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Proinflammatory Activity and the Sensitization of Depressive-Like Behavior during Maternal Separation

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Abstract

When guinea pig pups are isolated for a few hours in a novel environment, they exhibit a distinctive passive behavioral response that appears to be mediated by proinflammatory activity. Recently, we observed that pups separated on two consecutive days show an enhanced (sensitized) passive response on the second day. In Experiment 1, pups receiving intracerebroventricular infusion of 50 ng of the anti-inflammatory cytokine Interleukin-10 prior to a first separation failed to show a sensitized behavioral response to separation the next day. In Experiment 2, pups separated on Days 1 and 2, or just 2, showed an increase in passive responding during separation on Day 5. Pups injected with the bacterial antigen lipopolysacchride (LPS; 75µg/kg body weight, intraperitoneal) prior to separation on Day 1 showed an increase in passive behavior several days later not shown by pups injected with saline prior to Day 1 separation. However, injection of LPS without separation on the first day did not enhance responding during an initial separation on the second day. These results suggest that immune activation is necessary, but not sufficient, to account for the sensitization of passive behavior of isolated guinea pig pups the following day, that boosting proinflammatory activity during an initial separation may promote sensitization several days later, and that the sensitized response persists for at least several days.

Keywords

maternal separation; sensitization; passive behavior; sickness behavior; depressive-like behavior; cytokines; proinflammatory; Interleukin-10; lipopolysacchride; guinea pig

Stressful life experiences alter the activity of diverse neural and endocrine systems. Prominent among these changes are increases in activity of the hypothalamic-pituitary-adrenal (HPA) axis, sympathetic nervous system, amygdala, locus coeruleus, and extra-hypothalamic corticotropin-releasing factor (CRF) pathways (Stratakis & Chrousos, 1995; Rodrigues, LeDoux, & Sapolsky, 2009). In the past few decades, it has become clear that stressors also can activate proinflammatory signaling, resulting in physiological and behavioral responses similar to those of physically ill animals (e.g., altered production of liver proteins, fever, reduced responsiveness to the environment, sleepiness, postures that

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conserve heat; Black, 2003; Kubera, Holan, Mathison, & Maes, 2010; Maier & Watkins, 1998). The physiological reactions induced by stressors have been associated with various forms of psychopathology. For instance, episodes of adult depression often coincide with, or follow, periods of severe or prolonged stress (Bonde, 2008; Caspi et al., 2003; Chadda, Malhotra, Kaw, Singh, & Sethi, 2007).

Similar effects have been observed in children. Following isolation from primary caretakers, as was common practice in hospitals and other institutions in the middle of the last century, some children eventually lapse into a depressive-like state (i.e., “anaclitic depression”) characterized by weepiness and emotional withdrawal (Spitz, 1946). Moreover, early stressors, particularly disruption of attachment relationships (separation, abuse, neglect) repeatedly have been linked to an increased probability of acquiring a depressive disorder at a later age (Agid et al., 1999; Bernet & Stein, 1999; Furukawa et al., 1999; Gilman, Kawachi, Fitzmaurice, & Buka, 2003; Reinherz, Giaconia, Carmola Hauf, Wasserman, & Silverman, 1999; Takeuchi et al., 2002). This long-term effect has been proposed to involve a sensitization process, in which the early stress increases responsiveness to later stress challenges, so that relatively minor disturbances in adulthood can trigger a sustained and unregulated stress response that largely comprises a depressive episode (Gold, Goodwin & Chrousos, 1988). Studies suggest that the sensitization may be mediated by various physiological substrates, in particular activity of the amygdala and CRF (Anisman, Merali, & Stead, 2008; Gillespie & Nemeroff, 2007; Gold et al., 1988; Heim & Nemeroff, 1999; Jiménez-Vasquez, Mathé, Thomas, Riley & Ehlers, 2001; Ladd, Owens, & Nemeroff, 1996), though the full set of underlying systems involved, and how they interact, remains to be determined.

Early nonhuman primate studies showed that infants of some species of macaque monkeys exhibited a depressive-like response to protracted maternal separation that resembled the anaclitic depression of institutionalized children. After an initial period of active behavior termed “protest”, in which infants vocalized and displayed increased motor activity, a number of them would become quiet and passive, assume a hunched posture, and withdraw from interactions with the social and physical environment (Kaufman & Rosenblum, 1967; Mineka & Suomi, 1978). While in this “despair” stage of separation, the infant monkeys, like the human children they modeled, impressed observers as appearing *physically* ill (Rosenblum & Kaufman, 1967; Spitz, 1946). Studies with guinea pigs suggest that this observation might be accounted for by a stress-induced activation of proinflammatory activity (Hennessy, Deak & Schiml-Webb, 2001). That is, the stressor of the separation procedure may increase proinflammatory signaling, which in turn, induces behavioral changes (e.g., reduced responsiveness to the environment, hunching to conserve heat) that are characteristic of the despair stage.

Infant guinea pigs display a strong attraction or attachment to the mother (Hennessy, 2003; Hennessy & Ritchey, 1987; Jäckel & Trillmich, 2003), as well as a two-stage, active/passive response during separation that is reminiscent of the two-stage separation response of macaque monkeys. However, the guinea pig response unfolds in a much shorter span, i.e., hours rather than days or weeks. When first isolated in an unfamiliar enclosure, pups vocalize and tend to increase locomotor activity. After about an hour, vocalizations subside and pups enter a second, passive stage characterized by a crouched stance, prolonged eye-closure, and extensive piloerection (Hennessy, Long, Nigh, Williams, & Nolan, 1995). These responses do not occur if the mother accompanies the infant to the novel enclosure (Hennessy & Morris, 2005). As observed for separated monkey infants and children, the pup's appearance suggests physical illness. Several lines of evidence indicate that increased proinflammatory activity is a mediator of the passive response. First, pups injected with lipopolysacchride (LPS), which stimulates a potent inflammatory reaction, elicits the passive

response immediately following separation, when pups typically are still active (Hennessy, et al., 2004). Second, administration of compounds with anti-inflammatory activity [alpha-melanocyte-stimulating hormone, indomethacin, or the anti-inflammatory cytokine interleukin-10 (IL-10)] reduces the passive behavior pups show during a subsequent 3-hr separation (Hennessy et al., 2007b; Perkeybile, Schiml-Webb, O'Brien, Deak, & Hennessy, 2009; Schiml-Webb, Deak, Greenlee, Maken, & Hennessy, 2006). Third, 3 hr of separation induces tell-tale signs of immune activation, namely an elevation in core temperature (Hennessy, Deak, Schiml-Webb, Carlisle, & O'Brien, 2010) and increased expression of the proinflammatory cytokine, tumor necrosis factor-alpha, in spleen (Hennessy, Deak, Schiml-Webb, & Barnum, 2007).

Recently, we found that when pups were separated for 3 hr on 2 consecutive days, levels of passive behavior were greatly increased on the second day (Hennessy et al., 2010b). This behavioral sensitization was accompanied by a more-distinct increase in core temperature on Day 2. These results suggest that proinflammatory factors might contribute, not only to the initial passive response on Day 1, but also to the sensitized passive behavior on the second day. Therefore, the present study investigated the role of proinflammatory factors in the sensitization process (Experiments 1 and 2) as well as whether the behavioral sensitization would occur over a longer interval than a single day (Experiment 2).

General Method

Subjects

Albino guinea pigs (*Cavia porcellus*) were bred in our laboratory. Each mother and her litter were housed in opaque plastic cages (73 cm × 54 × cm 24 × cm) with wire fronts and sawdust bedding at ~70° F. Water and guinea pig chow were available *ad libitum*. Lights were maintained on a 12:12 light:dark cycle, with lights on at 0700 hr. Cages were changed twice per week. All procedures were in compliance with NIH guidelines and approved by the Wright State University Laboratory Animal Care and Use Committee. Following birth (Day 0), pups were maintained with their mothers for the duration of the experiments, being removed only for surgery for placement of intracerebroventricular (ICV) cannulae (Experiment 1), behavioral testing, and brief routine colony management procedures (such as weighing of pups). Testing was conducted near the time of natural weaning, which occurs around Day 25 (König, 1985; Schiml & Hennessy, 1990). At this age, guinea pigs show a strong attraction to the mother, as well as robust active and passive behavioral responses to separation (e.g., Hennessy, Young, O'Leary, & Maken, 2003; Hennessy et al., 1995).

Behavioral Testing

For assessing separation behavior, the pup was transferred from the home cage to a nearby testing room via a transport cage (< 10 s). In the testing room, the pup was placed into a clear, empty, plastic cage (47 × 24 × 20 cm) located on a table under full room lighting for 3 hr. A trained observer recorded behavior behind 1-way glass during Min 0-30, 60-90, and 150-180. For each 1-min interval, the observer noted whether the pup engaged in any of three characteristic passive responses: 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 1 s, and piloerection occurring over most of the visible body surface. Our measure of passive behavior was the number of 1-min intervals in which all three of these responses occurred. On occasion, pups were observed to lie down, supporting body weight with their trunk. For these instances, lying down was substituted for crouch, 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. Instances of the primary active separation behavior—whistle vocalizations—(Berryman, 1976), were detected with a microphone positioned over the test

cage and transmitted to the observer via headphones, who scored the absolute number with a hand-held counter. In each experiment, a single observer (not blinded) scored all tests of nearly all pups (20 of 23 pups of Experiment 1 by K.D.P.; 45 of 48 pups of Experiment 2 by J.D.C.) All observers were trained to at least 85% inter-observer reliability prior to the experiments. All separations began between 0700 and 0900 hr. The test cage was cleaned with detergent after each use.

Experiment 1

In this experiment, guinea pig pups separated on two consecutive days were infused through an ICV cannula with either the anti-inflammatory cytokine IL-10 or vehicle prior to the first separation. If proinflammatory responses on Day 1 contribute to the increased passive response on Day 2, treatment with the anti-inflammatory cytokine prior to the first separation might be expected to reduce or eliminate the enhanced responsiveness during the second separation.

Method

Subjects and Experimental Design—Twenty three guinea pig pups were assigned to one of two conditions. Twelve pups (6 males, 6 females) received IL-10 prior to their first separation and 11 pups (5 males, 6 females) received artificial cerebrospinal fluid (aCSF) vehicle. All separations occurred between Days 20 and 24. No more than one pup from a litter was assigned to either condition. Litters for the two conditions averaged 3.25 (IL-10) and 3.18 (aCSF) pups.

Surgery and Infusions—Between Days 16 and 19, pups underwent aseptic surgery for placement of an intracerebroventricular cannula. For consistency, the right lateral ventricle was always targeted. Pups were pretreated with atropine (0.05 mg/kg, IP) and anesthetized with isoflurane before being placed into a stereotaxic apparatus. The stereotaxic was equipped with a modified incisor bar/nose cone that delivered a constant dose of 3% isoflurane throughout the surgery. A local anesthetic was administered to the scalp (0.1 ml 0.25% bupivacaine) prior to the incision being made. Guide cannulae (26 gauge) were placed relative to bregma with coordinates of -3.0 mm anterior-posterior, -3.0 lateral, and -4.0 mm dorsal-ventral (from the skull; Luparello, 1967). A stainless steel screw was placed across the skull's sagittal suture adjacent to the guide cannula to help secure the cranioplastic cement that held the guide in place. All cannula supplies were sterile at the time of surgery and were purchased from Plastics One (Roanoke, VA). Following surgery, and again 12 hours later, pups were treated with buprenorphine [0.015 mg/0.5 ml, intraperitoneal (IP)] to control for post-operative pain. Each day post-surgery, pups were weighed and dummy cannulae (caps) were briefly removed so that the indwelling cannulae could be checked for patency. A recovery time of at least 4 days following surgery was allowed before behavioral testing.

Recombinant murine IL-10 (American Research Products) was prepared in aCSF. A dose of 50 ng was chosen because it was the median effective dose found previously to reduce vocalizations during a single 3 hr separation in guinea pig pups (Perkeybile et al., 2009). Aliquots of IL-10 and aCSF vehicle were stored at -80°C until just prior to administration. Infusions (5 μl volume) of drug or vehicle were made over the course of 2.5 min with a Hamilton syringe. After infusion, all pups were returned to the home cage for 1 hr prior to their first separation. To control for the several min of handling required for infusion before the first separation, all pups were handled for 2.5 min 1 hr prior to the second separation. All animals were killed after the second separation via carbon dioxide inhalation. Cannulae placement was then verified via infusion of dye (~ 50 μl India ink) through the cannula; only

data from animals in which dye was present in at least one lateral ventricle were included in the study.

Data Analysis—Because of non-normal distributions of scores, non-parametric tests (Mann-Whitney *U* tests and Wilcoxon-Matched Pairs, Signed Ranks Tests for between and within group comparisons, respectively) were used to assess passive behavior. Vocalizations were analyzed with a 2 (Condition) $\times$ 2 (Sex) $\times$ 2 (Day) analysis of variance (ANOVA) with the last factor treated as a repeated measure.

Results

There were no sex differences in the full passive response in either condition at either day; therefore, data from the two sexes were pooled. Sensitization of passive behavior (i.e., an increase from Day 1 to Day 2) was seen in those pups administered aCSF vehicle prior to the first separation ($p < 0.05$). There was no sensitization of the passive response in pups administered IL-10. (Fig. 1). Comparison between conditions at each day showed that on Day 2 the difference in passive behavior of aCSF and IL-10 pups approached significance ($p = 0.069$).

Pups vocalized at a high rate during both separations and this response was unaffected by repeated testing [aCSF, Day 1: $m = 2,789$ (± 598), Day 2: $m = 2,791$ (± 907); IL-10, Day 1: $m = 2,802$ (± 573), Day 2: $m = 3,040$ (± 868)]. The ANOVA for vocalizing yielded no significant main or interaction effects.

Overall, we found sensitization of passive, but not active, behavior across two daily separations in control animals. The sensitization did not occur, however, if pups were administered IL-10, a cytokine with potent anti-inflammatory properties, prior to the first separation.

Experiment 2

The results of Experiment 1 suggest that proinflammatory factors contribute to the sensitization of passive behavior seen across two daily separations in guinea pig pups. To further examine this question, Experiment 2 investigated the effect of injection of LPS. Specifically, the experiment assessed whether injection of LPS on Day 1 would increase the passive response to later separation. Because LPS is derived from the cell wall of gram negative bacteria, it elicits a robust proinflammatory response in the absence of a replicating pathogen. We were also interested whether sensitization could be demonstrated over more than a single day. Therefore, all pups were separated a final time on Day 5.

Method

Subjects and Experimental Conditions—Forty eight guinea pig pups were assigned to each of four experimental conditions (6 males, 6 females in each). The conditions differed in terms of treatment on the first day of the experiment (Day 21-23 of age). Twenty-four pups received an IP injection of saline vehicle and 24 received an IP injection of LPS (75 $\mu\text{g/kg}$ body weight). All pups were then returned to their home cage for 90 min, at which time half of each injection group was separated for 3 hr, whereas the other half of each injection group remained in the home cage. On Days 2 and 5, all 48 pups underwent 3-hr separations. This design afforded several means of assessing the effects of LPS and length of sensitization (Fig. 2). With one exception, no more than one pup from a litter was assigned to any condition (in one instance two pups from the same litter were tested one condition). Litter sizes ranged from 3.5 to 3.9 pups for the four conditions.

Data Analysis—For the two groups of subjects for which there were data on Day 1 (i.e., pups separated on Day 1), differences between groups and changes from Day 1 to Day 2 in passive behavior and vocalizations were analyzed with 2 (Injection) $\times$ 2 (Sex) $\times$ 2 (Day) ANOVAs with Day treated as a repeated measure. For Days 2 and 5, when all subjects were tested, behavior was assessed with 2 (Injection) $\times$ 2 (Separation) $\times$ 2 (Sex) $\times$ 2 (Day) repeated measures ANOVAs. Follow-up comparisons were conducted with simple main and interaction effect tests (Winer, 1971). For ANOVA of vocalizations on Days 2 and 5, data were subjected to a square root transformation prior to analysis due to heterogeneity of variance (raw data are displayed).

Results

Passive Behavior—As expected, LPS injection increased passive behavior. The ANOVA for Days 1 and 2 revealed significant effects of Injection, $F(1, 20) = 4.48, p < 0.05$, and the Injection $\times$ Day interaction, $F(1, 20) = 24.29, p < 0.01$. Follow-up of the interaction showed that there was a significant effect of Injection only on Day 1 ($p < 0.01$; Table 1), when LPS-injected animals exhibited the full passive response during nearly twice as many 1-min intervals as did saline-injected pups. Further, while animals injected with LPS and separated on Day 1 showed a decline in passive behavior from Day 1 to Day 2 ($p < 0.05$), pups that received saline before separation on Day 1 showed an increase (i.e., sensitization) in passive behavior on Day 2 ($p < 0.01$).

For Days 2 and 5, the ANOVA yielded significant effects for Day, $F(1, 40) = 48.96, p < 0.01$, Separation $\times$ Day, $F(1, 40) = 8.63, p < 0.01$, and Injection $\times$ Separation $\times$ Day, $F(1, 40) = 5.40, p < 0.05$. Separate follow-up tests on Day 2 and 5 were used to further analyze the significant 3-way interaction. On Day 2 there was a significant main effect of separation ($p < 0.01$). Pups separated on Day 1 exhibited passive behavior during more 1-min intervals on Day 2 than pups not separated on Day 1 (Fig. 3). There was no main effect found for Injection or for the Injection $\times$ Separation interaction on Day 2. Follow-up tests showed no significant main or interaction effects on Day 5.

Further follow-up comparisons were conducted to examine possible sensitization from Day 2 to Day 5 for each of the four Injection $\times$ Separation groups. A significant increase of the passive response from Day 2 to 5 was seen in both groups *not* separated on Day 1 regardless of injection condition (p 's < 0.01 ; Table 1). For those groups separated on Day 1, an increase in passive behavior from Day 2 to Day 5 was seen only in pups that were injected with LPS ($p < 0.01$). That is, if a pup's first separation occurred on Day 2, there was an increase (sensitization) of passive behavior during its second separation on Day 5. But if pups were separated on both Day 1 and Day 2, an increase in passive behavior from Day 2 to Day 5 occurred only if the Day 1 separation was accompanied by injection of LPS.

Vocalizations—Vocalizations tended to be low on Day 1 and to increase on Day 2 for pups injected with LPS prior to the first separation, and to be higher on Day 1 but to decrease on Day 2 for those pups injected with saline (Table 2). The ANOVA for the first two days yielded only an interaction of Injection by Day, $F(1, 20) = 4.71, p < 0.05$. Differences between injection conditions were not significant on either day. From Day 1 to Day 2, however, the increase in vocalizing for LPS-injected animals was significant ($p < 0.05$), but the decline for saline-injected animals was not. That is, the only increase from Day 1 to Day 2 in vocalizations was from the relatively suppressed level of LPS-injected animals. There was no evidence of sensitization.

For Days 2 and 5, the 4-way ANOVA for vocalizations yielded effects for Day, $F(1, 40) = 5.56, p < 0.05$, Separation $\times$ Day, $F(1, 40) = 5.00, p < 0.05$, and Separation $\times$ Sex $\times$ Day, $F(1, 40) = 10.17, p < 0.01$. Separate follow-up tests on Day 2 and 5 were used to further

analyze the significant 3-way interaction. On Day 2, there was a significant main effect of separation ($p < 0.05$). Pups that were separated on Day 1 vocalized more on Day 2 than those not separated on Day 1 [separated on Day 1: $m = 724$ (+/- 132); not separated on Day 1: $m = 502$ (+/- 140)]. Although this effect could reflect a sensitization of separation-induced vocalizing from Day 1 to Day 2, inspection of Table 2 suggests that the effect was primarily due to a “rebound” on Day 2 by the Separation/LPS group from the relatively low level of vocalizing following LPS injection on Day 1.

On Day 5, there was an interaction between Separation and Sex ($p < 0.01$). Males vocalized more on Day 5 if they had not been separated on Day 1 than if they had ($p < 0.05$; Table 3), whereas the pattern for females, though nonsignificant, was in the opposite direction. Comparisons between Days 2 and 5 for each of the four Separation $\times$ Sex groups yielded a significant effect only for the males separated on Day 1 ($p < 0.01$), which showed a decline in vocalizations from Day 2 to Day 5. In sum, while there was a somewhat complex pattern of vocalizing across days in the four groups, there was no clear evidence of sensitization of the vocalization response.

Discussion

In recent years, increased proinflammatory activity has been linked not only to stress, but also to some forms of depressive illness. Administration of the proinflammatory cytokine Interferon- α as a chemotherapy agent induces depressive symptoms in a significant proportion of patients (Bonaccorso et al., 2002). Further, medically healthy, but depressed, patients have been reported to exhibit a variety of inflammatory markers (Miller, Maletic, & Raison, 2009; Raison, Capuron, & Miller, 2006). A recent meta-analysis confirmed that many depressed individuals have higher circulating levels of the proinflammatory cytokines TNF- α and IL-6 (Dowlati et al., 2010). Increased proinflammatory activity can reduce serotonin synthesis and neural plasticity as well as increase neuroendocrine stress responsiveness, all of which are potential mediators of depression (Hayley, Poulter, Merali, & Anisman, 2005; Miura, et al., 2008).

If attachment disruption in human children is accompanied by enhanced proinflammatory activity, as appears to be the case in guinea pig pups (Hennessy, Schiml-Webb, & Deak, 2009), these findings raise the possibility of a link between the early enhancement of proinflammatory activity and inflammatory-mediated depressive symptoms in adulthood. In laboratory rats, a proinflammatory cascade during the preweaning period has been found to produce effects in later life like those associated with depression (i.e., reduced negative feedback of the HPA axis, enhanced CRF activity, enhanced anxiety-like behavior; Shanks, Larocque, & Meaney, 1995; Walker et al., 2009). Moreover, cytokine administration to adult rats can sensitize neuroendocrine stress effects and depressive-like behavior several weeks later (Anisman, Merali, & Hayley, 2003; Schimdt, Aguilera, Binnekade, & Tilders, 2003). Thus, the sensitization process through which early-life attachment disruption is thought to enhance later susceptibility for depression may involve a proinflammatory component (Hennessy, Deak, & Schiml-Webb, 2010). The current results suggesting proinflammatory contributions to the sensitization of depressive-like behavior in separated guinea pig pups support this hypothesis.

The finding that pups infused with IL-10 prior to their first separation failed to show increased passive behavior on Day 2 suggests that proinflammatory processes are *necessary* for the sensitization to occur. On the other hand, the inability of LPS injection in the absence of separation on Day 1 to increase passive responding on Day 2 indicates that a general increase in proinflammatory activity is not *sufficient* in and of itself to account for the sensitization effect. Yet among pups separated on Day 1, an increase in passive responding

from Day 2 to Day 5 was observed only in the group exposed to LPS on the first day. This finding suggests that augmenting proinflammatory activity during separation may enhance behavioral sensitization several days later, and provides at least preliminary evidence for cross-sensitization between the effects of immune activation with LPS and our maternal separation procedure.

Our initial observation that guinea pig pups show sensitization of passive behavior across two daily, 3-hr separations (Hennessy et al., 2010b) was replicated in both experiments of the present study. In Experiment 1, an increase in the passive response from Day 1 to Day 2 was seen in pups infused with vehicle prior to the first separation. In Experiment 2, a similar effect across separations on Days 1 and 2 was observed in pups that did not receive injection of LPS prior to the first separation. In addition, Experiment 2 extended the description of the prior observation in two ways. First, we found that the increase in passive responsiveness following two daily separations persists. In the Saline/Separation group, the level of passive behavior on Day 5 was comparable to that seen on Day 2. Second, we found that separations need not take place on consecutive days for sensitization to occur. Pups separated only on Days 2 and 5 exhibited an increase in the passive response over this time frame. These results are noteworthy because they illustrate that sensitization of passive behavioral responses is not a transitory phenomenon, but rather persists for days after the initial separation, thereby expanding the clinical relevance/implications of the guinea pig separation model.

Among control animals, those in Experiment 1 displayed less passive behavior and more active behavior than did those of Experiment 2. Variation in the absolute levels of these behaviors across different sets of observations is not uncommon (e.g., Hennessy et al., 2007b; Hennessy, Fitch, Jacobs, Deak, & Schiml-Webb, in press; Perkeybile et al., 2009). Probably more germane though is the different treatment of controls in Experiments 1 and 2. Those in Experiment 1 had undergone cannulation surgery and infusion of aCSF prior to testing, while those of Experiment 2 were unoperated and injected with saline. In another recent set of two experiments in which control pups had undergone these different procedures, we observed a very similar pattern of results (Hennessy et al., in press).

In an earlier study, infusion of IL-10 prior to separation reduced passive responding during that separation (Perkeybile et al., 2009). In Experiment 1 of the present study, this treatment prevented an increase in passive behavior from the first to the second separation, but did not reduce passive behavior during the first separation. This difference may have been influenced to some extent by a “floor effect” in the present study, as suggested by the low median levels of the full passive response in the controls of Experiment 1. Further examination of the individual component behaviors of the full passive response (crouch, eye-close, piloerection), each of which was observed during more 1-min intervals than was the combined measure, supports this conjecture. That is, piloerection was significantly reduced on Day 1 following IL-10 administration ($p < 0.05$, 1-tailed test). Another likely contributing factor to account for the discrepancy in Day 1 results is the difference in statistical power available in the two studies since Perkeybile et al (2009) examined the effect of IL-10 as a within-, rather than a between-, subjects variable, and with a much larger sample size ($n = 30$). Another point to be considered is that when maternal separation procedures produce effects with a gradual onset, these effects often appear due to the removal of regulatory influences on infant physiology that normally are provided by aspects of maternal stimulation (Hofer, 1987). While we cannot rule out such an interpretation of the second, passive stage of separation in guinea pig pups, it seems likely that the delayed onset rather is a result of the time required to mount a proinflammatory reaction sufficient to effect behavioral change (e.g., Deak, Bellamy, & Bordner, 2005; Thompson, Karpus, Van Eldic, 2008).

In conclusion, the present results suggest that stress-induced proinflammatory activity be considered in discussions of the mechanisms by which early psychosocial stressors sensitize neural systems to increase later susceptibility to depressive illness. Proinflammatory activity is known to interact with other hypothesized mediators of this sensitization. For instance, there are reciprocal influences between proinflammatory processes and HPA hormones (CRF, glucocorticoids, e.g., Karalis, Muglia, Bae, Hilderbrand, & Majzoub, 1997; Munhoz, Sorrells, Caso, Scavone, & Sapolsky, 2010; Paschos, Kolios, & Chatzaki, 2009), and proinflammatory activity in the amygdala may promote stress-related changes in behavior (e.g., Yamamoto et al., 2010)]. Thus, proinflammatory activity may be one component of a complex system mediating the sensitization effect.

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Figure 1.

Median number of 60-s intervals in which pups infused ICV with either IL-10 or aCSF vehicle exhibited full passive behavior during 90 min of observation (i.e., Min 0-30, 60-90, and 150-180) while separated on Days 1 and 2. * $p < 0.05$ vs Day 1.

Figure 2.
Design of Experiment 2 with primary comparisons of interest.

Figure 3.

Mean number of 60-s intervals in which pups that had been injected with LPS or injected with saline and separated or not separated, on Day 1 exhibited full passive behavior during 90 min of observation (i.e., Min 0-30, 60-90, and 150-180) while separated on Day 2. Vertical lines indicated standard errors of the means. ** $p < 0.01$.

Table 1

Mean (SE) number of 60-s intervals in which pups of Experiment 2 exhibited the full passive response during 90 min of observation.

Treatment on Day 1	Full Passive Response		
	Day1	Day2	Day5
Saline/ No Separation	-----	32.5 (3.6)	52.7(4.7) **
LPS/ No Separation	-----	35.7(4.7)	47.6(3.4) **
Saline/ Separation	31.4(5.4) **	51.2(5.2)	54.6 (4.8)
LPS/ Separation	58.1 (3.9) *††	47.6 (2.9)	56.0(3.9) **

*
 $p < 0.05$ vs Day 2

**
 $p < 0.01$ vs Day 2

††
 $P < 0.01$ vs Saline/Separation

Table 2

Mean (SE) number of vocalizations emitted in Experiment 2 on individual days.

Treatment on Day 1	Vocalizations		
	Day 1	Day 2	Day 5
Saline/ No Separation	-----	454 (120)	371 (140)
LPS/ No Separation	-----	549 (259)	572 (177)
Sal/ Separation	709 (278)	518 (163)	300 (113)
LPS/ Separation	474 (163)*	929 (196)	543 (203)

*
 $p < 0.05$ vs Day 2

Table 3

Mean (SE) number of vocalizations emitted on Day 5 by male and female pups that were and were not separated on Day 1 in Experiment 2.

Treatment on Day 1	Males	Females
No Separation	679 (198)	265 (77)
Separation	196 (87)*	646 (200)

*
 $p < 0.05$ vs No Separation