Published in final edited form as:

Physiol Behav. 2015 February ; 139: 34–40. doi:10.1016/j.physbeh.2014.11.030.

Naproxen Attenuates Sensitization of Depressive-Like Behavior and Fever during Maternal Separation

Michael B. Hennessy^a, Nathan P. Stafford^a, Brittany Yusko-Osborne^a, Patricia A. Schiml^a, Evan D. Xanthos^a, and Terrence Deak^b

^aDepartment of Psychology, Wright State University, Dayton, OH 45435

^bBehavioral Neuroscience Program, Department of Psychology, Binghamton University, Binghamton, NY 13902

Abstract

Early life stress can increase susceptibility for later development of depressive illness though a process thought to involve inflammatory mediators. Isolated guinea pig pups exhibit a passive, depressive-like behavioral response and fever that appear mediated by proinflammatory activity, and which sensitize with repeated separations. Treatment with an anti-inflammatory can attenuate the behavioral response during the initial separation and separation the following day. Here we used the cyclooxygenase inhibitor naproxen to examine the role of prostaglandins in mediating the depressive-like behavior and core body temperature of young guinea pigs during an initial separation, separation the next day, and separation 10 days after the first. The passive, depressive-like behavior as well as fever sensitized with repeated separation. Three days of injection with 14 mg/kg of naproxen prior to the initial separation reduced depressive-like behavior during all three separations. A 28 mg/kg dose of naproxen, however, had minimal effect on behavior. Fever during the early separations was moderated by naproxen, but only at the higher dose. These results suggest a role of prostaglandins in the behavioral and febrile response to maternal separation, and particularly in the sensitization of depressive-like behavior following repeated separation.

Keywords

maternal separation; sensitization; fever; depressive-like behavior; depression; prostaglandins; inflammatory; early stress; guinea pig

1. Introduction

Disruption of attachment relationships and other childhood stressors can increase vulnerability for developing mental disorders, such as depression, in adolescence or

© 2014 Elsevier Inc. All rights reserved.

Correspondence to: Michael B. Hennessy, PhD, Department of Psychology, 335 Fawcett Hall, Wright State University, Dayton, OH 45435, michael.hennessy@wright.edu, Tel: 937.775.2943.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

adulthood [1, 2, 3, 4]. Several lines of research suggest this effect can involve inflammatory mechanisms. First, it is well known that stressors can induce a systemic inflammatory reaction [5]. There also now is overwhelming evidence that systemic inflammation can promote depressive symptomatology [6, 7, 8]. Most importantly, an abundance of recent findings suggest that early-life psychosocial stress produces lasting elevations in inflammatory activity [e.g., 9, 10, 11, 12, 13, 14], and that these effects may be linked to later depression. For instance, Danese et al [15] found the severity of adult depression to be related to levels of the inflammatory marker, C-reactive protein, but only in individuals who had been exposed to childhood maltreatment. It has been suggested that early-life stressors may sensitize inflammatory processes, so that exposure to stressors in later life is more likely to evoke an inflammatory-mediated depressive episode [16, 17].

A variety of inflammatory factors have now been identified that influence neurotransmitter synthesis, release, and reuptake in ways that potentially could promote depression [8]. One class of mediators that may be particularly relevant for understanding lasting effects of early trauma are the prostaglandins, a group of lipid compounds derived from arachidonic acid largely through the action of cyclooxygenase (COX) isoenzymes. Prostaglandins not only subserve acute inflammatory reactions, but also appear to encourage the transition to, and maintenance of, chronic inflammation [18]. Further, COX inhibitors have been observed to reduce inflammatory mediated behavioral deficits, including depressive-like behavior, in rodents [19, 20, 21]. Heightened prostaglandin levels have been reported in depressed patients [22, 23], and COX inhibitors have been found to have an antidepressant effect [24, 25]. Finally, there is evidence that negative long-term biobehavioral consequences of early psychosocial stress are mediated by prostaglandins and can be blocked with anti-inflammatory treatment. Brenhouse and Andersen [26] found repeated periods of maternal separation in infant rats produced cognitive impairment as well as evidence of a reduction in parvalbumin-containing GABAergic interneurons and increased levels of COX expression in prefrontal cortex during adolescence, but these effects were reversed if animals were administered a COX inhibitor during the period between the early separations and later testing.

We examine the effect of maternal separation in the guinea pig, a precocial rodent in which the young display evidence of a selective filial attachment [e.g., 27, 28, 29], and the response to maternal separation resembles that of primate infants in a number of ways [30]. One of these similarities is that the behavioral reaction occurs in two stages. An initial active stage, marked primarily by vocalizing, is followed by a passive, depressive-like stage characterized by a crouched posture, prolonged eye-closure, and extensive piloerection [31]. These stages are reminiscent of the “protest” and “despair” of separated macaques [32], though the transition to the second stage develops over the course of hours rather than days. Several sets of findings indicate that this second stage of responsiveness in guinea pigs is mediated by inflammatory processes: (1) direct activation of an inflammatory reaction with lipopolysaccharide hastens the onset of the passive stage [33]; (2) separation elicits physiological signs of an inflammatory reaction, specifically fever and increased proinflammatory cytokine expression [34, 35]; and, (3) three different anti-inflammatory agents (including the COX inhibitor indomethacin) reduce the passive response to separation [36, 37, 38].

Both the passive behavioral response and the fever sensitize with repeated separations. Hennessy et al [39] found that pups separated on two consecutive days showed more of the depressive-like passive behavior and a more-distinct and rapid fever response during the second separation than during the first. Since an increase in fever likely reflects an increase in underlying proinflammatory signaling, the results suggest that inflammatory factors may have promoted the behavioral sensitization. To examine this possibility, pups were administered the anti-inflammatory cytokine Interleukin-10 (IL-10) through an intracerebroventricular cannula prior to the first of two separations that occurred on consecutive days. Whereas vehicle controls showed sensitization of depressive-like behavior during the second separation, pups receiving IL-10 prior to the first separation did not [40]. Subsequent work revealed that the sensitization would persist for more than just 24 hr. For instance, Schneider et al [41] found that pups separated on two consecutive days, and then again 10 days after the first, exhibited more of the passive depressive-like behavior during both of the later separations, as well as a larger and more-rapid fever response, particularly during the last separation.

In all, results to this point support the notion that sensitization of inflammatory factors contributes to the sensitization of the depressive-like response. However, we have only evaluated the effects of anti-inflammatory treatment on short-term behavioral sensitization (i.e., over a 24-hr period). Effects of this treatment on fever or its sensitization have not been investigated. In addition, nothing is known about which inflammatory factors might mediate the sensitization process. Therefore, in the present study we examined the role of prostaglandins in the short-term and more-prolonged sensitization of behavior and fever. The common, nonspecific COX inhibitor naproxen was administered prior to an initial separation. The pups were separated on two consecutive days and again 10 days after the first using the paradigm of Schneider et al [41]. We measured both passive depressive-like behavior and core temperature during all separations. For comparison, we also scored the primary behavior of the active stage of separation—vocalizing. Finally, we monitored locomotor activity to rule out the possibility that increases in core temperature were secondary to increased exertion rather than to true fever.

2. Method

2.1. Animals and experimental design

Albino Hartley guinea pigs (*Cavia porcellus*) were bred and housed in our laboratory. Each mother and litter were maintained in an opaque plastic cage (73 × 54 × 24 cm) with wire front and sawdust bedding. Food and water were available *ad libitum*. The colony room was kept at ~ 70° on a 12:12 light-dark schedule, with lights on at 0700 hr. Cages were changed twice per week. Pups were kept continuously with the mother with the exception of surgery, brief post-operative management procedures (e.g., inspection and weighing of pups), injections, and behavioral testing. All procedures were in compliance with NIH guidelines and were approved by the Wright State University Laboratory Animal Care and Use Committee.

In a first experiment, six male and six or seven female pups were tested in each of three experimental conditions. In the non-injected (NI) condition, pups were not injected prior to

testing. Those in the vehicle (VEH) condition received three daily, subcutaneous injections of saline vehicle all prior to the first of the three separations. In the naproxen (NAP) condition, pups were administered three subcutaneous injections of naproxen (14 mg/kg; Sigma Aldrich) in sterile saline with carboxymethyl cellulose (.25%) according to the same schedule as VEH pups. In light of core temperature results in the first experiment, a second experiment was conducted in which six male and six female pups were tested in the VEH and NAP conditions, but with the dose of naproxen increased to 28 mg/kg. Because there were no significant differences between NI and VEH animals in passive behavior or core temperature in the first experiment, only the VEH controls were included in the second experiment. No more than one pup from a litter was assigned to any condition in either experiment.

2.2. Surgery and Telemetry

Telemetry probes (PD 4000 Emitter, Philips Respironics) were surgically implanted into the abdominal cavity under isoflurane anesthesia (2-4%) using aseptic procedures between 15 and 18 days of age. Pups were treated with 0.05 mg/kg atropine (intraperitoneal) to reduce secretory activity. Buprenorphine (0.05 mg/100 g) was given subcutaneously immediately following surgery and again 24 hr later. At least 3 days intervened between surgery and behavioral testing. Core temperature was measured by the telemetry probe, which sent signals to a receiver plate (55 × 28 cm) placed under the cage during testing. The telemetry probe also signaled movement of the pup. The temperature and motor activity data were collected with computer software (Vital View) of the manufacturer, which aggregates data in 3-min bins.

2.3. Testing procedure

Pups in the VEH and NAP groups each received injections of their designated substance on 3 days at 24-hr intervals, with the last injection occurring 1 hr before the first separation test. In order to accommodate the scheduling of multiple 3-hr tests for each pup, the first test occurred between 19 and 23 days of age in the first experiment. (Mean age = 20.9 days for NI, 20.8 days for VEH, and 21.1 days for NAP). In the second experiment, the first test was between 21 and 23 day (Mean age = 22.2 for VEH and 21.7 for NAP). All pups were separated for 3 hr on three occasions. The second separation occurred 24 hr after the first, and the third was 10 days after the first. Natural weaning in the guinea pig is about 25 days of age so that the last separation occurred during what is best considered the juvenile or periadolescent period. For the first experiment, all testing began between 1600 and 1630 hr. In the second experiment, testing occurred during both morning and afternoon, but equal numbers of pups from the two conditions began at 0800, 1200, and 1400 hr. No apparent signs of discomfort or ill health were observed in any subjects following acute or repeated administration of the drug.

For testing, the pup was transported in a carrying cage to the adjoining observation room (~30 s) where it was placed alone into an empty, clear, plastic test cage (47 × 24 × 20 cm) positioned over the receiver plate. Core body temperature and activity were recorded for the next 3 hr. Additional behaviors were scored during Min 0-30, 60-90, and 150-180 from behind one-way glass by an observer trained to at least 85% inter-observer reliability. The

number of whistle vocalizations (the primary behavior of the active stage) was tallied using a hand-held counter. A microphone positioned over the cage broadcast the sound to the headphones of the observer. In addition, the observer scored the number of 1-min intervals in which pups exhibited each of the three passive, depressive-like behaviors characteristic of separated pups (Hennessy et al., 1995): (1) a distinctive crouched stance with feet pulled close to the body—this sometimes transitions into lying on the cage floor with the trunk supporting the body (either position was scored as “crouch”); (2) complete or near complete closure of at least one eye for at least 1 s; and, (3) piloerection over most of the body. The primary behavioral measure used in this study (full passive response) was the number of 1-min intervals in which all three passive behaviors were displayed.

2.4. Data analysis

To reduce temperature data, analyses were based on 15-min time blocks created by combining five consecutive 3-min bins of data. Activity data were also condensed into 5-min time blocks and used for visual comparison with temperature data. Analysis of variance (ANOVA) was the preferred means of statistical analysis. When sphericity was judged to be problematic by the Mauchly test, the Huynh-Feldt correction factor was used. Appropriate fractional degrees of freedom are shown for effects for which the correction factor was needed. Post-hoc analyses of significant interactions were conducted with tests for simple effects [42]. Nonparametric methods were used when data violated normality due to large numbers of scores of “zero” (full passive response in the first experiment). Mann-Whitney and Wilcoxon tests were used for paired comparisons between and within groups, respectively. The Friedman nonparametric ANOVA by ranks was used for multiple comparisons across days. Central tendency is represented with means for data appropriate for parametric analysis and with medians for the full passive response of the first experiment. A probability level of $p < 0.05$ (2-tailed) was accepted throughout. Portions of telemetry data were lost for one pup each in the NI and NAP conditions in the first experiment. These animals were dropped from analysis of temperature and activity scores.

3. Results

3.1. Body Temperature

Core body temperature in the first experiment showed a clear sensitization. A 3 (Condition) $\times 2$ (Sex) $\times 3$ (Separation Number) $\times 12$ (Time Block) ANOVA with Separation Number and Time Block treated as repeated measures yielded significant main effects of Separation, $F(2, 58) = 22.18, p < 0.001$, and Time Block, $F(4.64, 134.60) = 43.38, p < 0.001$. These main effects were qualified by a significant Separation Number $\times$ Time Block interaction, $F(9.65, 279.74) = 21.82, p < 0.001$. As is clear in the upper panel of Figure 1, this interaction was the manifestation of an increase in body temperature with repeated separation, particularly during early time blocks. That is, sensitization involved greater and more-rapid elevations in temperature with repeated separation. In addition, the ANOVA yielded a significant Sex $\times$ Time Block interaction, $F(4.64, 134.60) = 5.90, p < 0.001$, reflecting a pattern in which males had numerically higher body temperatures during the first five time blocks (1 hr, 15 min) and females had higher body temperatures during the last seven time blocks (1 hr, 45 min) regardless of experimental condition or separation number. Although

NAP pups had lower body temperatures than NI or VEH pups in 31 of the 36, 15-min time blocks across the three separations, there was no significant effect involving Condition. That is, the 14 mg/kg dose of naproxen had no verifiable effect on core body temperature.

For the second experiment, ANOVA again yielded significant main effects of Separation Number, $F(2, 40) = 5.03, p < 0.05$, and Time Block, $F(3.48, 69.63) = 16.92, p < 0.001$. However, there also was a significant interaction of Condition $\times$ Separation Number $\times$ Time Block, $F(6.62, 132.46) = 2.31, p < 0.05$, indicating an effect of the higher dose of naproxen on core temperature. As seen in the lower panel of Figure 1, the pattern was one of lower core temperature values for NAP animals than for VEH controls during the first separation and portions of the second. Tests for simple interactions (Condition $\times$ Separation Number) were significant for the first five time blocks (p 's < 0.01). However, follow-up simple main effect tests of these significant interactions revealed no significant difference between VEH and NAP animals at any single time block during any of the three separations. In short, while the pattern of lower core temperature in NAP animals during portions of the first two separations was significant, this difference could not be isolated to particular 15-min time blocks. We also calculated simple interaction effects (Separation Number $\times$ Time Block) to examine possible sensitization for the two experimental conditions. For VEH controls, there was no significant main or interaction effect for Separation Number; i.e., there was no indication of sensitization. This may have been due to separation without naproxen eliciting a particularly robust response during the first separation (Fig. 1, lower panel). For NAP animals, both the main effect of Separation Number and the interaction of Separation Number and Time Block were significant (p 's < 0.01). Core temperature sensitized, with higher core temperatures detected for each of the 12 time blocks in the third separation than in either of the first two separations.

3.2. Behavior

3.2.1. Activity—ANOVA of activity data in the first experiment yielded significant main effects of Separation Number, $F(2, 58) = 14.01, p < 0.001$ and Time Block, $F(4.39, 178.95) = 20.27, p < 0.001$, as well as a significant Separation Number $\times$ Time Block interaction, $F(7.49, 217.16) = 4.77, p < 0.001$. In addition, there was a significant interaction of Condition $\times$ Sex, $F(2, 29) = 3.83, p < 0.05$. The main purpose of collecting activity data was for comparison with temperature data to insure that increases in core temperature were not secondary to increased physical exertion. Therefore, we did not perform post-hoc tests on activity data, but plotted changes in activity across time blocks for each condition on each day in Figure 2 for visual comparison with core temperature changes in Figure 1. As can be seen in the upper panels of the two figures, activity declined across the three separations while core temperature increased.

In the second experiment, ANOVA of activity yielded only one significant effect, a main effect for Time Block, $F(6.21, 124.19) = 11.63, p < 0.001$. That is, the sensitization of core temperature across days seen in the VEH group was not associated with a sensitization of activity (Figs. 1 and 2, lower panels). Further, naproxen animals had numerically higher temperature values during the first separation of the second experiment, when temperature

was higher in controls. In sum, these comparisons indicate that increases in temperature in the two experiments cannot be accounted for by increases in physical activity.

3.2.2. Vocalization—In the first experiment, the 3 (Condition) $\times$ 2 (Sex) $\times$ 3 (Separation Number) ANOVA for vocalizing, the principal behavior of the active phase of separation, yielded only a significant main effect of Separation Number, $F(2, 62) = 12.41, p < 0.001$, with a decline in vocalizing by the third separation (Fig. 3 upper panel). Similarly, the main effect of Separation Number, $F(2, 40) = 13.90, p < 0.001$, was the only significant effect in the second experiment as well, again with a reduction in vocalizing by the third separation (Fig. 3, lower panel).

3.2.3. Passive behavior—In the first experiment, nonparametric tests were required because of a large number of scores of “zero” in the NAP group. Preliminary Mann-Whitney tests found no differences between male and female pups in any condition during any of the three separations. Therefore male and female pups were combined for additional analyses. Further, because all three conditions showed increasing patterns of passive behavior after the first separation the conditions were combined for the purpose of examining sensitization. Indeed, the full passive response, in contrast to vocalizations, increased (i.e., sensitized) across separations (Friedman test, $p < 0.005$). Follow-up Wilcoxon tests indicated that pups exhibited more of the full passive response during both the second ($p < 0.005$) and third separations ($p < 0.01$) than during the first (Fig 4, upper panel). As also illustrated in the upper panel of Figure 4, the median number of 1-min intervals in which the full passive response was observed in guinea pigs given 14 mg/kg naproxen prior to the initial separation was a fraction of that seen in the control groups. Because there was no significant difference in the amount of passive behavior displayed by NI and VEH controls during any separation, these groups were combined for comparison with the NAP condition. NAP animals exhibited less passive behavior than the combined controls during the first ($p = 0.005$), second ($p = 0.001$), and third ($p < 0.005$) separations.

A different pattern emerged for animals given the higher dose of naproxen. A 2 (Condition) $\times$ 2 (Sex) $\times$ 3 (Separation Number) ANOVA yielded a significant effect of Separation Number, $F(2, 40) = 10.31, p < 0.001$, reflecting a sensitization of the full passive response with repeated separations (Fig 4, lower panel). There were no other significant effects, including no effects of naproxen. In light of the robust effects of the lower dose in the first experiment, we decided to further probe the effect of the high dose by examining the three components of the full passive response (the crouched stance, eye closure, and extensive piloerection) in 2 (Condition) $\times$ 2 (Sex) $\times$ 3 (Separation Number) ANOVAs. There was one significant effect involving Condition, a Condition $\times$ Separation Number interaction for crouch, $F(2, 40) = 3.54, p < 0.05$ (Table 1). Simple main effect follow-up tests showed that VEH animals exhibited an increase in crouching with repeated separation ($p < 0.01$), whereas animals administered the 28 mg/kg dose of naproxen did not.

4.0. Discussion

In agreement with several recent reports [35, 41], we found that the passive depressive-like response of guinea pigs showed sensitization with repeated separation. The active behavior

of vocalizing did not sensitize, but rather declined, an effect previously demonstrated to be due to increased age rather than habituation [35, 41]. The sensitization of passive behavior was accompanied by a sensitization of fever, an indirect measure of proinflammatory activity, though in the second experiment only the NAP group showed this effect. Our results also extend previous findings that other anti-inflammatory agents (indomethacin, IL-10, and α -MSH) attenuate depressive-like behavior during separations immediately following drug administration [36, 37, 38], and that IL-10 can abolish sensitization of depressive-like behavior during a second separation the following day [40].

The main novel finding of the current study, however, was that 14 mg/kg of the reversible COX inhibitor naproxen reduced depressive-like behavior during separation a full 10 days following the first separation and last injection, a time at which the drug would have long been metabolized and cleared from the body (half life of naproxen in the guinea pig $\approx$ 9 hr) [43]. We also found that naproxen moderated the febrile response to separation. This effect appeared less robust than the behavioral effect, requiring a higher dose of naproxen, and only was apparent during the initial one or two separations. Because prostaglandins play a central role in the generation of fever [44], the moderating effect of naproxen on core temperature observed here reinforces the conclusion that the elevation of temperature during separation constitutes true fever. The reduction of the passive, depressive-like response in guinea pigs receiving naproxen indicates prostaglandin involvement in the behavioral effect as well. Our earlier study with the COX inhibitor indomethacin [36] provided evidence for prostaglandin mediation of depressive-like behavior during an initial separation experience. The current results both support this conclusion and point to prostaglandin mediation of the sensitization of the depressive-like behavior with repeated separation.

Although the 28 mg/kg dose of naproxen moderated fever, it produced only a minimal reduction of depressive-like behavior, i.e., inhibited sensitization of one of the three components of the full passive response. Naproxen is a nonspecific COX inhibitor, blocking both the COX-1 and COX-2 isoforms [45]. Whereas COX-2's effects are primarily proinflammatory, COX-1 catalyzes the production of cytoprotective prostaglandins, so that most negative side effects of therapy with naproxen are thought to be due to COX-1 inhibition [46]. It is possible, therefore, that the higher dose of naproxen produced an action via COX-1 that negated or offset its anti-inflammatory effect on behavior. Also of potential relevance is the apparent U-shaped association between prostaglandins and memory, with evidence for both high levels of prostaglandins, as well as inhibition of prostaglandins below non-inflammatory basal levels, impairing memory formation [47]. If a similar function were to exist for prostaglandin effects on behavior during separation, both very high and very low levels of prostaglandin activity could have increased depressive-like behavior in our paradigm. That is, the 14 mg/kg dose may have blocked synthesis of very high, stress-induced prostaglandin levels that had increased depressive-like behavior, and the 28 mg/kg dose may have reduced prostaglandins to levels low enough to again increase depressive-like behavior.

In addition to their acute effects, prostaglandins promote chronic inflammation by several means, including amplification of proinflammatory signaling through increased gene expression, of for instance, the IL-1 receptor [18]. The longer term sensitization of

depressive-like behavior in the present study may have been mediated by such actions. In human populations, chronic inflammation in adolescence or adulthood has often been found in those experiencing early life stress [11, 12, 13, 14, 15, 48] and is thought to promote the onset of depressive illness [17]. The current results suggest that prostaglandins may play a role in the initiation of this process.

Acknowledgements

The authors thank Kate Berberich, Alex Oxner, and Tang Watanasriyakul for their technical assistance. N.P.S. is now at the Department of Psychology, University of New Hampshire. B.Y.-O. is now at the Department of Psychology, University of Delaware. The research was funded by grant MH068228 from the National Institute of Mental Health. The sponsor did not contribute to the experimental design, data collection, analysis, interpretation, writing of the report, or decision to submit the report for publication.

References

- [1]. Agid O, Shapira B, Zislin J, Ritsner M, Hanin B, Murad H, Troudart T, Bloch M, Heresco-Levy U, Lerer B. Environment and vulnerability to major psychiatric illness: a case control study of early parental loss in major depression, bipolar disorder and schizophrenia. *Molecular Psychiatry*. 1999; 4:163–172. [PubMed: 10208448]
- [2]. Bernet CZ, Stein MB. Relationship of childhood maltreatment to the onset and course of major depression in adulthood. *Depression and Anxiety*. 1999; 9:169–174. [PubMed: 10431682]
- [3]. Hornung OP, Heim CM. Gene-environment interactions and intermediate phenotypes: early trauma and depression. *Frontiers in Endocrinology*. 2014; 5 Article 14.
- [4]. Pechtel P, Pizzagalli DA. Effects of early life stress on cognitive and affective function: an integrated review of human literature. *Psychopharmacology*. 2011; 214:55–70. [PubMed: 20865251]
- [5]. Maier SF, Watkins LR. Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychological Review*. 1998; 105:83–107. [PubMed: 9450372]
- [6]. Dantzer R, O'Connor JC, Lawson MA, Kelley KW. Inflammation-associated depression: from serotonin to kynurenine. *Psychoneuroendocrinology*. 2011; 36:426–436. [PubMed: 21041030]
- [7]. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim E, Lanctôt K. A meta-analysis of cytokines in major depression. *Biological Psychiatry*. 2010; 67:446–457. [PubMed: 20015486]
- [8]. Miller AH, Haroon E, Raison CL, Felger JC. Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. *Depression and Anxiety*. 2013; 30:297–306. [PubMed: 23468190]
- [9]. Bertone-Johnson ER, Whitcomb BW, Missmer SA, Karlson EW, Rich-Edwards JW. Inflammation and early-life abuse in women. *American Journal of Preventive Medicine*. 2012; 43:611–620. [PubMed: 23159256]
- [10]. Carpenter LL, Gawuga CE, Tyrka AR, Lee JK, Anderson GM, Price LH. Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. *Neuropsychopharmacology*. 2010; 35:2617–2623.
- [11]. Gouin J-P, Glaser R, Malarkey WB, Beversdorf D, Kiecolt-Glaser JK. Childhood abuse and inflammatory responses to daily stressors. *Annals of Behavioral Medicine*. 2012; 44:287–292. [PubMed: 22714139]
- [12]. Miller GE, Chen E. Harsh family climate in early life presages the emergence of a proinflammatory phenotype in adolescence. *Psychological Science*. 2010; 21:848–856. [PubMed: 20431047]
- [13]. Slopen N, Kubzansky LD, McLaughlin KA, Koenen KC. Childhood adversity and inflammatory processes in youth: a prospective study. *Psychoneuroendocrinology*. 2013; 38:188–200. [PubMed: 22727478]

- [14]. Zeugmann S, Buehrsch N, Bajouj M, Heuser I, Anghelescu I, Quante A. Childhood maltreatment and adult proinflammatory status in patients with major depression. *Psychiatria Danubina*. 2013; 25:227–235. [PubMed: 24048389]
- [15]. Danese A, Moffitt TE, Pariante CM, Ambler A, Poulton R, Caspi A. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. *Archives of General Psychiatry*. 2008; 65:409–416. [PubMed: 18391129]
- [16]. Hennessy MB, Deak T, Schiml-Webb PA. Early attachment-figure separation and increased risk for later depression: potential mediation by proinflammatory processes. *Neuroscience and Biobehavioral Reviews*. 2010; 34:782–790. [PubMed: 20359585]
- [17]. Slavich GM, Irwin MR. From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. *Psychological Bulletin*. 2014; 140:774–815. [PubMed: 24417575]
- [18]. Aoki T, Narumiya S. Prostaglandins and chronic inflammation. *Trends in Pharmacological Sciences*. 2012; 33:304–311. [PubMed: 22464140]
- [19]. De Paiva VN, Lima SNP, Fernandes MM, Soncini R, Andrade CAF, Giusti-Paiva A. Prostaglandins mediate depressive-like behaviour induced by endotoxin in mice. *Behavioural Brain Research*. 2010; 215:146–151. [PubMed: 20654654]
- [20]. Jain NK, Kulkarni SK, Singh A. Lipopolysaccharide-mediated immobility in mice: reversal by cyclooxygenase enzyme inhibitors. *Methods and Findings in Experimental and Clinical Pharmacology*. 2001; 23:441–444. [PubMed: 11838318]
- [21]. Salah LA, Hamza M, El Gayar NH, Abd El-Samad AA, Nasr EA, Massoud SI. Ibuprofen suppresses depressive like behavior induced by BCG inoculation in mice: role of nitric oxide and prostaglandin. *Pharmacology, Biochemistry and Behavior*. 2014 (in press).
- [22]. Nishino S, Ueno R, Ohishi K, Sakai T, Hayaishi O. Salivary prostaglandin concentrations: possible state indicators for major depression. *American Journal of Psychiatry*. 1989; 146:365–368. [PubMed: 2627202]
- [23]. Ohishi K, Ueno R, Nishino S, Sakai T, Hayaishi O. Increased level of salivary prostaglandins in patients with major depression. *Biological Psychiatry*. 1988; 23:326–334. [PubMed: 3422573]
- [24]. Mendlewicz J, Kriwin P, Oswald P, Souery D, Alboni S, Brunello N. Shortened onset of action of antidepressants in major depression using acetylsalicylic acid augmentation: a pilot open-label study. *International Clinical Psychopharmacology*. 2006; 21:227–231. [PubMed: 16687994]
- [25]. Müller N, Schwarz MJ, Dehning S, Douhe A, Ceroveckí A, Goldstein-Müller B, Spellmann I, Hetzel G, Maino K, Kleindienst N, Möller H-J, Arolt V, Riedel M. The cyclooxygenase-2 inhibitor celecoxib has therapeutic effects in major depression: results of a double-blind, randomized, placebo controlled, add-on pilot study to reboxetine. *Molecular Psychiatry*. 2006; 11:680–684. [PubMed: 16491133]
- [26]. Brenhouse H, Anderson SL. Nonsteroidal anti-inflammatory treatment prevents delayed effects of early life stress. *Biological Psychiatry*. 2011; 70:434–440. [PubMed: 21679927]
- [27]. Hennessy MB, Ritchey RL. Hormonal and behavioral attachment responses in infant guinea pigs. *Developmental Psychobiology*. 1987; 20:613–625. [PubMed: 3691968]
- [28]. Jäckel M, Trillmich F. Olfactory individual recognition of mothers by young guinea pigs (*Cavia porcellus*). *Ethology*. 2003; 109:197–208.
- [29]. Pettijohn TF. Attachment and separation distress in the infant guinea pig. *Developmental Psychobiology*. 1979; 12:73–91. [PubMed: 573221]
- [30]. Hennessy MB. Enduring maternal influences in a precocial rodent. *Developmental Psychobiology*. 2003; 42:225–336. [PubMed: 12621648]
- [31]. Hennessy MB, Long SJ, Nigh CK, Williams WT, Nolan DJ. Effects of peripherally administered corticotropin-releasing factor (CRF) and a CRF antagonist: Does peripheral CRF activity mediate behavior of guinea pig pups during isolation? *Behavioral Neuroscience*. 1995; 109:1137–1145. [PubMed: 8748963]
- [32]. Mineka S, Suomi SJ. Social separation in monkeys. *Psychological Bulletin*. 1978; 85:1376–1400. [PubMed: 104324]

- [33]. Hennessy MB, Deak T, Schiml-Webb PA, Wilson SE, Greenlee TM, McCall E. Responses of guinea pig pups during isolation in a novel environment may represent stress-induced sickness behaviors. *Physiology and Behavior*. 2004; 81:5–13. [PubMed: 15059678]
- [34]. Hennessy, MB.; Deak, T.; Schiml-Webb, PA.; Barnum, CJ. Immune influences on behavior and endocrine activity in early-experience and maternal separation paradigms. In: Czerbska, MT., editor. *Psychoneuroendocrinology Research Trends*. Nova Science Publishers; Hauppauge, NY: 2007. p. 293-319.
- [35]. Yusko B, Hawk K, Schiml PA, Deak T, Hennessy MB. Sensitization of depressive-like behavior during repeated maternal separation is associated with more-rapid increase in core temperature and reduced plasma cortisol levels. *Physiology and Behavior*. 2012; 105:861–867. [PubMed: 22079581]
- [36]. Hennessy MB, Schiml-Webb PA, Miller EE, Maken DS, Bullinger KL, Deak T. Anti-inflammatory agents attenuate the passive responses of guinea pig pups: Evidence for stress-induced sickness behavior during maternal separation. *Psychoneuroendocrinology*. 2007; 32:508–515. [PubMed: 17462831]
- [37]. Perkeybile AM, Schiml-Webb PA, O'Brien E, Deak T, Hennessy MB. Anti-inflammatory influences on behavioral, but not cortisol, responses during maternal separation. *Psychoneuroendocrinology*. 2009; 34:1101–1108. [PubMed: 19324498]
- [38]. Schiml-Webb PA, Deak T, Greenlee TM, Maken DS, Hennessy MB. Alpha-melanocyte stimulating hormone reduces putative stress-induced sickness behaviors in isolated guinea pig pups. *Behavioural Brain Research*. 2006; 168:326–330. [PubMed: 16214237]
- [39]. Hennessy MB, Deak T, Schiml-Webb PA, Carlisle CW, O'Brien E. Maternal separation produces, and a second separation enhances, core temperature and passive behavioral responses in guinea pig pups. *Physiology and Behavior*. 2010; 100:305–310. [PubMed: 20206192]
- [40]. Hennessy MB, Paik KD, Caraway JD, Schiml-Webb PA, Deak T. Proinflammatory activity and the sensitization of depressive-like behavior during maternal separation. *Behavioral Neuroscience*. 2011; 125:426–433. [PubMed: 21500883]
- [41]. Schneider RL, Schiml PA, Deak T, Hennessy MB. Persistent sensitization of depressive-like behavior and thermogenic response during maternal separation in pre- and post-weaning guinea pigs. *Developmental Psychobiology*. 2012; 54:514–522.
- [42]. Winer, BJ. *Statistical Principles in Experimental Design*. McGraw-Hill; New York: 1971.
- [43]. Runkel R, Chaplin M, Boost G, Segre E, Forchielli E. Absorption, distribution, metabolism, and excretion of naproxen in various laboratory animals and human subjects. *Journal of Pharmaceutical Sciences*. 1972; 61:703–707. [PubMed: 4624708]
- [44]. Blatteis CM. The onset of fever: new insights into its mechanism. *Progress in Brain Research*. 2007; 162:3–14. [PubMed: 17645911]
- [45]. Hecken A, Schwartz JI, Depré M, Lepeleire I, Dallob A, Tanaka W, et al. Comparative inhibitory activity of rofecoxib, meloxicam, diclofenac, ibuprofen, and naproxen on COX-2 versus COX-1 in healthy volunteers. *J Clin Pharmacol*. 2000; 40:1109–1120. [PubMed: 11028250]
- [46]. Süleyman H, Demircan B, Karagöz Y. Anti-inflammatory and side effects of cyclooxygenase inhibitors. *Pharmacological Reports*. 2007; 59:247–258. [PubMed: 17652824]
- [47]. Hein AM, Stutzman DL, Bland ST, Barrientos RM, Watkins LR, Rudy JW, Maier SF. Prostaglandins are necessary and sufficient to induce contextual fear learning impairments after interleukin-1 beta injections into the dorsal hippocampus. *Neuroscience*. 2007; 150:754–763. [PubMed: 18035502]
- [48]. Pace TWW, Mletzko TC, Alagbe O, Musselman DL, Nemeroff CB, Miller AHn, Heim CM. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. *American Journal of Psychiatry*. 2006; 163:1630–1633. [PubMed: 16946190]

Highlights

- Guinea pig depressive-like response sensitized with repeated maternal separations
- Febrile response also sensitized with repeated maternal separation
- 14 mg/kg of naproxen reduced behavioral response to early and later separations
- 28 mg/kg of naproxen reduced febrile response to early separations
- Prostaglandins may mediate sensitization of maternal separation effects

Figure 1.

Mean core body temperature of young guinea pigs during 12, 15-min time blocks during each of three, 3-hr separations occurring on two consecutive days and then 10 days after the first. Animals were either not injected, injected with saline vehicle, or injected with naproxen. During the first experiment (upper panel) the dose of naproxen was 14 mg/kg. During the second separation (lower panel) the dose of naproxen was 28 mg/kg. Core temperature sensitized with repeated separations across all groups in the first experiment, and in naproxen-treated animals in the second. Naproxen reduced core temperature in the second experiment, but not the first.

Figure 2.

Mean activity counts for young guinea pigs during 12, 15-min time blocks during each of three, 3-hr separations occurring on two consecutive days and then 10 days after the first. Animals were either not injected, injected with saline vehicle, or injected with naproxen. During the first experiment (upper panel) the dose of naproxen was 14 mg/kg. During the second separation (lower panel) the dose of naproxen was 28 mg/kg.

Figure 3.

Mean number of vocalizations of young guinea pigs during each of three, 3-hr separations occurring on two consecutive days and then 10 days after the first. Animals were either not injected, injected with saline vehicle, or injected with naproxen. During the first experiment (upper panel) the dose of naproxen was 14 mg/kg. During the second separation (lower panel) the dose of naproxen was 28 mg/kg. In both experiments, there was a significant reduction in vocalizing across days. Vertical lines represent standard errors of the means.

Figure 4.

Number of 1-min intervals in which young guinea pigs exhibited the depressive-like full passive response during each of three, 3-hr separations occurring on two consecutive days and then 10 days after the first. Animals were either not injected, injected with saline vehicle, or injected with naproxen. During the first experiment (upper panel) the dose of naproxen was 14 mg/kg. During the second separation (lower panel) the dose of naproxen was 28 mg/kg. Because of non-normal distribution of scores, central tendency is represented as medians for the first experiment. In the second experiment means and standard errors (vertical lines) are presented. The full passive response sensitized in both experiments and was significantly lower in the naproxen group than in the controls during each separation of the first experiment.

Table 1

Mean ($\pm$ SE) number of 1-min intervals that guinea pigs in the VEH and NAP conditions exhibited the crouched stance during the three separation tests of the second experiment.

Condition	Separation					
	1		2		3	
	<i>M</i>	<i>SE</i>	<i>M</i>	<i>SE</i>	<i>M</i>	<i>SE</i>
VEH*	33.2	5.7	42.4	5.7	63.8	3.8
NAP	36.8	6.6	47.9	6.1	45.2	4.5

* Values increase with repeated separation, $p < 0.01$