of ANOVA Results for Group X Day Analysis of Average Velocity

Effect	Sum of Squares	df	Mean Squares	F	p	eta^2	Power
Group	245.84	2	122.92	2.64	0.09	0.15	0.48
Error	1,395.75	30	46.52				
Time	1,316.94	2	658.47	24.06	0.00*	0.45	0.99
Group X Time	344.20	4	86.05	3.15	0.02	0.17	0.79
Error	1,641.77	60	27.36				

*$p<0.000001$

Table 4. Summary of Fisher LSD *post hoc* Between-Group Comparisons for Average Velocity (Least Conservative Analysis; Means Rounded to 0.10)

Group/Day	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Means	16.1	30.4	29.0	25.9	30.6	30.8	24.8	29.9	29.6
(1) NTS Day 1		*	*	*	*	*	*	*	*
(2) NTS Day 2	*		†	†	†	†	*	†	†
(3) NTS Day 3	*	†		†	†	†	†	†	†
(4) C Day 1	*	†	†		*	*	†	†	†
(5) C Day 2	*	†	†	*		†	*	†	†
(6) C Day 3	*	†	†	*	†		*	†	†
(7) TS Day 1	*	*	†	†	*	*		*	*
(8) TS Day 2	*	†	†	†	†	†	*		†
(9) TS Day 3	*	†	†	†	†	†	*	†	

*p<0.05 †p>0.05

Table 5. Summary of Bonerroni *post hoc* Between-Group Comparisons for Distance (Most Conservative Analysis; Means Rounded to Integers)

Group/Day	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Means	311	508	555	791	681	540	515	574	571
(1) NTS Day 1		†	†	*	*	*	†	†	†
(2) NTS Day 2	†		†	†	†	†	†	†	†
(3) NTS Day 3	†	†		†	†	†	†	†	†
(4) C Day 1	*	†	†		†	†	†	†	†
(5) C Day 2	*	†	†	†		†	†	†	†
(6) C Day 3	*	†	†	†	†		†	†	†
(7) TS Day 1	†	†	†	†	†	†		†	†
(8) TS Day 2	†	†	†	†	†	†	†		†
(9) TS Day 3	†	†	†	†	†	†	†	†	

*$p<0.05$ †$p>0.05$

Table 6. Summary of Bonerroni *post hoc* Between-Group Comparisons for Average Velocity (Most Conservative Analysis; Means Rounded to 0.10)

Group/Day	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Means	16.1	30.4	29.0	25.9	30.6	30.8	24.8	29.9	29.6
(1) NTS Day 1		*	*	*	*	*	*	*	*
(2) NTS Day 2	*		†	†	†	†	†	†	†
(3) NTS Day 3	*	†		†	†	†	†	†	†
(4) C Day 1	*	†	†		†	†	†	†	†
(5) C Day 2	*	†	†	†		†	†	†	†
(6) C Day 3	*	†	†	†	†		†	†	†
(7) TS Day 1	*	†	†	†	†	†		†	†
(8) TS Day 2	*	†	†	†	†	†	†		†
(9) TS Day 3	*	†	†	†	†	†	†	†	

*$p<0.05$ †$p>0.05$

Distance Traveled

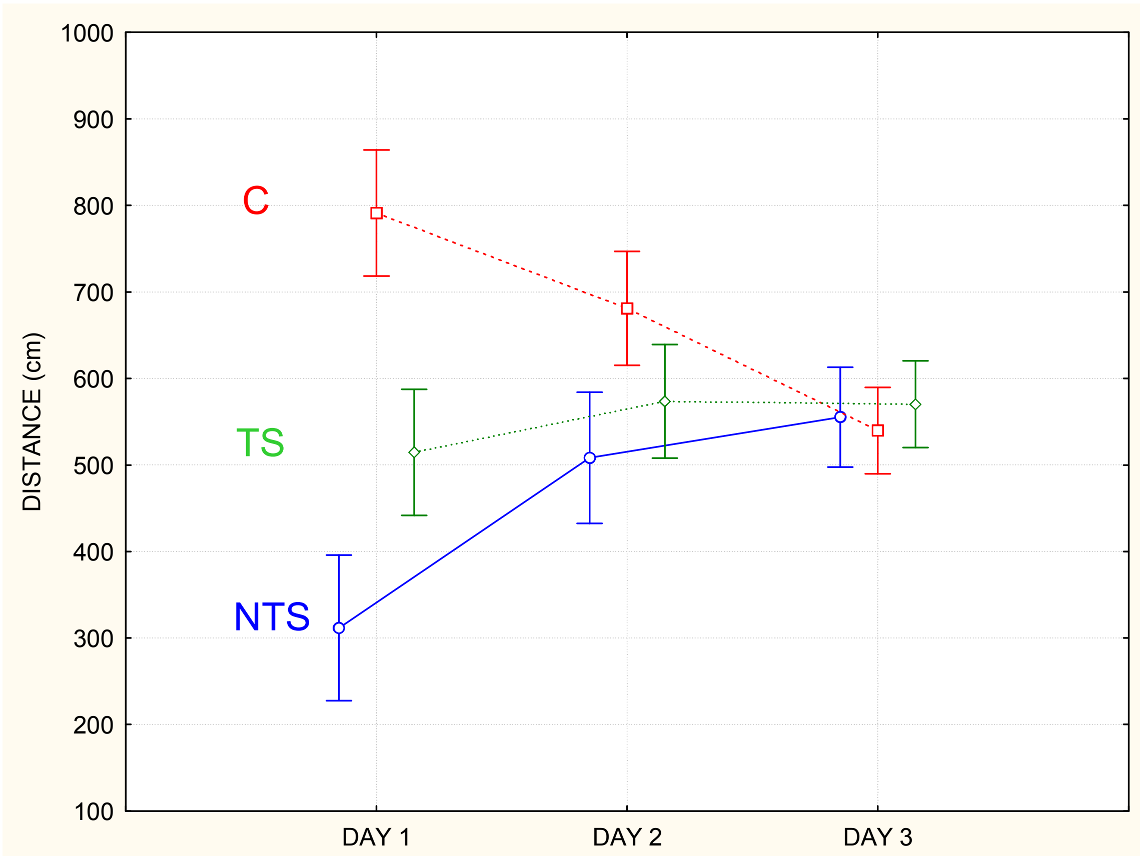

Figure 2. Distance traveled in five minutes by the experimental and control groups during open field testing repeated over three consecutive days.

Average Velocity

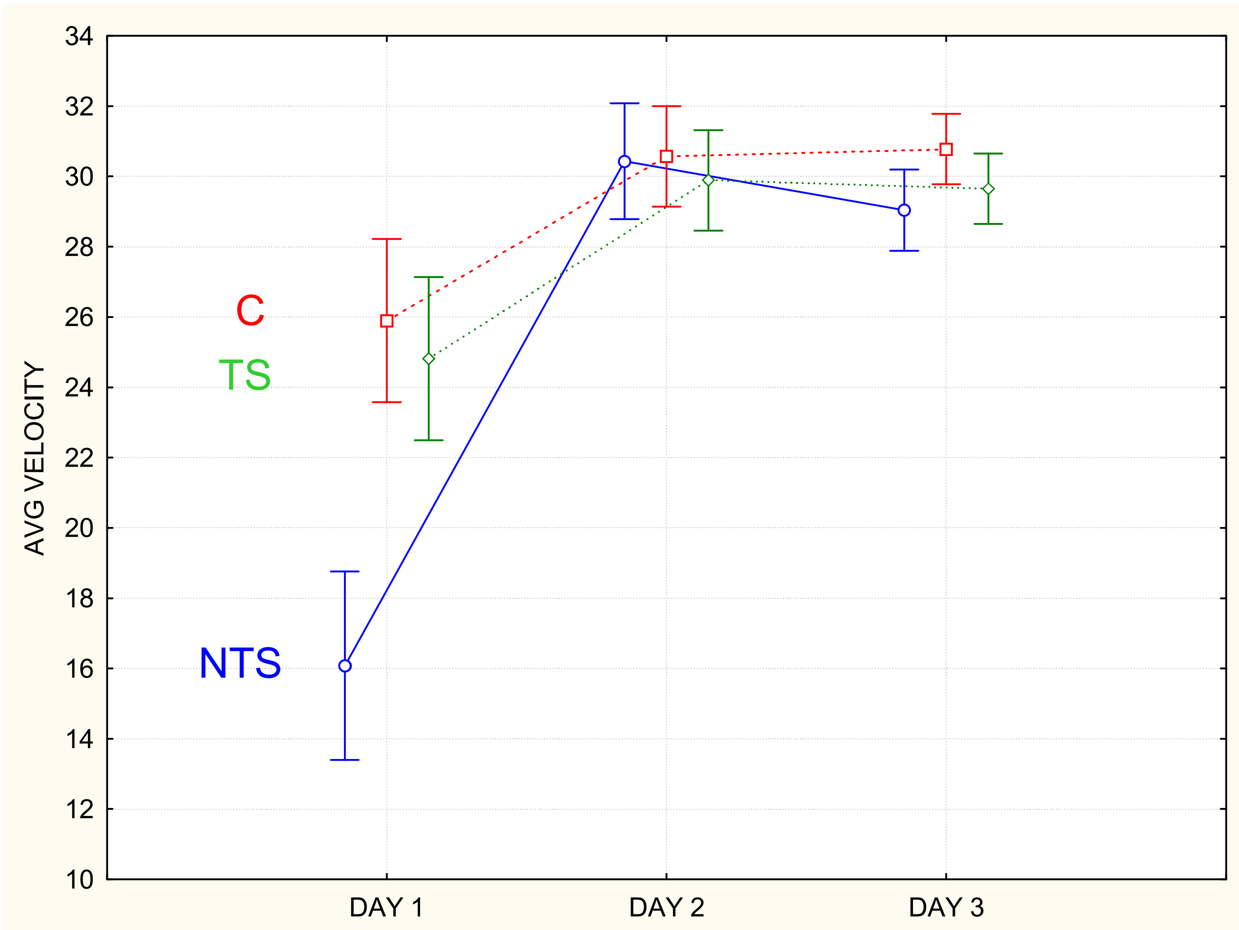

Figure 3. Average velocity during ambulation by the experimental and control groups during a five-minute open field testing repeated over three consecutive days.

Discussion

The hypotheses of the present study were confirmed and the findings replicate similar studies examining the effects of neonatal-maternal separation on anxiety later in the neuropsychological development of the organism. In the present study, an anxiety-attenuating effect of relatively brief tactile stimulation during maternal separation as revealed in statistically significant differences between TS and NTS animals at critical points in the repeated exposure to the open field environment. That is, TS subjects were stimulated during only 10-min of each of the 3-hr periods of separation representing only 17% of the time that the dam was away from her pups. It would be interesting to speculate whether or not TS subjects would be identical to controls if a longer period of stimulation was employed, e.g., 30-min. If the data of the present study reveal an incomplete attenuation of the emotional consequences of maternal separation, could an extended period of stimulation completely attenuate the anxiety revealed in open field challenges by offspring of dams who were separated from them during a critical developmental period.

Another important finding of the present study is that all subjects converged in both distance traveled and velocity of travel by the third day of testing. This finding raises questions about the depth of the anxiety-inducing effect created by the maternal separation experience. In other words, while the separation had distinct and significant inhibitory effects on open field exploratory behavior in the first day of testing, presumably an indication of a sensitized nervous system with a reduced threshold for the elicitation of anxiety, the inhibitory effect completely habituated with only two repeated exposures to the novel (and potentially threatening) environment of the open field.

Future studies should examine if repeated interactions with different novel exposures replicate the findings of the first day of open field in this experiment. For instance, a wheel-running test and a Y-maze could be used consecutively after the open field test, given that each would provide a novel experience.

Indeed one of the most interesting findings of this study is that all subjects habituated to the repeated exposure of the novel (open field) testing environment despite highly significant differences in the initial reactions to that environment! Perhaps this finding underscores the resilience of an organism when a potentially threatening situation relatively quickly proves to be non-threatening. In other words, in the absence of threat with the passage of time, even an animal biologically primed for vulnerability to anxiety by virtue of early developmental experiences will rapidly habituate unless a threat is experienced or another organism is "modeling" fearful behaviors at the time of exposure to the environment.

Further complicating this is the fact that physiological responses to stress have necessary evolutionary value. The fight or flight response attests to this. Consequently, one can argue that mild stressors, such as handling, can be beneficial (Daskalakis, Kaperoni, Koros, Kloet, & Kitraki, 2009). In the present study, though, 3 hours of MS was sufficiently severe in eliciting anxiety-like behaviors in the rats. The implications of these results can parallel those in the clinical setting since reversing the adverse effect of phobic and other anxiety-based disorders developed as a consequence of child neglect and abuse is important.

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APPENDIX A

APPENDIX A

MATERNAL INTERACTION DATASHEET

DAM OBSERVATIONS

LG = Licking/Grooming ABN = Arched back nursing B = "blanket" - passive nursing mother on side CP = Carrying pups DO = Dam off O = Other

	LG				ABN				B				CP				DO				O			
	Prior MS		After MS		Prior MS		After MS		Prior MS		After MS		Prior MS		After MS		Prior MS		After MS		Prior MS		After MS	
	TS	NTS	TS	NTS	TS	NTS	TS	NTS	TS	NTS	TS	NTS	TS	NTS	TS	NTS	TS	NTS	TS	NTS	TS	NTS	TS	NTS
PND 1																								
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PND 16																								
PND 17																								
PND 18																								
TOTAL																								

Please circle the behavior that was observed the most each day (if more than one behavior is observed).

Pre-MS, record what was observed right before dam was taken out of cage. Post-MS, record 10 minutes of behavior.

GENERAL NOTES:

BIOGRAPHICAL SKETCH

Nancy I. Salinas was born in Reynosa, Mexico on May 13, 1981. Her family immigrated to McAllen, Texas on 1988. For most of the following 10 years, her family migrated to various parts of the country as farm workers.

She attended the McAllen School District until her freshman year and graduated from PSJA Memorial High School with academic recognition in the year 2000. From 2002-2005 she attended South Texas College where she earned an Associate of Arts Degree in Psychology. In 2008, a Bachelor of Science in Psychology and Sociology was conferred to Salinas by the University of Texas Pan American. While completing her undergraduate degree, she served as President, Vice President and Treasurer for the National Honor Society in Psychology (Psi Chi). In addition, she received the UTPA International Study Abroad Scholarship (2009) and The International Women's Board Scholarship (2009). During her undergraduate years at UTPA she was also nominated for the Excellence Award for the Social and Behavioral Science College (2007) and awarded the Undergraduate Research Initiative Grant (2005). In the fall of 2008, she pursued her master's degree in Experimental Psychology, with a concentration in Applied Behavior Analysis at UTPA.

Salinas currently lives in McAllen, TX with her 7 year old daughter, Jacky Weaver. All correspondence should be directed to P.O. Box 4853, Edinburg, TX 78541.