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Persisiting Sensitization of Depressive-Like Behavior and Thermogenic Response During Maternal Separation in Pre- and Post Weaning Guinea Pigs

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PERSISTING SENSITIZATION OF DEPRESSIVE-LIKE BEHAVIOR AND THERMOGENIC RESPONSE DURING MATERNAL SEPARATION IN PRE- AND POST-WEANING GUINEA PIGS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Randi Lynn Schneider ENTITLED Persisting Sensitization of Depressive-like Behavior and Thermogenic Response During Maternal Separation in Pre- and Post-weaning Guinea Pigs BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science

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ABSTRACT

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Persisting Sensitization of Depressive-like Behavior and Thermogenic Response During Maternal Separation in Pre- and Post-weaning Guinea Pigs.

Early attachment disruption is thought to promote later onset of depressive illness through a process involving sensitization. Maternal separation in guinea pig pups (~21 days of age) produces depressive-like behavior and core body temperature fluctuations that appear to be mediated by proinflammatory activity. These responses are enhanced during repeated separations over several days. Here, enhanced depressive-like behavior and core body temperature responses were observed from the early pre-weaning to the periadolescent period (~10-40 days of age) and persisted for more than a week. The greatest temperature response was observed during the final separation. These results demonstrate persisting sensitization of behavioral and thermogenic responses to maternal separation over the age range in which these responses are known to occur. Further, the findings are consistent with the hypothesis that proinflammatory activity contributes to the sensitization response and suggest that the impact of early attachment disruption on susceptibility to depression involves proinflammatory processes.

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I. INTRODUCTION

Protracted separation from the mother has profound effects on the behavior of children. Initial crying and attempts to re-establish contact give way to more serious consequences. If separation persists for a matter of days, as was commonplace for institutionalized children in the middle of the last century, the child may begin to exhibit signs of depression, including inactivity, withdrawal from environmental interactions, and persistent expressions of grief (Robertson, 1953; Spitz, 1946). Over the years it has become increasingly clear that early separation and other forms of attachment disruption (e.g., abuse, neglect) also enhance vulnerability for later depressive disorders, perhaps by sensitizing elements of the stress response (e.g., amygdala, CRF and HPA activity) to future stressful circumstances (Gillespie & Nemeroff, 2007; Gold, Goodwin, & Chrousos, 1988; Schulkin, McEwen & Gold, 1994). In other species in which infants exhibit filial attachment, prolonged separation can also elicit a depressive-like response. In some macaque monkeys, separated infants may eventually become passive, disengage from social interactions, assume a hunched posture, and display facial expressions suggesting sadness (Kaufman & Rosenblum, 1967; Mineka & Suomi, 1978).

Such responses are not unique to primates. Guinea pig pups display evidence of a selective filial attachment (Hennessy, 2003; Jackel & Trillmich, 2003; Pettijohn, 1979) and display a two-stage, active/passive response to separation (Hennessy, Long, Nigh, Williams, & Nolan, 1995). When isolated in a novel environment, pups initially vocalize at a high rate and tend to increase their activity. But after about an hour, they quiet down, typically show little apparent attention to their surroundings, and adopt a characteristic crouched stance, with closed eyes, and extensive piloerection. These responses are inhibited if the mother is present in the novel environment (Hennessy & Morris, 2005). Although weaning naturally occurs around day 25, guinea pigs will continue to show these active and passive separation behaviors up to at least 40 days of age (i.e., periadolescence), particularly if the young have been continuously housed with their mother (Hennessy & Morris, 2005). Behaviors like those seen during the second, passive stage of separation can be mediated by proinflammatory activity. Exposure to pathogens and some stressors can induce a systemic inflammatory response that consists, in part, of release of proinflammatory cytokines, fever, altered synthesis of liver proteins, and behavioral changes including reduced activity, sleepiness, and other reactions that appear to support induction of fever (e.g., hunched stance and piloerection; Black, 2003; Hart, 1988; Maier & Watkins, 1998). Evidence indicates that the passive, second stage of separation in guinea pigs is, indeed, mediated by proinflammatory processes. The passive response to separation can be reduced by administration of any of several anti-inflammatory agents (Hennessy, Schiml-Webb, Miller, Maken, Bullinger, & Deak, 2007b; Perkeybile, Schiml-Webb, O'Brien, Deak, & Hennessy, 2009; Schiml-Webb, Deak, Greenlee, Maken, & Hennessy, 2006); direct activation of a systemic inflammatory response produces the same constellation of crouching, eye-closing, and piloerection as does separation (Hennessy, Deak, Schiml-Webb, Wilson, Greenlee, & McCall, 2004) and separation increases physiological indicants of an inflammatory response: increased expression of the proinflammatory cytokine, tumor necrosis factor-alpha, in spleen (Hennessy, Deak, Schiml-Webb, & Barnum, 2007a) and a rise in core temperature

(Hennessy et al., 2004), suggesting fever. These findings may be relevant to the aforementioned effects of separation in children because proinflammatory activity appears to promote forms of human depressive illness (Anisman, 2009; Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Miura, Ozaki, Sawada, Isobe, Ohta, & Nagatsu, 2008; Raison, Capuron & Miller, 2006).

The passive response of young guinea pigs has also been found to show sensitization. When pups were separated on two or three occasions over not more than 5 days, levels of passive behavior increased from the initial, to the subsequent, separations (Hennessy, Deak, Schiml-Webb, Carlisle, & O'Brien, 2010b; Hennessy, Paik Caraway, Schiml, & Deak, in press). Proinflammatory activity may be involved in this sensitization because administration of an anti-inflammatory prior to the initial separation was found to block the increase in passive behavior during separation the following day (Hennessy et al., in press). Behavioral effects of repeated separation were accompanied by corresponding changes in thermogenic response: core temperature showed a pronounced initial elevation and more rapid decline during separations 1 to 4 days later than during the first separation experience (Hennessy et al., 2010b; Yusko et al, unpublished). Thus, core temperature may reflect proinflammatory mechanisms underlying the behavioral sensitization.

If the increase in passive responding to repeated separations bears any resemblance to the sensitization process that is thought to link early attachment disruption and later onset of depression in humans, then one might expect the sensitization effect to continue beyond a few days. One purpose of the present study was to extend the interval between separations. The behavior of young guinea pigs was examined during separations on 2 consecutive days and then again 8 days later. To assess the correspondence of behavior and core temperature, I also monitored continuous changes in core temperature of animals during all separations with a telemetry device. A second purpose was to evaluate possible changes with age in the strength of the sensitization response. That is, I asked whether there was a sensitive period during which the effect was most robust, or if the response was relatively constant across the age range at which the passive response has been observed. In previous studies, testing began about 3 weeks of age. In the present study, guinea pigs were administered multiple separations beginning at either 10/11, 20/21, or 30/31 days of age. The range was determined by concerns regarding the youngest age at which surgery to implant a telemetry device could be conducted and the oldest age at which animals have been observed to show the passive response (i.e., the last separation for the oldest group was about 40 days of age). To account for changes in response measures due to increasing age, I also compared the effects of a single separation at 10/11, 20/21, 30/31, and 40/41 days of age.

II. METHODS

Animals

Albino guinea pigs (*Cavia porcellus*) of the Hartley strain were bred in our laboratory. Following birth (Day 0), each mother was housed with her litter in an opaque plastic cage (73cm x 54cm x 24cm) with a wire front and sawdust bedding. Water and guinea pig chow were available ad libitum. Lights were maintained on a 12:12 light:dark cycle with lights on at 07:00 h. All procedures were approved by the Wright State University Animal Care and Use Committee. Offspring remained with their mothers and littermates from birth (Day 0) until the end of testing, being removed only for surgery, behavioral testing, and maintenance procedures (e.g. cage changes, weighing of pups). Separate groups of 8 animals (4 males, 4 females) were tested in each of four experimental conditions. In three of these conditions, guinea pigs underwent multiple separations beginning at either Day 10/11 (MSEP10), Day 20/21 (MSEP20) or Day 30/31 (MSEP30). In a final condition, animals experienced a single separation on Day 40/41 (SSEP40). In each of the first three conditions, animals were separated on two consecutive days, and again 8 days later. In this way, the age of the last separation for one group was the age of the first separation for the following group (Fig. 1). No more than one pup from a litter was assigned to a condition.

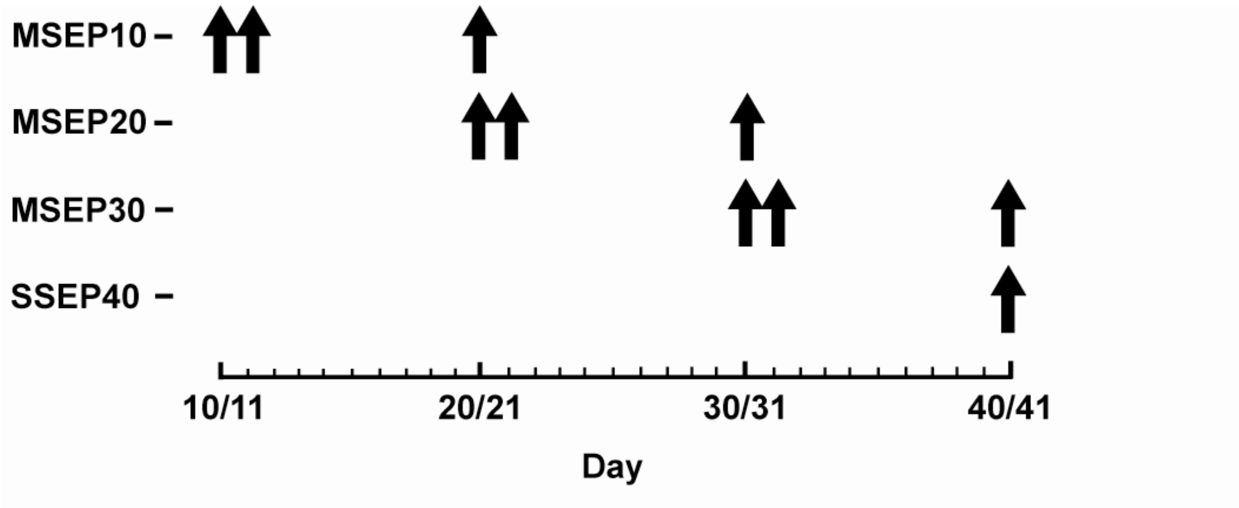

Figure 1. Illustration of the study design. Each arrow represents a single separation.

Surgery.

To obtain core temperature and activity data, a telemetry probe (PD4000 Emitter, Mini-Mitter, Bend, OR) was surgically implanted in the peritoneal cavity 3 to 5 days prior to testing. Surgery was performed using aseptic conditions. Guinea pigs were anesthetized using isoflurane, with atropine (0.05mg/kg, i.p.) given to reduce secretory activity. A section of the abdomen was shaved and swabbed with antiseptic solution, and a small incision (~1 cm) was then made through the skin and abdominal wall. The sterilized probe (23mm x 8mm diameter) was inserted into the cavity and sutured to the inside of the abdominal wall. The incision through the wall was sutured shut and the overlying skin was closed using standard laboratory wound clips. Buprenorphine (0.015mg/0.05ml) was given immediately following surgery and again 24 hr later for post operative pain (1.0ml/kg). Animals gained weight readily after surgery and behavior appeared normal.

Test Procedure

For testing, the animal was removed from its home cage and taken quietly in a carrying cage to the nearby testing room where it was placed into a clear, empty plastic cage (47cm x 24cm x 20cm) for 3 hr. The test cage was positioned on an energizer/receiver platform (also from Mini-Mitter) for remote telemetry. To prevent escape, the cage was covered with plastic lid that permitted ample passage of light. Temperature data were collected using Vital View (Mini-Mitter) software run by a remote computer. These data were processed by the software as mean values for each 3-min interval. Movement of the animal was also detected with the telemetry system in 3-min bins across the 3-hr test. The primary purpose of assessing activity was to evaluate whether any observed changes in body temperature were associated with physical exertion.

An observer behind one-way glass recorded other active and passive behaviors during Min 0-30, 60-90, and 150-180. The active behavior scored at this time was the characteristic “whistle” vocalization (Berryman, 1976) of pups separated from their mother. A microphone placed over the cage transmitted sound to the head phones of the observer, who scored vocalizations on a hand counter. The number of 1-min intervals in which the following passive behaviors occurred also was recorded: a crouched stance in which the body is held close to the floor, complete or near complete closure of one or both eyes for at least 1s, and piloerection occurring over most of the visible body surface. The animal was considered to exhibit “full passive” behavior when all three responses were shown within the 1-min interval. On occasion, pups were observed to completely lie down, with the trunk supported by the cage floor. Lying down could be substituted for crouching, i.e., the “full passive” response was scored if eye-closure, piloerection, and either crouch or lying down were observed in the same 1-min interval. Scoring was done by a trained observer (inter-observer reliability of at least 85%). All testing began between 07:00h and 11:00h. Test cages were cleaned with detergent after each test.

Data Analysis

For core temperature and activity counts, data across five consecutive 3-min bins were averaged to generate scores for 15-min time blocks. Data were analyzed with analysis of variance (ANOVA) procedures. To compare the groups starting repeated separations at different ages, we used 3 (Age Group) x 2 (Gender) x 3 (Separation) x 12 (Time Block) ANOVAs with the last two factors treated as repeated measures for core temperature and activity counts. For full passive response and vocalizations, 3 (Age Group) x 2 (Gender) x 3 (Separation) ANOVAs were performed. To examine the effect of age on response to initial separation, data collected during the first separation of each of the four groups was analyzed with either a 4 (Age Group) x 2 (Gender) x 12 (Time Block) ANOVA (core temperature and activity counts) or a 4 (Age Group) x 2 (Gender) ANOVA (full passive response and vocalizations). Significant effects were followed with simple main effect tests (Winer, 1971) and Newman-Keuls paired comparisons as appropriate. When sphericity was judged to be problematic by Mauchly’s test, the Huynh-Feldt correction factor was used. A $p < 0.05$ level of significance (2-tailed) was accepted throughout.

III. RESULTS

Core Temperature.

The ANOVA examining the effect of multiple separations in the three age groups yielded significant effects of Group, F (2, 17) = 5.82, $p < 0.05$, Separation, F (2, 34) = 13.84, $p < 0.01$, Time Block, F (3.7, 62.4) = 22.78, $p < 0.01$, and Separation x Time Block, F (22, 374) = 7.46, $p < 0.01$. For the Group effect, Newman-Keuls tests revealed that mean temperature was significantly higher for the MSEP30 group (M = 39.31 +/- 0.06) than for the MSEP10 (M = 39.08 +/- 0.06, $p < 0.05$) and MSEP20 groups (M = 39.07 +/- 0.06, $p < 0.05$) . That is, animals starting testing at 30 days of age had a significantly higher mean core temperature across separations than animals beginning testing at 10 and 20 days of age. For the significant two-way interaction, simple main effect tests showed that core temperature during the three separations differed for Time Blocks 1-11 (p's < 0.05; Fig. 2). Newman-Keuls tests showed that for Time Blocks 1-5, core temperature was higher during the third separation than during the two previous separations (p's < 0.05), which occurred 8 and 9 days earlier. For Time Blocks 6-10, core temperature during the second separation was significantly lower than during the first and third separations (p's < 0.05). There were no significant paired comparisons for Time Block 11. In sum, test animals showed a reduction in core temperature during the latter portion of their second separation, and a substantially enhanced temperature during the initial portion of their third separation, as compared to their first. Of particular interest here, this pattern did not depend on the age at which testing began.

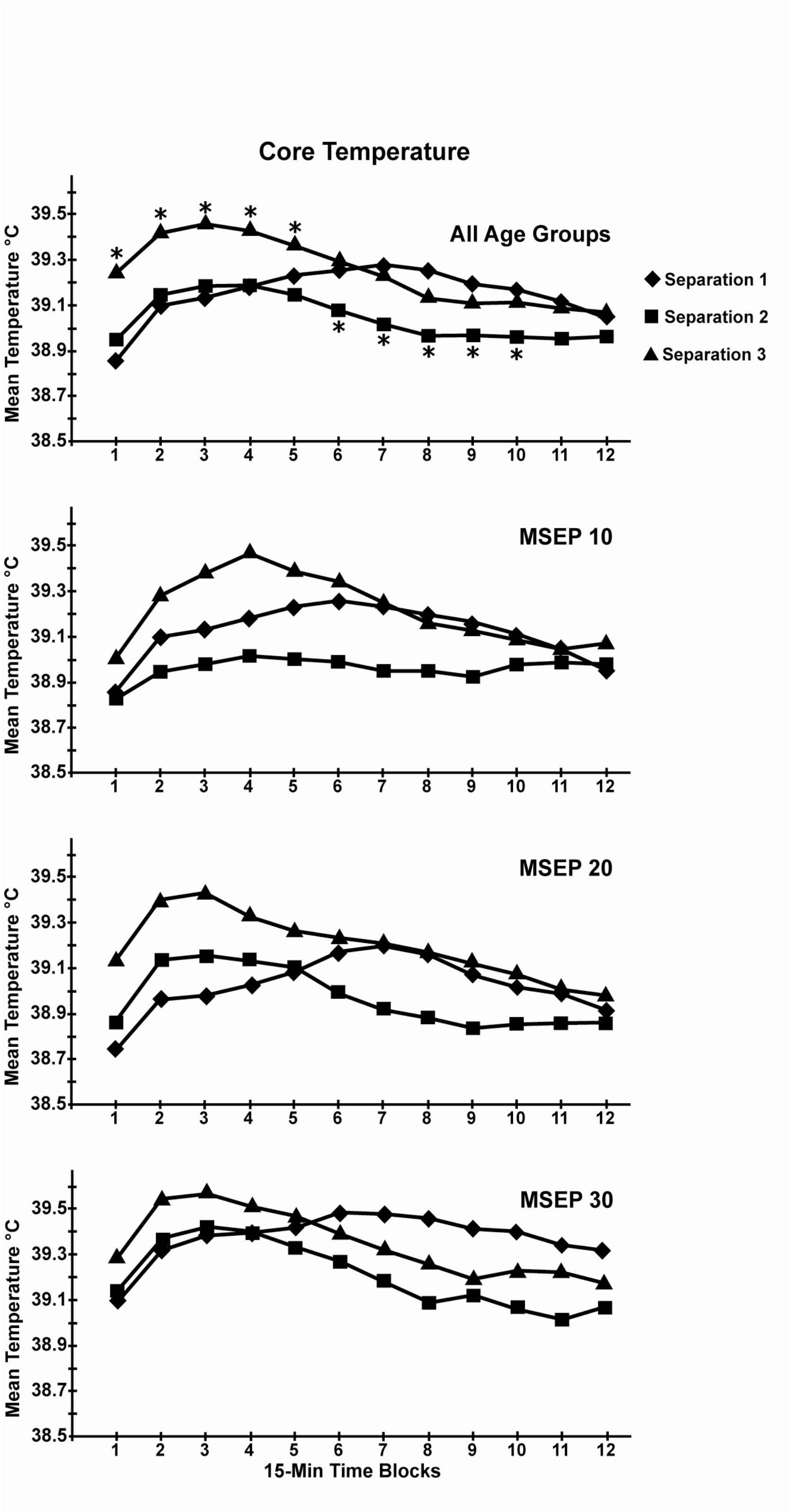

Figure 2.The upper panel illustrates the mean core temperature across all age groups undergoing multiple separations. For comparison, mean core temperature for each of the individual age groups is depicted in the remaining panels.

*$p < 0.05$ vs each of other two groups

Table 1. Mean and standard error for each dependent measure during initial separation at each age.

Age (Days)	10/11		20/21		30/31		40/41	
	m	*se*	*m*	*se*	*m*	*se*	*m*	*se*
Temperature	39.07	0.09	39.02	0.06	39.37*	0.08	39.31*	0.07
Activity Counts	46.38	8.51	35.21	7.61	29.26	5.35	18.43	5.53
Depressive-Like Behavior	4.50	1.83	16.38	5.79	14.38	5.94	22.13	8.33
Vocalizations	3036†	520	1880	582	1185	555	391	191

*$p < 0.05$ vs youngest two age groups

† $p < 0.05$ vs oldest two age groups

The ANOVA for age effects yielded significant outcomes for Age Group, F (3, 24) = 4.68, $p = 0.01$, and Time Block, F (4.9, 117.7) = 14.45, $p < 0.01$. Post-hoc tests revealed that core temperature during a first separation on Day 30/31 and 40/41 was significantly higher than core temperature during initial separation on Day 10/11 and 20/21 (*p's* < 0.05; Table 1). The Time Block effect reflects an initial rise and then decline in temperature during the 3-hr test (Fig. 3, upper panel).

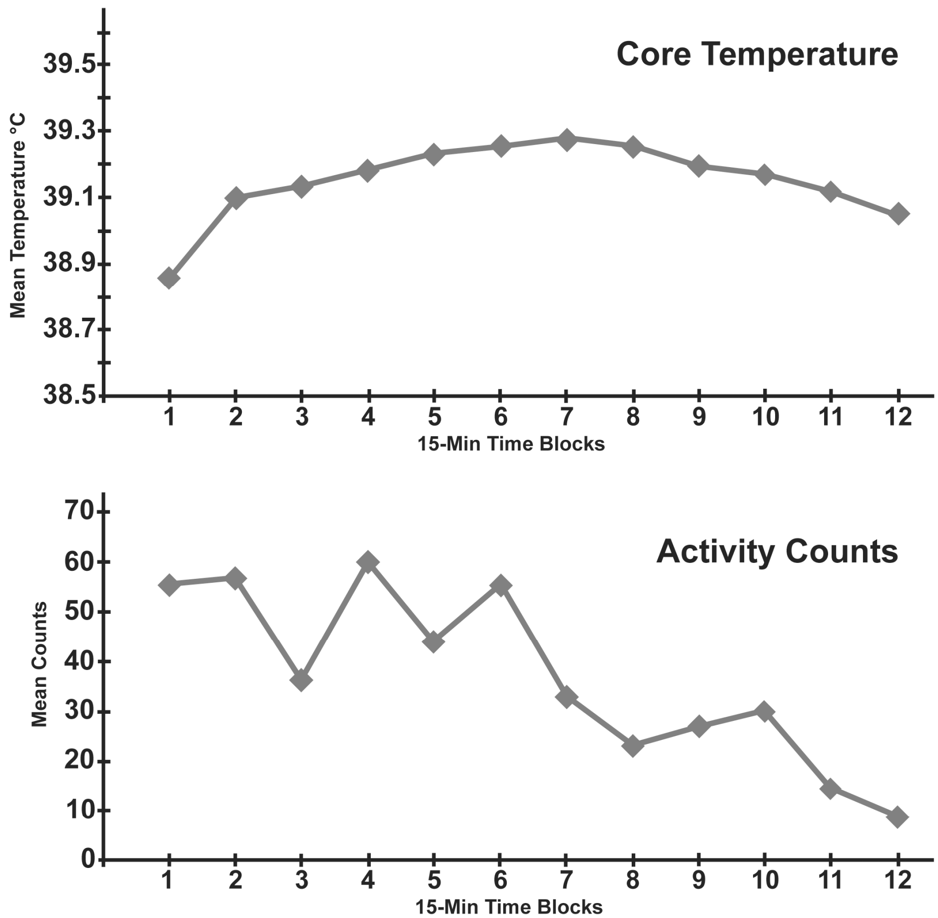

Figure 3. The top panel shows mean core temperature during the initial separation for all age groups undergoing multiple separations. The bottom panel shows mean activity counts during the initial separation for all age groups undergoing multiple separations.

Behavior.

Activity Counts. The ANOVA for multiple separations yielded significant effects for Separation, F (2, 34) = 18.72, $p < 0.01$, Time Block, F (7.5, 126.9) = 11.05, $p < 0.01$, and Separation x Time Block, F (22, 374) = 1.89, $p < 0.01$. The pattern of change in activity counts during each of the three separations showed little or no correspondence to changes in core temperature (Fig. 4). Because my interest in activity was primarily to determine if such correspondence existed, specific post-hoc tests were not performed. The ANOVA for age effects revealed a significant effect of Time Block, F (6.9, 167.7) = 5.97, $p < 0.01$ (Fig. 3, lower panel). Although mean activity counts decreased with age (Table 1), the effect of Age Group was only marginally significant ($p < 0.08$).

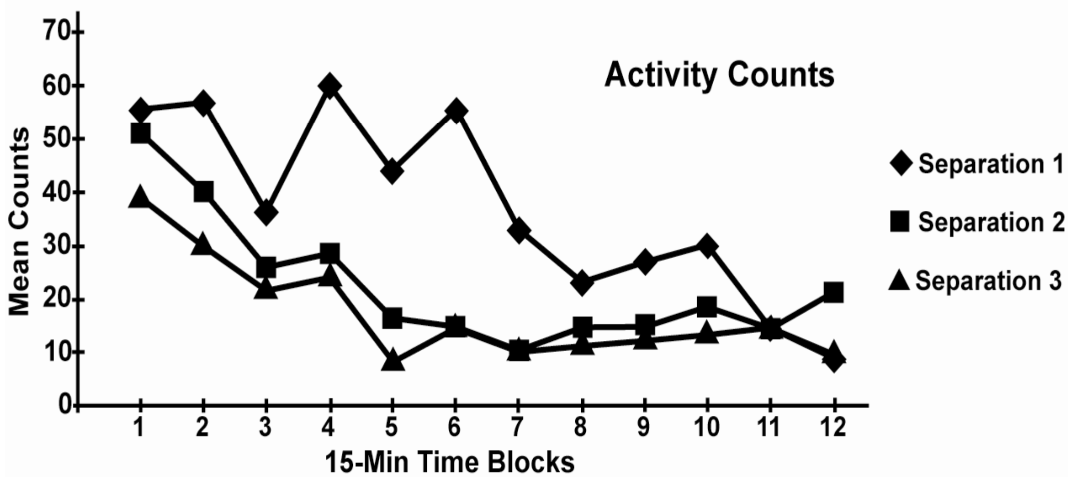

Figure 4. Mean activity counts across all age groups undergoing multiple separations.

Vocalizations. The ANOVA for multiple separations revealed main effects of Age Group, F (2, 18) = 3.89, $p < 0.05$, and Separation, F (2, 36) = 8.32, $p < 0.01$. For the Age Group effect, a Newman-Keuls test showed that animals beginning testing at the youngest age vocalized significantly more across separations than those animals beginning testing at the oldest age (MSEP10 M = 2443 +/- 137; MSEP30 M = 943+/-

333, $p < 0.05$). For the Separation effect, animals vocalized significantly more during their first separation than during their third ($p < 0.05$; Fig. 5, upper panel).

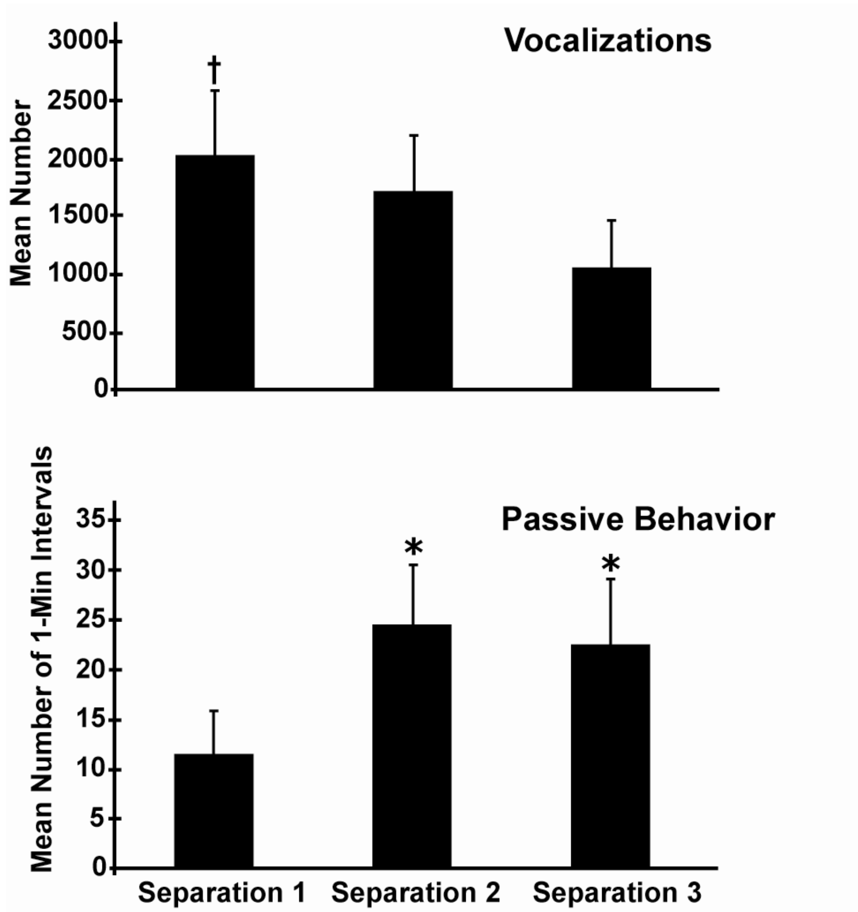

Figure 5. The upper panel illustrates the mean number of vocalizations emitted by animals across age groups during repeated separations. The bottom panel shows the mean number of 1-min intervals of passive depressive-like behavior exhibited by animals across age groups during repeated separations. Vertical lines represent standard errors of the means.

† $p < 0.05$ vs Separation 3

* $p < 0.05$ vs Separation 1

The ANOVA for age effects yielded a significant main effect of Age Group, F (3, 28) = 5.28, $p < 0.01$. Animals tested on Day 10/11 vocalized significantly more than animals tested on Day 30/31 and 40/41 (p's < 0.05; Table 1).

Passive Responses. The ANOVA for multiple separations yielded only a significant main effect of Separation, F (2, 36) = 10.02, $p < 0.01$. Animals exhibited more full passive behavior during their second separation, as well as their third, which occurred 8 days later, than they did during their first (Fig. 5, lower panel). The absence of an interaction with Age Group indicates that animals of these age groups showed comparable increases of full passive behavior during later separations. The ANOVA for age effects revealed no significant effects, although as seen in Table 1, considerably more passive behavior was observed at the oldest age than at the youngest.

IV. DISCUSSION

Early disruption of attachment relationships is thought to increase risk for later depressive illness through a sensitization process in which later exposure to other stressors is amplified as a result of the early experience (Gillespie & Nemeroff, 2007; Gold, et al., 1988; Schulkin, et al., 1994). Previous work in the guinea pig showed that a 3-hr separation near the age of weaning sensitized passive, depressive-like behavior the following day as well as several days later (Hennessy et al., 2010b; Yusko et al, unpublished). The present study shows that the enhancement of passive behavior with repeated separations occurs across a wide age range and can persist for at least 8 days following the second separation. This study also examined the effect of a single separation across a wide age range in order to assess possible confounds between age effects and effects of repeated separations. Analysis revealed no significant changes with age in levels of passive behavior. Nonetheless, comparison of Tables 1 and 2 shows that absolute mean levels of passive behavior during the third separation of the youngest and oldest age groups approximate the mean value obtained for animals separated for a first time at the same age. Therefore, conclusions regarding actual sensitization during the final separation of these two groups must remain tentative pending further study. On the other hand, the passive behavior of animals in the middle age group (beginning testing at 20/21 days of age) during their third separation was elevated above the level of guinea pigs first separated at this age. The mean difference was about as great as that between the first and third separation for the intermediate age group. Thus, I conclude that sensitization of

passive, depressive-like behavior can persist for more than a week, at least for guinea pigs of this age.

Table 2. Mean number of 1-min intervals that the depressive-like response was observed in each group undergoing repeated separations.

	Separation		
Group	1	2	3
MSEP10	4.50	19.50	19.88
MSEP20	16.38	30.75	28.13
MSEP30	14.38	23.75	21.13

Earlier studies showed that core body temperature also changed with repeated separations, being higher during the initial portion of the test and exhibiting a greater subsequent decline. (Hennessy et al., 2010b; Yusko et al, unpublished). The higher temperature during the early portion of the subsequent separations could not be attributed to differences in temperature at the time the later separations began, but rather was a difference in response to the separations, per se (Yusko et al., unpublished). This study found a similar pattern of response during separation, although the heightened initial response occurred only during the third separation, and the decline was significant only during the second separation. The Age Group x Separation interaction for core temperature was nonsignificant indicating that this pattern was consistent across the age ranges tested. Nevertheless, inspection of Figure 2 shows that pups beginning testing at the same approximate age as in earlier studies (MSEP20) show a pattern that is quite similar to those previous studies, including an increase in temperature during the initial segment of the second separation. This suggests that the difference in the pattern for Day 2 in the current study (across all age groups) versus previous studies is driven primarily by the MSEP10 and MSEP30 groups. Perhaps the most interesting aspect of the temperature data, however, was that a third separation occurring more than a week after the second produced such a clear elevation during the first 75 min of the 3-hr test. Further, core temperature during the remainder of the test never dipped below levels of the earlier two. Therefore, core temperature, as well as passive, depressive-like behavior, showed clear sensitization with repeated separations.

Vocalizations declined with repeated separations. The finding that there also was a reduction with increasing age in vocalizing during an initial separation suggests that the effect of repeated separations may be due largely to an age effect. This interpretation is in agreement with previous work showing a reduction in vocalizing during separations across this age range (Hennessy & Morris, 2005;Yusko et al., in preparation). Activity counts also generally decreased with multiple separations in the present study and showed no apparent relation to concurrent changes in core temperature. The selective sensitization of passive behavior and core temperature, but not active behavior, suggests a common underlying mechanism for the sensitized responses. I suggest this mechanism involves a stress-induced increase in proinflammatory activity. Proinflammatory activity can be enhanced by stressors (Maier & Watkins, 1998), induces fever (Blattein, Quan, Xin, & Ungar, 1990) and mediates the passive responses of guinea pig pups during separation (Hennessy, Schiml-Webb, & Deak, 2009). Furthermore, inhibiting proinflammatory activity before an initial separation blocks sensitization to separation the following day (Hennessy et al., in press). It would appear then that the initial separation experience either potentiates proinflammatory signaling, or increases sensitivity to this signaling, during later separations (Hennessy, Deak, & Schiml-Webb, 2010a). My results indicate that following two 3-hr separations, sensitization persists for more than a week, and based on the temperature results, may even increase during this period.

There is a growing literature indicating a role for proinflammatory factors in the onset of forms of depressive illness (Anisman, 2009; Dantzer, et al., 2008; Miura, et al., 2008; Raison, et al., 2006). Moreover, there recently has been increasing evidence for links between early trauma, enhanced later proinflammatory activity, and depression. Danese, Moffitt, Pariante, Ambler, Poulton and Caspi (2008) observed that depression was associated with increased proinflammatory activity in their sample, but only among those subjects experiencing early-life maltreatment. These depression-proinflammatory associations do not appear to be the result of the depression associated with early maltreatment then leading to an inflammatory response because non-depressed adolescents and adults reporting early life adversity or a harsh early family climate were also found to exhibit exaggerated proinflammatory responses to later challenge (Carpenter, Cawuga, Tyrka, Lee, Anderson, Price, 2010; Miller & Chen, 2010). Further, the enhanced proinflammatory response was observed to increase in magnitude over time (Miller & Chen, 2010), suggesting a continued sensitization of this response. In sum, both human and animal studies point to a role for sensitization of proinflammatory activity as a possible mechanism linking early life events and the later development of psychopathology.

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