# Maternal separation increases later immobility during forced swim in guinea pig pups: evidence for sensitization of a depressive-like state

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## Abstract

Early-life stress is thought to increase later vulnerability for developing depressive illness by sensitizing underlying stress-responsive systems. Guinea pig pups separated from their mother and isolated in a novel cage for 3 hr exhibit a sensitized depressive-like behavioral response when separated again the following day as well as weeks later. The behavioral response and its sensitization appear to be mediated by inflammatory factors. To determine if this sensitization is specific to the separation response or if it reflects a broader underlying depressive-like state, guinea pig pups that had either been separated for 3 hr or remained with their mothers were observed in the forced swim test the following 3 days. Earlier separation was found to increase the duration of immobility, a measure sensitive to antidepressant treatment. These results support the use of the guinea pig as a model for examining mechanisms of inflammatory-mediated sensitization of depression following stress in early life.

## 1 | INTRODUCTION

It is now indisputable that early trauma increases vulnerability for developing depression and other forms of psychopathology for many individuals in later life (e.g., Heim & Binder, 2012; Mandelli, Petrelli, & Serretti, 2015). It appears that social or attachment-related stressors, such as neglect or abuse, may be particularly potent (Brown, Harris, & Copeland, 1977; Mandelli et al., 2015; McCory, De Brito, & Viding, 2011). The mechanisms underlying these effects are only beginning to be understood. However, the process commonly is conceived as involving sensitization of some stress-related physiological

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system(s) (e.g., hypothalamic-pituitary-adrenal, inflammatory), with the early trauma increasing responsiveness so that exposure to additional stressors in adulthood elicits enhanced and/or unregulated stress responses that precipitate, or actually constitute, the depressive episode (Gold, Goodwin, & Chrousos, 1988; Heim, Newport, Mletzko, Miller, & Nemeroff, 2008).

The guinea pig offers a potential animal model for examining such sensitization effects. Guinea pigs exhibit evidence of filial attachment (Hennessy & Ritchey, 1987; Jäckel & Trillmich, 2003; Porter, Berryman, & Fullerton, 1973). When separated from the attachment figure in a novel environment, pups initially vocalize to re-establish contact with the mother, but after an hour or more, begin to quiet and adopt a characteristic crouched posture with closed eyes and extensive piloerection (Hennessy, Long, Nigh, Williams, & Nolan, 1995). This response is reminiscent of the depressive or "despair" response evinced by human or macaque infants separated for prolonged periods (Kaufman & Rosenblum, 1967; Spitz, 1946). Moreover, the response sensitizes so that pups exhibit more of the depressive-like behavior when a second separation is imposed 24 hr after the first. Once established, the enhanced response persists days or even weeks past weaning (Schneider, Schiml, Deak, & Hennessy, 2012; and unpublished observations).

Although we have demonstrated that the depressive-like response to separation sensitizes with repeated separation, the model would be strengthened if we could show that the sensitized response generalizes to other depression-related paradigms. Human depression is not a single behavior pattern, but rather an internal state manifested by a variety of behaviors. It should be possible, therefore, to show that previous separation increases some other depression-related behavior. Furthermore, our previous findings lacked what has been termed "trans-situationality" (Maier & Watkins, 2005). That is, because we assessed sensitization in the same context as that in which the previous separation occurred, we cannot be sure that the increased later depressive-like behavior fully reflected a change in internal state, as opposed to conditioning to the environmental surroundings in which the previous separation occurred. The present study addressed these issues by asking whether previous maternal separation would increase depressive-like responding in an entirely different context. A number of depression-related paradigms have been developed for rodents, but these have been limited largely to rats and mice. The most widely used of these is the forced swim test (Porsolt, Le Pichon, & Jalfre, 1977). When placed in a vessel of water from which they cannot escape, rats and mice eventually stop swimming and attempting to climb the walls, and instead become immobile and float. Immobility in this test (either increased duration or reduced latency) has high predictive validity in that it is selectively responsive to a wide-range of antidepressant compounds (Cryan, Valentino, & Lucki, 2005; Czéh, Fuchs, Wiborg, & Simon, 2016). The forced swim test recently has been validated in the guinea pig by two studies demonstrating delayed onset of immobility following administration of a variety of antidepressants but not control substances (Rex, Voight, Wicke, & Fink, 2008; Wicke, Rex, Jongen-Relo, Groth, & Gross, 2007). Therefore, in the present experiment, we examined whether the separation procedure known to increase depressive-like behavior in guinea pig pups during a second separation the following day would also increase immobility during later testing in the forced swim.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals and experimental conditions

Albino Hartley guinea pigs (*Cavia porcellus*) were bred and housed in our laboratory from breeders purchased from Hilltop Lab Animal, Inc., Scottdale, PA. Following birth (Day 0) mother and litter were maintained in an opaque plastic cage (73 × 54 × 24 cm) with a wire front and sawdust bedding. Food and water were available ad libitum. The colony room was kept at ~70°F on a 12:12 light/dark schedule with lights on at 0700 hr. Pups were housed continuously with the mother with the exception of behavioral testing to be described below. Sixteen pups (8 male, 8 female) were assigned to each of two conditions: separated (SEP) and non-separated (NSEP). No more than one pup from a litter was assigned to either condition. All procedures were in compliance with PHS guidelines and were approved by the Wright State University Laboratory Animal Care and Use Committee.

### 2.2 | Behavioral testing

**2.2.1 | Separation—**Consistent with past studies (e.g., Hennessy, Paik, Caraway, Schiml, & Deak, 2011), SEP pups were isolated in an empty, clear, plastic test cage (47 × 24 × 20 cm) for 3 hr on one occasion between 21 and 23 days of age. The pup was gently removed from the home cage and transported to a test room in the same laboratory suite, where it was monitored from behind one-way glass during Min 0–30, 60–90, and 150–180 by an observer trained to 85% inter-observer reliability. Behaviors recorded included the number of 1-min intervals the pup engaged in any of the three passive, depressive-like behavioral categories characteristic of separated pups: (1) a distinctive crouched stance with feet pulled close to the body—sometimes transitioning into lying on the cage floor with the trunk supporting the body (crouch); (2) complete or near complete closure of one or both eyes for greater than 1 s (eye-close); (3) piloerection over more than half of the body (piloerection). The number of 1-min intervals in which a pup displayed all three passive behaviors was scored as "full passive" behavior. In addition, "whistle" vocalizations, typical of the initial active phase of separation, were tallied using a handheld counter. A microphone positioned within 15 cm of the test cage broadcast the sound to headphones to assist the observer. NSEP pups were simply left in their home cages. All separations were begun between 0800 and 0900 hr.

**2.2.2 | Forced swim test—**Animals in both SEP and NSEP conditions underwent three forced-swim trials at 24-hr intervals. The first trial occurred 24 hr following initiation of separation for SEP pups and at a comparable age for NSEP animals (*M* age = 22.7 days for SEP and 23.0 days for NSEP). A clear, glass cylinder (20 × 45 cm) was filled to a depth of 21 cm with 30 ± 1 °C fresh water. At this depth, no pup could touch bottom with its nose above water. The test procedures were based on those described for the guinea pig (Wicke et al., 2007), but with modification. Those investigators exposed the animals to the water for 5 min on a training day 24 hr prior to a single 5-min test session. However, because immobility during an initial trial has been shown to be sensitive to antidepressants in rats and mice (e.g., Overstreet, Keeney, & Hogg, 2004; Parale & Kulkarni, 1986), we scored behavior during the first as well as second 5-min trial. Further, because pilot testing revealed that duration of immobility was substantially lower in our guinea pigs than in those of Wicke et al. (2007), but that immobility increased over days, we also scored behavior during a

third, 5-min trial 24 hr after the second. During these three trials, behavior was recorded with a Logitech c920 webcam positioned directly above the cylinder and later scored with a custom behavior observation program. The primary behavior of interest was immobility, defined as the guinea pig maintaining a near stationary posture with its front paws no more than slightly moving so as to keep its nose above water and its hind legs spread so as to maintain balance. Both the total duration of immobility and latency to onset were scored. Total climbing time and swimming time also were monitored. Climbing was defined as the pup actively attempting to scale the glass walls with its front paws extending above the water. Swimming was identified as the pup pedaling through the water with all four paws under the surface. Immediately following each forced swim test, the pup was placed in a drying cage on a heating pad for 30 min before being returned to the home cage. The drying cage contained two clean, dry towels positioned under a 120-W infrared heat lamp.

### 2.3 | Data analysis

For duration of immobility, climbing, and swimming, data were analyzed with 2 (Condition) × 2 (Sex) × 3 (Trial) analyses of variance (ANOVAs) with Trial treated as a repeated measure. Due to lack of normal distribution of latency scores, non-parametric tests were used. Mann–Whitney $U$ tests examined differences across variables of Condition and Sex on each trial. Friedman ANOVA then assessed effects of Day. Spearman-rho correlation coefficients were used to assess the relation between the number of 1-min intervals full passive behavior was observed during separation and duration of immobility during each day of forced swim testing, as well as during all days combined. A probability level of $p < .05$ was accepted throughout.

## 3 | RESULTS AND DISCUSSION

During separation, pups showed the typical pattern of initially vocalizing at a high rate before quieting and increasingly exhibiting the passive, depressive-like behaviors (Table 1).

For forced swim, changes across trials (main effects) were seen for three of the four measures (Figure 1). Time (s) spent immobile increased, $F(2, 56) = 35.34$, $p < .001$, whereas latency to onset of immobility decreased, $p < .001$. Of the two active behavior measures, time spent swimming also declined with repeated testing, $F(2, 56) = 14.66$, $p < .001$. Of most interest here, however, was a main effect of Condition for immobility duration, $F(1, 28) = 4.59$, $p < .05$. Pups that previously had been separated were immobile longer than NSEP pups across trials. No other main or interaction effects were significant. There was no significant correlation between the duration of passive behavior during separation and immobility duration during forced swim.

In this experiment, a single 3-hr period of isolation in a novel cage increased immobility during forced swim across the following 3 days. This effect does not appear to have been due to physical exertion during the separation period. First, pups were relatively inactive during separation, spending considerable time exhibiting the passive, depressive-like behaviors. Second, the difference between groups did not diminish with greater time since the separation period. Rather the difference was maintained across the 3 days of forced swim testing and, while the interaction between Condition and Trial was not significant, the

absolute difference between the two groups actually increased with repeated forced swim trials.

In altricial rats and mice, effects of early manipulations of pups can often be traced to changes in treatment of the pup by the mother upon return to the nest (e.g., Parent et al., 2005). By contrast, maternal behavior in the precocial guinea pig is extremely passive, with negligible active behavior directed toward the pup beyond one week of age (Hennessy & Jenkins, 1994; König, 1985). Thus, at the age of pups tested here, the chance that maternal treatment of the pup following return to the home cage affected forced swim performance seems remote.

It appears instead that the stress of the separation procedure affected later forced swim performance. Separation of the sort conducted here is known to increase hypothalamic-pituitary-adrenal, sympathetic, and amygdala activity as well as central monoamine turnover related to stress (Harvey, Moore, Lucot, & Hennessy, 1994; Hennessy, Tamborski, Schiml, & Lucot, 1989; Maken, Weinberg, Cool, & Hennessy, 2010; Tamborski, Lucot, & Hennessy, 1990). Earlier studies of the passive, depressive-like response of separated guinea pig pups indicate that the mechanism involves stress-induced inflammatory activity. Stressors can induce a systemic inflammatory response (Maier & Watkins, 1998; Wohleb, McKim, Sheridan, & Godbout, 2015), and anti-inflammatory treatment of guinea pig pups reduces the depressive-like response to an initial separation that occurs immediately following administration (Hennessy et al., 2015; Perkeybile, Schiml-Webb, O'Brien, Deak, & Hennessy, 2009) as well as during sensitization of the response at the time of additional separations a day or more later (Hennessy et al., 2011; Hennessy et al., 2015). These findings jibe well with those of recent human studies. Inflammatory activity has not only been found to induce depressive symptomology (Bull et al., 2009), but may play a key role in the depression of those who were exposed to neglect, abuse, and other severe stressors in childhood (Slavich & Irwin, 2014).

Interpretation of results in the forced swim has been a subject of considerable debate. The idea often promoted in the literature that immobility reflects something akin to despair, or represents a true animal model of depression, has come under considerable recent criticism (Czéh et al., 2016; Molendijk & de Kloet, 2015). What is not debated is that the forced swim is an effective antidepressant screen with good predictive validity (Cryan et al., 2005; Hendriksen & Groenink, 2015). Thus, our results are best interpreted as generalization of the depressive-like response that guinea pig pups exhibit during separation to another depression-related paradigm, or to a behavioral state associated with depression, but not to another animal model of depression. In the current study, we used guinea pig pups close to the natural weaning age (~25 days), whereas the forced swim is most often examined with older rats and mice. Yet, the two extant studies demonstrating selective responsiveness to antidepressants in guinea pigs both used prepubertal animals. In one, males as young as 50 days were tested (Rex et al., 2008). In the other, ages were not provided, but the weights of the animals fell in the range of guinea pigs of the age tested here (Hennessy, Becker, & O'Neil, 1991; Wicke et al., 2007).

We saw no indication of sex differences in the forced swim or in the response to separation, which is consistent with the great majority of our previous, related studies. However, it would be premature to conclude that sex differences do not exist in the responses of guinea pig pups to separation, or the sensitization of these responses to a depressive-like state. The current experiment's sample sizes of eight males and eight females are among the largest we have included in such studies. More typically, sample sizes have been limited to five or six animals of each sex. While a greater focus on sex-differences is a goal for future work, studies to date have generally been underpowered to sensitively detect differences between males and females.

While much has been learned about the relation of early-life stress, inflammation, and later depression, much more remains hypothetical and in need of further study. The guinea pig may be particularly well-suited for this task. The evidence of filial attachment from an early age, the similarity of many of its responses during separation with those of non-human primates (Hennessy, 2003), and the sensitization of depressive-like behavior during repeated separations all favor the guinea pig over traditional rat and mouse models. The present results indicate that the sensitization of passive, depressive-like behavior following maternal separation in the guinea pig is not specific to future separation events, but instead generalizes to an entirely different context that is selectively sensitive to antidepressant treatments. This finding provides a critical, additional piece of evidence supporting the validity of guinea pig studies in pursuing the developmental processes by which early-life stress enhances susceptibility for depressive illness at later ages.

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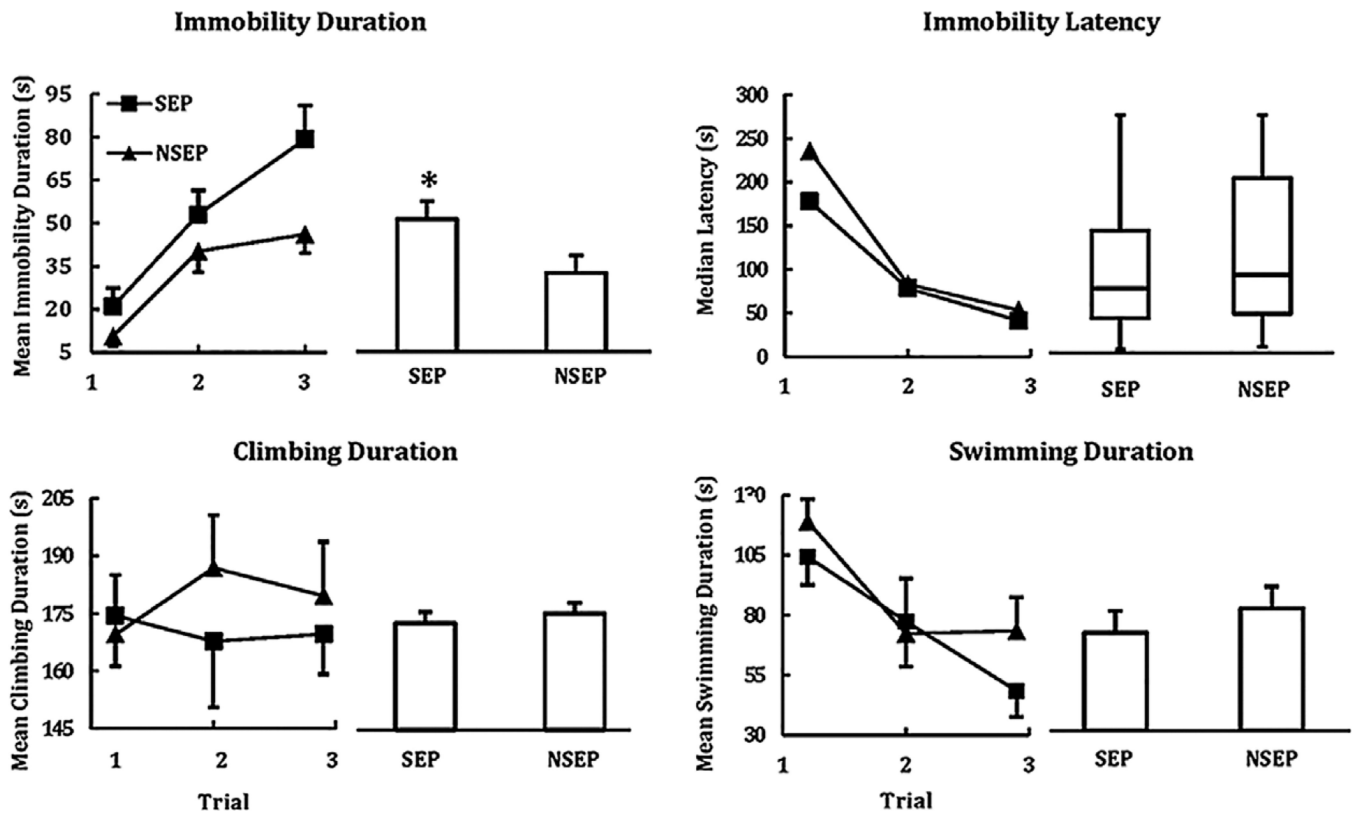
**FIGURE 1.**

Mean duration in s that previously separated and non-separated guinea pig pups spent immobile, climbing, and swimming; and median latency in s for pups to become immobile, during each of 3 days of forced swim, as well as averaged across all 3 days (histograms). Standard errors of the means are indicated with vertical lines. Box and whisker plots indicate the 10th, 25th, 50th, 75th, and 90th percentiles. Changes across days were significant for immobility duration, immobility latency, and swimming duration. *$p$ < .05 versus NSEP

**TABLE 1**

Median levels of behavior during separation

| | Min 0–30 | Min 60–90 | Min 150–180 |
|---|---|---|---|
| Vocalization[a] | 704 | 106 | 0 |
| Crouch[b] | 1 | 0 | 28.5 |
| Eye-close[b] | 0 | 0 | 18.5 |
| Piloerection[b] | 12.5 | 17.5 | 30 |
| Full passive[b] | 0 | 0 | 15 |

[a]Frequency.

[b]Number of 1 min intervals.