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Sensitization of depressive-like behavior during repeated maternal separation is associated with more-rapid increase in core body temperature and reduced plasma cortisol levels

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ABSTRACT

Infant guinea pigs exhibit a 2-stage response to maternal separation: an initial active stage, characterized by vocalizing, and a second passive stage marked by depressive-like behavior (hunched posture, prolonged eye-closure, extensive piloerection) that appears to be mediated by proinflammatory activity. Recently we found that pups showed an enhanced (i.e., sensitized) depressive-like behavioral response during repeated separation. Further, core body temperature was higher during the beginning of a second separation compared to the first, suggesting a more-rapid stress-induced febrile response to separation the second day, though the possibility that temperature was already elevated prior to the second separation could not be ruled out. Therefore, the present study examined temperature prior to, and during, 2 daily separations. We also examined the temperature response to a third separation conducted 3 days after the second, and assessed the effect of repeated separation on plasma cortisol levels. Core temperature did not differ just prior to the separations, but showed a more-rapid increase and then decline during both a second and third separation than during a first. Temperature responses were not associated with changes in motor activity. Depressive-like behavior was greater during the second and third separations. Pups separated a first time showed a larger plasma cortisol response at the conclusion of separation than did animals of the same age separated a third time. In all, the results indicate that the sensitization of depressive-like behavior during repeated separations over several days is accompanied by a more-rapid febrile response that may be related to a reduction of glucocorticoid suppression.

1. Introduction

When children or infant monkeys are separated from their mothers for a protracted period, an initial stage of active distress is often followed by a second passive, depressive-like stage of response [34,40,46,50]. Over the years, it has become clear that prolonged separation and other forms of attachment disruption in children (e.g., abuse, neglect, death of a parent) also increases the vulnerability for developing a depressive or anxiety disorder at a later age [1,4,9,45]. In nonhuman primates, seemingly parallel long-term effects of early separation on later behavior have been observed [15]. The mechanism through which attachment disruption confers vulnerability for later psychopathology remains unknown, but has been hypothesized to involve a sensitization process—for instance of corticotropin-releasing factor (CRF) secretion or amygdala activity—so that reaction to later loss and other adverse circumstances is magnified as a result of the early attachment disruption [14,16,19,48].

Guinea pigs are laboratory rodents that display good evidence for a specific filial attachment process, as well as similarities to primates in their responses to social separation [20,32,44]. Of particular interest here, guinea pigs exhibit a two-stage, active/passive behavioral response to maternal separation procedures. Upon isolation in a novel enclosure, pups initially vocalize at a high rate and tend to move about the enclosure. But after an hour or so, they quiet and assume a hunched or crouched posture, with eyes closed and piloerection over most of the body [26]. These effects do not occur if the mother is with the pup in the test enclosure. It appears that the stressor of being separated from the mother in an unfamiliar environment activates proinflammatory signaling that mediates the passive stage.

In recent years, it has become clear that stressors can often induce a systemic proinflammatory reaction (i.e., "stress-induced sickness") [36] that promotes fever and behaviors that appear to support fever (e.g., hunched posture, piloerection) or conserve energy (e.g., sleepiness) [18]. Three lines of evidence support the hypothesis that the passive response in guinea pigs is mediated by proinflammatory signaling stimulated by the stressor of the separation procedure: (a) pups injected with lipopolysaccharide (LPS), which elicits a potent proinflammatory reaction, exhibited high levels of crouching, eye-

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closure, and piloerection [24]; (b) any of three anti-inflammatory compounds reduced the passive behavioral response to separation [29,43,47]; and, (c) separation induced two signs of systemic inflammation—an increase in the expression of the proinflammatory cytokine tumor-necrosis factor alpha in spleen [22] and an increase in core temperature [23,24], suggesting fever. These findings are of particular interest in light of mounting evidence that proinflammatory processes contribute to forms of human depressive illness [12], and may be a major mechanism through which stressors precipitate depressive episodes [41].

There is increasing support for the notion that behaviors induced by proinflammatory activity in laboratory animals share common features with depression. For instance, proinflammatory stimulation in rats and mice increases depressive-like behavior in conventional depression testing paradigms such as tail suspension and the sucrose preference test of anhedonia [12]. Mice deficient in proinflammatory signaling show reduced impact of stress on sucrose consumption and other depression-related measures [17], and antidepressant medication reduces some proinflammatory-induced behavioral change [54]. Thus, in addition to the face validity deriving from the similarity of the second stage of separation in guinea pigs and primates, there is sufficient reason to consider that the behavior of separated guinea pigs might inform our understanding of how early separation and other forms of early stress increase vulnerability for later depression.

We recently observed that the passive behavioral response of guinea pigs sensitizes with repeated separations. Pups exhibited an increase in passive behavior during a second 3-h separation that followed a first separation by 1 or 4 days [23,28]. Further, central infusion of interleukin-10—a cytokine with anti-inflammatory properties—before an initial separation blocked the increase in passive behavior during a separation the following day [28]. This finding suggests that proinflammatory activity not only mediates the initial response to separation, but also may contribute to the sensitization of the behavioral response with repeated separations. It may be, for instance, that the proinflammatory reaction is enhanced with repeated separations, which in turn, promotes the sensitization of the depressive-like behavior [21]. This possibility is consistent with another recent finding that core body temperature was higher during the initial portion of a second daily, 3-h separation, and lower in the latter portion than was observed on the previous day [23]. This suggested a shorter-latency febrile response during the second separation. However, because temperature was examined only during the separation, it is unclear whether the initial rise in temperature on the second day was due to an actual elevation in the temperature response during the second separation or to a difference between days in temperature at the time the second separation began (e.g., a persistent elevation in basal temperature or an anticipatory response to subtle environmental cues on the second day). Further, because temperature was observed only on two consecutive days, it is unclear whether the increased thermogenic response was a transitory phenomenon or a more-persistent effect.

Therefore in the present study, we used a telemetry device to measure temperature for a 1-h period just prior to, as well as during, three, 3-h separations. Tests occurred on two consecutive days as well as 3 days after the second. Control measures were included to differentiate febrile responses from other changes in core temperature (e.g., activity-induced thermogenic responses). We again assessed sensitization of the passive behavioral response and, unlike our earlier study of behavior over this time span, included age-matched controls separated a first time at the same mean age as pups undergoing their third separation. Finally, because of the hypothesized role of proinflammatory activity in the sensitization process, together with the well-established suppressive action of glucocorticoids on inflammatory responses, we also evaluated the effect of single versus repeated separations on plasma cortisol. Our separation procedure reliably activates the hypothalamic–pituitary–adrenal (HPA) axis of guinea pig pups [27,37], but HPA activity has not previously been examined in the context of this sensitization paradigm.

2. Method

2.1. Animals

Albino guinea pigs (*Cavia porcellus*) of the Hartley strain were bred and housed in our laboratory. Mother and litter were maintained together for the duration of the experiment in a cage (73 × 54 × 24 cm), which had a wire front and sawdust bedding. Food and water were continuously supplied. The colony room was maintained on a 12-h light:dark cycle, with lights on at 07:00 h. All procedures were conducted in accordance with the Wright State University Animal Care and Use Committee.

Three groups of pups were used for this study. The SEP1 and SEP3 groups received surgery to implant a telemetry device and were administered one and three, 3-h separation tests, respectively, with the last test of SEP3 occurring at the same approximate age as the single test of SEP1. For SEP3, pups were separated on two consecutive days, and then again three days later (i.e. Days 1, 2, and 5). The SURG group received the same surgery to implant a telemetry device, but unlike the other groups, did not undergo separation testing. SURG pups served as a control for assessing the effect of the separation procedure on plasma cortisol levels. Each group was comprised of 11 or 12 pups with approximately equal numbers of males and females (SEP1: 6 males, 6 females; SEP3: 6 males, 6 females; SURG: 6 males, 5 females). No more than one pup from a litter was assigned to any condition. When a SEP1 and SEP3 pup were drawn from the same litter, they were always separated on different days.

2.2. Surgery and telemetry

Telemetry probes (PD 4000 Emitter from Mini-Mitter Company) were surgically implanted into the abdominal cavity under isoflurane anesthesia (2–4%) using aseptic procedures between 16 and 19 days of age. Pups were treated with 0.05 mg/kg atropine (intraperitoneal) to reduce secretory activity. Buprenorphine (0.015 mg/0.05 ml) was given subcutaneously immediately following surgery and again 24 h later. At least three days intervened between surgery and any experimental manipulation.

Core temperature was measured by the probe, which sent signals to a receiver plate (54.6 × 27.9 cm) placed under the cage during testing. The telemetry device also signaled movement of the pup. Temperature and motor activity data were recorded using computer software (Vital View) of the manufacturer, which collects data in 3-min bins.

2.3. Testing procedure

Because of constraints involved in scheduling 4-h tests with a single receiver plate, testing occurred over a range of several days. That is, for SEP3 the first test was between 21 and 25 days of age, with subsequent tests occurring exactly 1 and 4 days after the first. Pups in the other two groups were tested over a similar range at the oldest age, so that the average age of SEP3 pups at the time of their final test and blood sample collection (27.3 days) approximated the ages of pups tested and/or sampled in the SEP1 (27.7 days) and SURG (27.8 days) groups. The “pre-separation” phase of testing was designed to continuously monitor the core temperature of the pup in the home cage without the potential confound of passive heat exchange from physical contact with mother or littermates. To begin this phase, mother and litter were moved to a new home cage that was identical to the previous cage except for a white, plastic, mesh divider that separated its width into 45-cm and 28-cm sections. Mother and litter were placed into the larger section to allow habituation to the presence of the divider for at least 1 h. The cage was then moved to