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Behavioral effects of peripheral corticotropin-releasing factor during maternal separation may be mediated by proinflammatory activity

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Summary

When guinea pig pups are separated from their mothers in a novel environment, an initial period of active behavior (vocalizing, locomotor activity) wanes after an hour or so and is replaced by a second, passive stage characterized by a crouched stance, closed eyes, and extensive piloerection. If pups are given a peripheral injection of 7–14 μ g of corticotropin-releasing factor (CRF) prior to testing, the passive behaviors occur immediately upon separation. We found that intracerebroventricular infusion of 1–10 μ g of CRF did not increase passive behavior relative to vehicle infusion, but that peripheral injection of the anti-inflammatory cytokine, Interleukin-10, attenuated the passive behavior induced by peripheral CRF injection. These results together with previous findings suggest that peripheral CRF administration affects behavior of separated guinea pig pups through a mechanism that involves peripheral proinflammatory activity. The possible role of endogenous peripheral CRF in the behavioral response of untreated pups during maternal separation is considered.

Keywords

Corticotropin-releasing factor; Corticotropin-releasing hormone; Maternal separation; Sickness behavior; Stress-induced sickness behavior; Proinflammatory cytokines; Interleukin-10; Guinea pig

1. Introduction

It is well established that corticotropin-releasing factor (CRF) is a primary mediator of the body's diverse responses to stressors. CRF neurons in the CNS drive not only pituitary-adrenal activity, but also responses of sympathetic, locus coeruleus/noradrenergic, and gastrointestinal systems (Dunn & Berridge, 1990; Owens & Nemeroff, 1991). Central CRF activity also appears to underlie widespread behavioral effects associated with stress or anxiety in laboratory animals, including emergence into, and activity in, an open field

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(Takahashi et al., 1989), activity on an elevated plus maze (Heinrichs et al., 1992; Skutella et al., 1994), shock-induced freezing (Swiergiel et al., 1993), conditioned defeat (Jasnow et al., 1999), appetite suppression (Krahn et al., 1986), and the ultrasonic vocalization response of rat pups to maternal separation (Harvey & Hennessy, 1995; Insel & Harbaugh, 1989). CRF also appears to be widely distributed in the periphery, including the gastrointestinal tract, pancreas, liver, lungs, reproductive organs, placenta, immune system, skin, sympathetic ganglia, and adrenal medulla (Dufau et al., 1993; Emeric-Sauval, 1986; Krukoff, 1986; Nazarloo et al., 2006). In the periphery, CRF and the closely related urocortins influence a range of functions including gastrointestinal motility (Stengel & Tache', 2009), inflammation (Karalis et al., 1997; Paschos et al., 2009), and reproductive (Dufau et al., 1993) and cardiovascular (Coste et al., 2002) activity. Peripheral CRF activity, like CRF in the CNS, may be an important mediator of stress responses. CRF is co-released with catecholamines from the adrenal medulla following hemorrhage or splanchnic nerve stimulation (Bruhn et al., 1987; Edwards & Jones, 1988) and peripheral CRF signaling plays a key role in stress-related gastrointestinal disorders, notably inflammatory conditions (Paschos et al., 2009; Stengel & Tache', 2009; Zheng et al., 2009).

Although CRF is unlikely to cross the blood-brain barrier into the brain (Banks & Kastin, 1985; Martins et al., 1996), peripherally administered CRF occasionally has been found to produce behavioral effects. In one study, a subcutaneous (SC) injection of a dose of CRF that had no impact on circulating glucocorticoid levels facilitated retention of passive avoidance learning in rats (Veldhuis & DeWeid, 1984). In another, intracardial CRF reduced rats' tail flick response to heat (Ayesta & Nikolarakis, 1989). Peripheral injection of CRF also markedly affects the behavior of preweaning guinea pigs. When injected SC with 7–14 µg of CRF and isolated in a novel cage, pups showed a dramatic reduction in the vocalizing and locomotor activity that is characteristic of the immediate reaction to the separation procedure (Hennessy et al., 1991). This response suppression could not be duplicated with injection of ACTH, nor reversed with naloxone (Hennessy et al., 1991), suggesting that it was not mediated by other hormones of the HPA axis. Further, the behavior suppression did not appear to be the result of emission of incompatible behaviors, including stress-induced "freezing" (Becker & Hennessy, 1993), nor did it appear due to motor incapacity (Becker & Hennessy, 1993), or CRF-induced hypotension (Hennessy et al., 1995). The effect of CRF injection was blocked by peripheral administration of a peptide CRF-receptor antagonist (Hennessy et al., 1995), indicating that it was mediated by CRF receptors.

At about this time we also noted that CRF induced a distinct passive behavioral reaction during the inhibition of active behavior (Becker & Hennessy, 1993). Specifically, pups adopted a characteristic crouched posture; their eyes were often closed; and they exhibited extensive piloerection. Our interest in the passive response increased as a result of two additional results. First, it was found that pups displayed this passive behavior not only immediately following separation when injected with CRF, but also during more-prolonged periods of separation (> 1 hr) under non-drug conditions (Hennessy et al., 1995). Second, pups injected peripherally with a non-specific, peptide CRF-receptor antagonist exhibited an increase in active behavior, and fewer pups exhibited any passive behavior, during a 1-hr test (McInturf & Hennessy, 1996). Because the peptide antagonist was unlikely to cross the blood-brain barrier, this finding suggested that *endogenous peripheral* CRF might be part of the mechanism that normally shifts pups from an initial active, to a subsequent passive, phase of behavioral response during separation.

Additional study of the passive behavior during prolonged separation under non-drug conditions indicated that proinflammatory factors contributed to the response. Specifically, it was found that: (1) direct activation of a proinflammatory response produced the passive response immediately following separation, much as did CRF injection (Hennessy et al.,

2004); (2) any of three anti-inflammatory agents reduced the passive behavior during prolonged (3-hr) separation (Hennessy et al., 2007b; Perkeybile et al., 2009; Schiml-Webb et al., 2006); and, (3) an increase in the expression of the pro-inflammatory cytokine, tumor necrosis- α , was observed in spleen over the course of a 3-hr separation (Hennessy et al., 2007a). These results, together with findings indicating that CRF in the periphery has various proinflammatory effects (e.g., Ilias & Mastorakos, 2003; Karalis et al., 1997; Paschos et al., 2009), raised the possibility that CRF injection induced passive behavior through a proinflammatory mechanism. Peripheral proinflammatory activity can induce central proinflammatory activity through various pathways (Quan & Banks, 2007). Central proinflammatory activity, in turn, is known to produce behavioral changes characterized by inactivity and reduced engagement with the environment (Dantzer et al., 2008). We then found that administration of α -melanocyte stimulating hormone (α -MSH)—a peptide with broad anti-inflammatory effects (Cantania & Lipton, 1993; Lipton & Cantania, 1997)—significantly reduced passive behavior of guinea pig pups injected with CRF (Schiml-Webb et al., 2009). While these findings implicated a proinflammatory mechanism for CRF's effect, α -MSH is known to have a variety of other physiological actions (Gonzalez et al., 1996; Panksepp & Abbott, 1990; Rao et al., 2003), leaving open alternative interpretations.

The primary purpose of the present experiments was to examine the means by which peripheral CRF administration induced the passive behavior response in separated guinea pig pups. Experiment 1 evaluated the effect of ICV infusion of CRF on behavior during separation. If peripherally administered CRF crosses into the brain to induce passive behavior, ICV CRF might also be expected to induce passive behavior. Experiment 2 provided a second test of the hypothesis that peripheral CRF acts through a proinflammatory mechanism. The ability of a peripheral injection of Interleukin-10 (IL-10) to attenuate the behavioral effect of SC CRF injection was assessed. IL-10 is a cytokine with potent anti-inflammatory actions. It has been found to reduce the behavioral response to proinflammatory activation produced by injection of lipopolysaccharide (Bluthe' et al., 1999; Nava et al., 1997). Further, Perkeybile et al (2009) recently found central IL-10 to blunt the passive behavior of guinea pig pups during prolonged separation under non-drug conditions.

2. Method

2.1. Subjects

Albino guinea pigs (*Cavia porcellus*) were bred in our laboratory. Each mother and her litter were housed in opaque plastic cages (73 cm $\times$ 54 $\times$ cm 24 $\times$ cm) with wire fronts 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 0700 hr. Cages were changed twice per week. All procedures were 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 ICV cannulae (Experiment 1), behavioral testing, and brief routine colony management procedures (such as weighing of pups). No more than one pup per litter was assigned to the same condition in either experiment. As in earlier studies (e.g., Hennessy et al., 1992; Hennessy et al., 1991; Schiml-Webb et al., 2009) testing was conducted near the time of natural weaning, which occurs around Day 25 (König, 1985; Schiml & Hennessy, 1990). It should be noted, however, that young guinea pigs continue to show a strong attraction to the mother, as well as behavioral and physiological reactions to separation, for weeks thereafter (Hennessy et al., 1996; Hennessy & Morris, 2005; Hennessy et al., 2003).

2.2. Experimental Design

2.2.1. Experiment 1—Pups were assigned to one of three separate groups receiving doses of ICV CRF ranging up to a dose known to induce passive behavior when injected peripherally. These groups received either 1 μ g (n = 8; 4 males, 4 females), 5 μ g (n = 7; 3 males, 4 females), or 10 μ g (n = 9; 5 males, 4 females) of CRF (rat/human, Sigma-Aldrich) 20 min prior to a 1-hr test. Pups were returned to the home cage during the 20-min interim. In each of the groups, pups were tested twice: once with CRF and once with artificial cerebrospinal fluid (aCSF) vehicle. Within each group, the order in which pups received CRF or vehicle was counter-balanced as closely as possible given that an odd number of pups was tested in some groups. The first test occurred between Day 20–24 and the second test was administered at least 3 days later, but no later than Day 27.

2.2.2. Experiment 2—This experiment examined the ability of the anti-inflammatory cytokine IL-10 to moderate passive behavior induced by peripheral injection of CRF. There were two groups of pups. One group (n = 12; 6 males, 6 females) received SC injections of 10 μ g of CRF in saline (.2 ml volume), whereas the other (n = 11; 5 males, 6 females) received SC injections of just the saline vehicle. Pups of both groups were given an intraperitoneal (IP) injection of 1 μ g of IL-10 (American Research Products) in saline (.1 ml volume) on one occasion and an IP injection of the saline vehicle on the other. IP injection of IL-10 or saline occurred 90 min prior to the 1-hr test; SC injection of CRF or vehicle occurred 60 min before the test. Order in which the animals received IL-10 or its vehicle was counter-balanced within each group. Pups were returned to the home cage following each injection. The first test was administered at Day 18–21, and the second test was given at least 3 days later, but no later than Day 25.

2.3. Cannulation and Infusion Procedures

Between Days 16–19 (Day 20 in one case), each pup in Experiment 1 underwent surgery for placement of an indwelling cannula aimed at the right lateral ventricle. Pups were pretreated with atropine (0.05 mg/kg, IP) and anesthetized with isoflurane before being placed into a stereotaxic apparatus. Surgery was performed with the skull oriented level in the 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 (0.25 mg/0.1 ml 0.25% bupivacaine, SC) prior to the scalp 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 cannulae 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, IP) to control for post-operative pain. Each day post-surgery, pups were weighed, and dummy cannulae (caps) were removed so that the indwelling cannulae could be checked for patency; these procedures required that pups be removed from the home cage for no more than 5 minutes. A recovery time of at least three days was allowed before behavioral testing. Infusions (5 μ l volume) of drug or vehicle were made over the course of 2.5 min. All animals were killed after the second behavioral test via carbon dioxide inhalation. Cannulae placement was then verified via infusion of dye (~50 μ l 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.

2.4. Behavioral Testing

For testing, pups were carried in a transport cage (< 10 s) from the colony room to the testing room, where they were placed into an empty, clear plastic cage (47 × 24 × 20 cm) on a table top under full room lighting and observed from a blind for the 60-min test period. A trained observer recorded the number of 1-min intervals in which pups exhibited the characteristic crouched posture in which the feet are tucked beneath the body, complete or near complete closure of one or both eyes (> 1 s), and extensive piloerection (over half the body), as well as the number of intervals in which the pups simultaneously exhibited all three passive behaviors (designated “full passive” response). Because single instances of crouch, eye-close, and piloerection typically occur over an extended period of time, these behaviors were scored with one-zero sampling as in previous studies (Schiml-Webb, et al. 2006). To assess active behavior, all occurrences of the whistle vocalization (Berryman, 1976) and line-crossings (the number of times pups crossed lines on the cage floor that divided it into four equal sections) were recorded. Vocalizations were scored with a hand-held counter; other behaviors were scored with pencil and paper. The test cage was cleaned with detergent prior to each use. Testing occurred during the light phase of the cycle and was initiated at approximately the same time of day (3-hr span) to control for circadian variation. Inter-observer reliability was 85% or above within each behavioral category.

2.5. Analyses

One pup in the 10 µg CRF group of Experiment 1 was dropped due to error in scoring. Because of large numbers of scores of “zero” for measures in both experiments, and for consistency in presentation, non-parametric analyses were used throughout. Between-subjects comparisons were made with Kruskal-Wallis analyses of variance by ranks for multiple comparisons and Mann-Whitney *U* tests for paired comparisons. Within-subjects paired comparisons were conducted with Wilcoxon matched-pairs, signed-ranks tests. Preliminary analyses found that males and females did not differ in their patterns of response for any measure in either experiment; therefore, data were pooled across gender. A two-tailed level of significance of $p < 0.05$ was used throughout.

3. Results

3.1. Experiment 1: Central CRF infusion

As expected, little passive behavior was observed during the relatively brief 1-hr tests when pups were infused with vehicle. As seen in Table 1, animals assigned to the 10 µg dose group appeared to show somewhat more passive behavior both when given CRF and when administered vehicle. Kruskal-Wallis tests showed that, when given CRF, effects of dose (1 vs 5 vs 10 µg) reached significance for both piloerection ($p < 0.05$) and full passive response ($p < 0.05$) and approached significance for crouch ($p < 0.06$) and eye closure ($p < 0.06$). There was no difference among these same groups when administered aCSF vehicle. However, when direct comparisons were made between CRF and aCSF at each dose level, no significant effects of CRF were obtained (p 's > 0.17).

Active behavior, which peaks shortly after separation (Hennessy et al., 1995), frequently was observed during the 1-hr tests. There was no significant difference among the three groups either when given various doses of CRF or when administered its vehicle (Table 2). Direct comparison between tests following CRF and aCSF revealed no influence of CRF at any dose level for either vocalizing or line-crossing. Because the comparisons among dose groups were nonsignificant, we also pooled data across dose groups and compared effects of CRF to those of aCSF. These comparisons were nonsignificant for both vocalizing and locomotor activity.

3.2. Experiment 2: IL-10 and peripheral CRF

SC injection of 10 μ g CRF markedly enhanced passive behavior relative to SC saline (Fig. 1). For the group of pups that also received an IP saline injection, CRF increased crouching ($p < 0.05$), eye-closing ($p < 0.01$), piloerection ($p < 0.05$), and the full passive response ($p < 0.05$). For the animals that also received IL-10, SC CRF increased eye-closing ($p < 0.01$), piloerection ($p < 0.01$) and full passive response ($p < 0.05$) as compared to SC saline. The increase in crouching was not significant. Further, the effect of CRF was reduced by IL-10. That is, when given IP IL-10 in addition to CRF, levels of crouching ($p = 0.05$) and the full passive response ($p < 0.05$) were significantly lower than when given IP saline vehicle together with CRF. Differences in eye-closing and piloerection were not significant. The influence of IL-10 was specific to the pups administered CRF. Among those pups that received SC saline rather than CRF, IP IL-10 did not significantly affect any measure of passive behavior.

The active behaviors of vocalizing and line-crossing were significantly reduced by CRF (p 's < 0.01), but only if the pups received IP saline instead of IL-10. [Although the difference in the median level of vocalizations shown by pups when injected with IL-10 is large (Fig. 2), 5 of 11 pups in the SC saline group emitted no vocalizations.] However, direct comparison of tests with IL-10 and its vehicle showed that IL-10 had no significant effect on either vocalizing or line-crossing in either the group of pups injected on both occasions with CRF, or in the group injected with its vehicle.

4. Discussion

In Experiment 1, CRF was infused directly into the brain to determine if the effect of peripherally administered CRF on the passive responses might be accounted for by the peptide acting at a central site. Our results suggest otherwise. Although comparison among doses of CRF produced significant effects on passive behavior, direct comparisons of the passive behavior of pups when given CRF and when given aCSF vehicle revealed no significant differences for any of four measures of passive behavior at any of three doses of CRF. It is possible that at least part of the explanation for the dose effect on passive behavior is that pups in the 10 μ g dose group tended to exhibit more passive behavior even under control conditions. Whereas the effect of "dose" was not significant when pups were infused with vehicle, median levels of each of the measures of passive behavior were higher for pups in the 10 μ g dose group than in the other two groups (Table 1). In sum, the effect on passive behavior of ICV CRF, at doses ranging up to the effective peripheral dose, was minimal at best. Although we cannot unequivocally rule out the possibility ICV CRF failed to reach the critical central receptors in our guinea pigs, this possibility seems remote since ICV administration of CRF has proven to be a reliable means of accessing receptors mediating behavioral effects across a range of rodent and other vertebrate species (De Pedro et al., 1992; DeVries et al., 2002; Dirks et al., 2002; Insel & Harbaugh, 1989; Kalin et al., 1983b; Krahn et al., 1986; Lowry & Moore, 1991; Parrott, 1990; Winslow et al., 1989). Overall, our results suggest that it is very unlikely that the marked increase in passive behavior observed when pups were given a peripheral injection of 10 μ g of CRF can be accounted for by some of the peptide passing to a central site of action. In fact, work in mice indicates that while the blood-brain barrier prevents passage of CRF from periphery to brain, significant passage from brain to periphery does occur (Martins et al., 1996). Thus, the dose effect of CRF observed in Experiment 1 of our study could potentially have been due to CRF passing to a peripheral site of action.

CRF administered through an indwelling ICV cannula had no significant impact on either vocalizing or line-crossing in Experiment 1. This finding is consistent with the results of an earlier study of active behavior (Hennessy et al., 1992) conducted before the passive stage

had been documented. Further, the control pups of Experiment 1 exhibited more active, and somewhat less passive, behavior than those of Experiment 2. While the reason for this variation is unclear, it likely relates to the different procedures of the two experiments (surgery vs no surgery; central infusion vs two peripheral injections).

Increased proinflammatory activity elicits passive behavior across a wide range of vertebrate species (Hart, 1988; Johnson et al., 1993) and is associated with major depressive illness in humans (Dantzer et al., 2008; Dowlati et al., 2010). When encountering pathogens such as bacteria, the body's first line of defense is a non-specific immune reaction known as the acute phase response, or just "sickness" (Baumann & Gauldie, 1994), in which macrophages and other cells of the immune system secrete an array of proinflammatory cytokines. These peptides orchestrate various physiological as well as behavioral adjustments that help promote recovery. Peripheral proinflammatory activity appears to induce central proinflammatory activity to produce behavioral change (Dantzer et al., 2008; Maier & Watkins, 1998). There is now wide acceptance of an assortment of ways in which this peripheral-to-brain signal might be generated, including the passage of cytokines through transport mechanisms or in areas in which the blood-brain barrier is absent (i.e., the circumventricular organs), by means of a signal carried by vagal afferents, or by communication across the endothelial walls of central blood vessels (Quan & Banks, 2007). Importantly, stressors such as electric shock can induce aspects of an acute phase response, including behavioral components (i.e., "stress-induced sickness"; Deak et al., 1997; Maier & Watkins, 1995, 1998). As described in the Introduction, several lines of evidence indicate that the passive responses that guinea pig pups exhibit several hours following separation under non-drug conditions are mediated by proinflammatory activity induced by the stressor of separation in a novel environment.

CRF injection produces the same pattern of passive behavior in guinea pig pups as does either prolonged separation or direct stimulation of proinflammatory activity with injection of lipopolysaccharide (Hennessy et al., 2004). Similarly, in rhesus macaques, intravenous CRF produces sickness-like (i.e., lying down) behavior (Kalin et al., 1983a). There also is increasing evidence that peripheral CRF stimulates a variety of proinflammatory responses (Baker et al., 2003; Chowdrey et al., 1984; Hagen et al., 1993; Karalis et al., 1997; Leu & Singh, 1992; Paschos et al., 2009; Singh & Leu, 1990; Stengel & Tache', 2009; Webster et al., 1997). Our earlier study (Schiml-Webb et al., 2009) showed that α -MSH, which has various biological effects, including potent anti-inflammatory action (Cantania & Lipton, 1993; Lipton & Cantania, 1997), blunted the impact of peripheral CRF on the passive behavior of guinea pig pups. IL-10 is a cytokine with robust anti-inflammatory properties, and we are aware of no reports that IL-10 affects behavior other than by reducing proinflammatory activity. IP injection of 1 μ g of IL-10 significantly reduced passive behavior in CRF-injected pups. One of three individual passive behaviors—crouch—was modestly reduced, and the full passive response, which requires pups to exhibit each of the three individual behaviors in the same 1-min interval showed a much clearer, and greater percent, reduction. It seems then that the primary effect of IL-10 was to prevent the most extreme behavioral reaction, as reflected by the "full passive" measure, to CRF injection. Because the known common action of α -MSH and IL-10 is to reduce inflammation, the similar behavioral effects in the present study and that by Schiml-Webb et al (2009) seem most likely due to this common action and not to a secondary effect of one or the other compound. In sum, the results of the two studies provide convergent evidence that at least a portion of the passive behavior elicited by peripheral CRF injection in guinea pig pups is mediated by a proinflammatory reaction.

The findings that anti-inflammatory peptides can reduce both the passive behavior that arises gradually over a 3-hr separation in otherwise untreated pups, as well as the passive

response that CRF-injected pups exhibit immediately upon separation, raise the possibility that endogenous peripheral CRF stimulates a proinflammatory cascade, which then effects the onset of passive behavior during prolonged separation of guinea pig pups under non-drug conditions. This possibility is consistent with the observation that a peripheral injection of a peptide CRF-receptor antagonist reduces the number of pups that begin to show passive behavior within a 1-hr period (McInturf & Hennessy, 1996). Such an effect would not be expected if the effect of CRF injection on passive behavior was due to a pharmacological action by, for instance, evoking cytokine release as a result of inducing physical malaise. Further, the absence of a hypotensive effect of our CRF injection procedure [Hennessy et al., 1995; hypotension is a known pharmacological action associated with peripheral CRF administration (e.g., Corder et al., 1992) that could affect behavior], as well as the recognition (e.g., Bruhn et al, 1987) that concentrations of CRF in local pools where peripheral CRF is released in close proximity to its receptors may be quite high, all indicate the hypothesis that endogenous peripheral CRF affects behavior during prolonged separation through a proinflammatory mechanism should be given serious consideration.

The idea that perception of a psychological stressor (absence of the mother in a novel environment) affects behavior in part by producing a peripheral signal (release of CRF) which then feeds back to the CNS, seems circuitous, but is consistent with other findings. For instance, evidence indicates that peripheral release of CRF contributes to gastrointestinal effects of psychosocial stressors (Zheng et al., 2009). Gastrointestinal bacterial infections induce anxiety, apparently by means of a vagal signal to stress-related brain regions (Goehler et al., 2007), and peripheral administration of a peptide CRF-receptor antagonist has been reported to reduce anxiety in patients with irritable bowel syndrome (Sagami et al., 2004).

In the case of the separated guinea pig, the initial response to separation includes not only active behavior, but also increased hypothalamic-pituitary-adrenal activity and sympathetic activation (Hennessy et al., 1989), the latter of which may stimulate peripheral CRF secretion in the pup. Indeed, the sympathetic nervous system is thought to be a primary source of immunologically active CRF in the periphery (Webster et al., 1997). The adrenal medulla seems of particular interest because it not only appears to release appreciable quantities of CRF in the vicinity of CRF receptors during stressor exposure (Bruhn et al., 1987), but also was recently found to exhibit stress-induced proinflammatory cytokine activation (Deak et al, unpublished results). The gradual emergence of passive behavior about an hour after separation ensues may reflect the time required for CRF to induce a proinflammatory response via de novo synthesis of cytokines, which then signals the CNS to influence behavior. Thus, while it is premature to suggest a site at which CRF stimulates a proinflammatory response that ultimately is responsible for the behavior effects we observe, the adrenal medulla in particular appears to warrant further study.

The hypothesized brain-to-periphery-to-brain process, and the lag in onset time of passive behavioral effects it entails, could account in large part for the onset of the second, passive stage of the separated guinea pig pup's response. More generally, this process suggests a possible mechanism for "conservation-withdrawal" interpretations of the passive behavior of separated macaque monkeys (Kaufman & Rosenblum, 1967) as well as for more-recent hypotheses concerning the transition from panic-like, to depressive-like, behavior during social isolation (Panksepp, 1998). In all, the present findings together with those of other recent studies (e.g., Goehler et al., 2007; Sagami et al., 2004; Zheng et al., 2009) suggest that, as connections between peripheral immunological and peptide function and CNS activity become more fully understood, mechanisms of behavior may increasingly be found to include unexpected forms of communication between peripheral and CNS components.

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Figure 1.

Median number {and semi-interquartile range} of 1-min intervals during which separated guinea pig pups injected SC with CRF or saline vehicle exhibited the four measures of passive behavior when they also were injected IP with IL-10 and when they were injected IP with saline vehicle. * $p < 0.05$; ** $p < 0.01$.

Figure 2. Median number {and semi-interquartile range} of vocalizations and line-crossings exhibited by separated guinea pig pups injected SC with CRF or saline vehicle they also were injected IP with IL-10 and when they were injected IP with saline vehicle. ** $p < 0.01$.

Table 1

Median level (and semi-interquartile range) of passive behavior measures following ICV infusion of aCSF and different doses of CRF

Dose Group	Passive Behavior		
	Crouch	Eye-close	Piloerection
1 μ g			
CRF	2.5 (10.2)	0.0 (0.0)	0.0 (16.4)
aCSF	4.8 (17.7)	0.0 (4.1)	0.0 (10.4)
5 μ g			
CRF	6.1 (6.0)	0.0 (0.5)	0.0 (1.5)
aCSF	5.0 (4.5)	0.0 (0.0)	0.0 (4.0)
10 μ g			
CRF	33.0 (18.8)	1.0 (2.2)	12.5 (7.7)
aCSF	16.3 (12.1)	0.5 (4.2)	9.5 (14.2)

Table 2

Median level (and semi-interquartile range) of active behavior measures following ICV infusion of aCSF and different doses of CRF

Dose Group	Active Behavior	
	Vocalization	Line-Cross
1 μ g		
	CRF 4,276.0 (2,522.4)	149.5 (102.4)
aCSF	4,234.5 (3,254.7)	126.5 (195.5)
5 μ g		
	CRF 1,928 (955.5)	176.0 (71.0)
aCSF	1,747 (2,029.5)	226.0 (112.5)
10 μ g		
	CRF 2,275 (1,862.5)	56.0 (46.5)
aCSF	2,104 (2,021.0)	52.0 (59.1)