Developmental Psychobiology

Maternal separation followed by isolation-housing differentially affects prepulse inhibition of the acoustic startle response in C57BL/6 mice

Journal:	*Developmental Psychobiology*
Manuscript ID	DEV-15-125.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Bailoo, Jeremy; The University of Bern, Switzerland, Division of Animal Welfare Varholick, Justin; University of Bern, Division of Animal Welfare Garza, Xavier; The University of North Carolina at Greensboro, Psychology Jordan, Richard; The University of North Carolina at Greensboro, Psychology Hintze, Sara; The University of Bern, Switzerland, Division of Animal Welfare
Keywords:	maternal separation, early handling, match/mismatch hypothesis, isolation-housing, prepulse inhibition

Maternal separation followed by isolation-housing differentially affects prepulse inhibition of the acoustic startle response in C57BL/6 mice.

Jeremy D. Bailoo[1], Justin A. Varholick[1], Xavier J. Garza[2], Richard L. Jordan[2] and Sara Hintze[1]

[1]Division of Animal Welfare, The University of Bern, Bern, Switzerland, 3012, [2]Department of Psychology, The University of North Carolina-Greensboro, Greensboro, NC 27412.

Short title: MATERNAL SEPARATION AFFECTS PREPULSE INHIBITON

Corresponding Author:
Jeremy D. Bailoo, Ph.D.
Division of Animal Welfare
The University of Bern,
Bern, Switzerland 3012
Ph: 919-308-9069
jeremy.bailoo@vetsuisse.unibe.ch

The current study examined the effect of early-life stress in C57BL/6 offspring reared under four conditions: typical animal facility rearing (AFR, Control), early handling (EH, daily 15 min. separation from dam), maternal separation (MS, daily 4 hr. separation from dam), and maternal and peer separation (MPS, daily 4 hr. separation from dam and from littermates). After weaning, mice across these four conditions were either housed socially (2 - 3/cage) or in isolation (1/cage) and then tested for prepulse inhibition in adulthood. Isolation-housed MPS subjects displayed greater deficits in prepulse inhibition relative to socially-housed MPS subjects while socially-housed AFR subjects displayed greater deficits in prepulse inhibition relative to isolation-housed AFR subjects. The results indicate that these treatment conditions represent a potentially valuable model for evaluating the match/mismatch hypothesis in regards to neuropsychiatric dysfunction.

Keywords: maternal separation, early handling, match/mismatch hypothesis, isolation-housing, prepulse inhibition

Exposure to chronic stress during development has been associated with an increased risk for neuropsychiatric dysfunction in later life (de Kloet, Joëls, & Holsboer, 2005). Two primary and seemingly contradictory hypotheses have been used in the evaluation of such risk; the cumulative stress hypothesis and the match/mismatch hypothesis. The more traditional of these hypotheses, the cumulative stress hypothesis, states that exposure to consecutive stressors across development increases allostatic load, vulnerability to aversive challenges, and susceptibility to neuropsychiatric dysfunction in later life (McEwen, 2003). Conversely, the match/mismatch hypothesis states that an individual who has experienced high levels of stress early in development is better able to cope with stressors later in life compared to an individual who has experienced no or low levels of early-life stress, and therefore, is at a decreased risk for neuropsychiatric dysfunction (Schmidt, 2011).

Rodents have a long history of use in modeling such neuropsychiatric risks (Pryce, Rüedi-Bettschen, Dettling, & Feldon, 2002) and although data from such models are not always consistent (Lehmann & Feldon, 2000), one model that is generally thought to be predictive of vulnerable phenotypes is maternal separation (Branchi & Cirulli, 2014). Brief periods (~15 minutes) of dam-pup separation (i.e., early handling, EH) may lead to offspring exhibiting decreased reactivity of the hypothalamic pituitary adrenal (HPA) axis to stress-inducing situations in later adult life (c.f., Kaffman & Meaney, 2007). However, this result is only observed if the comparison group is not handled until weaning (Levine, 2002). If the comparison group is a typical animal facility reared (AFR) group, no differences in stress reactivity are observed between these groups (Levine, 2002).

Longer periods of dam-pup separation (between 3 - 6 hours), conversely, produce an exaggerated HPA axis response to a stressor in later adult life (Meaney, 2001). The two most common forms of these longer periods of separation are maternal separation (MS) of the dam from the pups, and maternal and peer separation (MPS) of the dam from the pups in addition to the littermates from one another. The effects of MS/MPS on HPA axis reactivity to stressors are not consistent throughout the literature. Such inconsistencies are thought to be a consequence of methodological differences between laboratories, including but not limited to the timing, duration, and number of MS/MPS episodes (for review, c.f., Millstein & Holmes, 2007).

Both EH and MS/MPS lead to increases in maternal care for approximately 1 - 2 hours after reuniting the pups with the dam (Liu et al., 1997; Macrí, Mason, & Würbel, 2004). It is this increase in maternal behavior that has traditionally been associated with the series of downstream effects that mediate the response of the pups to stressors as adults (Meaney, 2001; Smotherman & Bell, 1980), although some have challenged this premise (Macrì & Würbel, 2006).

To date, only one study has included and systematically compared the effects of these three most common treatment conditions (EH, MS, MPS) on maternal care and adult offspring behavior in a single experiment (Bailoo, Jordan, Garza, & Tyler, 2013). In this study, we demonstrated that one consequence of MS/MPS is an increase in maternal care in the immediate reunion phase relative to EH/AFR groups. Thus, while MS/MPS groups are generally thought to be associated with poorer outcomes because the longer periods of separation deprive the pups of maternal care (Caldji, Francis, Sharma, Plotsky, & Meaney, 2000), the results of our original study suggested that groups receiving the highest levels of maternal care were largely comprised

of the MS/MPS groups and displayed decreased "anxiety-like behavior" in an open field compared to groups that received lower levels of maternal care (largely comprised of AFR/EH groups).

In the current study, we extended the results of our previous work by investigating the development of an endophenotype related to neuropsychiatric dysfunction, prepulse inhibition of the acoustic startle response (Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001). Prepulse inhibition of the startle response is a neurological phenomenon, in which a weaker sensory stimulus inhibits the reaction of an organism to a subsequent strong and typically startling stimulus (a.k.a., sensorimotor gating) (Ison & Hoffman, 1983). Disruption of prepulse inhibition is noted in humans with symptoms of neuropsychiatric dysfunction and has previously been modeled in mice using manipulations occurring at two points in development, pre-weaning (maternal separation) and post-weaning (isolation-housing) (for review, c.f., Braff, Geyer, & Swerdlow, 2001).

Some studies have hypothesized that the application of both paradigms successively may lead to potentiated deficits in prepulse inhibition in later adult life (Matsumoto et al., 2011; Weiss, Domeney, Moreau, Russig, & Feldon, 2001). However, no study to date has evaluated the additive or interactive effects of the most common dam-pup separation paradigms (EH, MS, MPS) in conjunction with post-weaning housing (social- vs. isolation-housing); a gap in the literature which this study addresses. Additionally, an AFR control group was included, as this is the most common reference group used in these investigations.

The successive application of these manipulations permits for the evaluation of both the cumulative stress and the match/mismatch hypotheses. Specifically, if the match/mismatch

hypothesis is supported, then based on the existing maternal behavior data (Bailoo et al., 2013), it was predicted that MS/MPS subjects housed in social isolation post-weaning would show an increased susceptibility for neuropsychiatric dysfunction (operationally defined here as disruption of the startle response and prepulse inhibition of the startle response) while AFR/EH subjects housed in social isolation post-weaning would show a decreased susceptibility for neuropsychiatric dysfunction. Conversely, if the cumulative stress hypothesis is supported, AFR/EH subjects housed in social isolation post-weaning would show the greatest susceptibility for neuropsychiatric dysfunction.

Method

General Husbandry Procedures

Subjects were housed in 29 x 19 x 12 cm polypropylene cages on a 14:10 light/dark cycle with lights on at 14:00. Temperature was maintained at 21°C and humidity at 50 %. Subjects were provided with food and water *ad libitum*, nesting material, and Harlan Aspen Sani-Chips bedding approximately 1.3 cm deep. Weekly cage changes occurred between 14:00 and 15:00.

Breeding Subjects

Ten female and five male C57BL/6 mice were purchased from Harlan Laboratories, Frederick, MD, USA. Using a common breeding strategy, these ten females each produced three litters. The first two litters were used for training purposes with students and for piloting a behavioral test battery. Experimental subjects were produced by breeding different pairs of animals from the third litter of animals and their offspring onwards. Breeding for at least three generations was performed to reduce or remove experimental artifacts which may have arisen as

a consequence of differential rearing, husbandry, and the laboratory environment at Harlan Laboratories.

Experimental Subjects

Forty-four litters were bred across 11 cohorts and assigned via a pseudo-random manner to one of the four groups described below. Forty-one of these litters were primiparous. Assignment was such that there was always a cohort of litters representing each of the four groups at any given time. The average litter size was six, with a minimum of four and a maximum of eight offspring. One randomly selected male and female from each of the 44 litters was used in another study (Bailoo et al., 2013). The *remaining* offspring of these litters were used in this project (c.f., Table 1).

Insert Table 1 Here

Maternal Separation Procedures

Dam-offspring separations occurred from post-natal day (PND) 2 to 14 (day of birth was PND 0) and were performed by the same two experimenters. First, the dam was removed from the home-cage and placed into a clean cage with bedding. Then, pups were individually removed from the home-cage and placed into a clean cage with bedding. After pup removal, the dam was replaced into the home-cage for the duration of the separation.

MS pups were separated from the dam for 240 minutes (between 0900 and 1300), and MPS pups were separated from both the dam and their littermates for 240 minutes (between 0900 and 1300). Both MS and MPS pups were placed into a standard (29 x 19 x 12 cm) polypropylene cage. For the MPS group, frosted Plexiglas® partitions were placed within the cage to create eight separate compartments, one for each pup (Millstein, Ralph, Yang, &

Holmes, 2006). This partition eliminated tactile and visual interactions between littermates. Using infrared heating lamps, pup cages were maintained at 31°C (± 2°C) for the 240 minute separation groups (MS and MPS) in order to prevent thermoregulatory distress. Pups in the EH group were separated from the dam for 15 minutes in the same manner as the MS group (between 12:45 and 13:00) but were not placed under heating lamps. All separation procedures ended at 13:00, one hour before lights on.

For reunion, the dam was removed from the home-cage and temporarily placed into a clean cage with bedding (the same cage used previously). Then, the pups followed by the dam were replaced into the home-cage. An AFR control group was not separated from the dam but received the same weekly cage changes as the other three groups.

Weekly cage changes began when the pups were seven days old (PND 7). The dam was removed and placed in a clean cage with bedding. Some soiled bedding from the home-cage was sprinkled into a new cage and the nest from the home-cage was relocated (same side/area) to this new cage. Pups were then individually placed in the relocated nest. The dam was then placed in the new home-cage. This process took less than one minute. Regular cage changes occurred on PND 7 and 14 between 1400 and 1500 hours.

Maternal Behavior

The maternal behavior of the dams in each treatment group was characterized and has been detailed elsewhere (Bailoo et al., 2013). Briefly, maternal behaviors were recorded for one hour both before and after each separation period every other day from PND 2 to 14 during the dark phase under a 50 W infrared lamp using a closed-circuit camera connected to a high definition video recorder. All recordings for all groups occurred at the same time during the

light/dark cycle. Maternal behaviors were scored using Noldus Observer 5.1 on an ethogram of nursing postures and parental care behaviors adapted from Stern & Johnson (1989) and Shoji & Kato (2006).

Post-weaning Housing

Subjects were weaned on PND 21. Animals that were not used in the original study (Bailoo et al., 2013) were randomly allocated to either social-housing (2 - 3 subjects/cage with their same sex, same group siblings) or isolation-housing (1 subject/cage) for the duration of the experiment. When fewer than three extra animals per litter per sex were present, assignment to social-housing was given priority. Cage changes continued to occur once per week thereafter.

Sensorimotor Gating Procedures

Startle response was measured using the SR-LAB (San Diego Instruments, San Diego, CA, USA) startle response measurement system, including software (Paylor & Crawley, 1997). In this system, an acrylic cylinder (inner diameter 4 cm, length 13 cm) for holding the mouse was mounted on a Plexiglas® platform with a piezoelectric accelerometer unit attached below the acrylic cylinder. The piezoelectric unit transduced vibrations created by mouse body movements into signals that were rectified and stored by a microcomputer and then converted into a signal proportional to response amplitude. The acrylic cylinder and platform were located in a sound-attenuated chamber with a loudspeaker located 33 cm above the cylinder and house-light. Baseline values of startle between the two SR-LAB chambers used in this study were equated using the SR-LAB Standardization Unit at the onset of the experiment.

Subjects were tested individually by one of the original experimenters in one of the two chambers in a predefined pseudo-random manner between PND 60 - 70. Following a 5 minute

acclimation period in the cylinder, individual subjects were presented 50 trials over a 12.39 minute session. Each session consisted of five different trial types presented in pseudo-random order in 10 blocks. Three of the five trial types consisted of a 20 ms prepulse stimulus (72-, 76-, 84-dB white noise) presented so that the onset of the prepulse stimulus occurred 100 ms before the onset of the 40 ms, 120-dB white-noise startle stimulus. The fourth of the five trial types involved the presentation of the startle stimulus alone, and the fifth trial type was background only (65-dB) to establish baseline movement in the test chamber. The average inter-trial interval was 15 s (9 - 23 s range). The amplitude of the startle response was measured every 1 ms for 65 ms starting with the onset of the startle stimulus. When a startle stimulus (120-dB white noise on a 65-dB white noise background) follows a prepulse, the amplitude of the startle response is reduced, compared with its amplitude when the startle stimulus is presented without a prepulse. This amplitude reduction is called prepulse inhibition (our primary outcome variable) and is the percentage reduction of the mean startle amplitude for the prepulse trial expressed as the percentage reduction of the mean startle amplitude for startle-alone trial:

$$\left(\frac{\text{Mean Startle Amplitude (120-dB)} - \text{Mean Prepulse (either 76-, 80-, 84-dB)}}{\text{Mean Startle Amplitude (120-dB)}}\right) \times 100$$

. Secondary outcome variables included baseline startle response amplitude at 68-dB and acoustic startle response amplitude at 120-dB.

Statistical Analyses

All statistical analyses were performed with IBM SPSS Statistics (version 23) using the MIXED procedure. Assumptions of normality of error distribution, homogeneity of variance, and parameter linearity were examined graphically. No transformation of data was required based on

these inspections. Predictors used in all models were sex (male, female), pre-weaning group (AFR, EH, MS, MPS), post-weaning housing (socially-housed, isolation-housed), and decibel level (respectively 68-, 76-, 80-, 84-, 120-dB, at level 1 to account for repeated measurement). For all models built, 1) individual animals nested within litter, and 2) chambers were included as random effects to accommodate for dependencies in the experimental design. Inclusion of these random effects allowed us to partition the variation associated with each of these variables, and to obtain a treatment effect estimate that was independent of these variables. Subject weight was also included as a covariate (control factor) in the model, as the intensity of the startle response is affected by body weight (Blaszczyk & Tajchert, 1996). In all analyses, the full factorial model was the best model (based on ΔAIC and ΔBIC). P-values below 0.05 were considered statistically significant, and significant main effects and interactions were probed with Bonferroni corrected *post hoc* comparisons.

Results

Prepulse Inhibition of the Startle Response

A main effect of decibel level was observed, indicating that irrespective of sex, pre-weaning group, and post-weaning housing, as prepulse intensity increased, prepulse inhibition of the startle response also increased, ($F_{2,169}$) = 49.84, p = 0.00001 (Figure 1).

Insert Figure 1 here.

A main effect of sex was also observed, indicating that regardless of prepulse level, males displayed lower levels of prepulse inhibition of the startle response compared to females, ($F_{1,39}$) = 8.378, p = 0.006 (Figure 2).

Insert Figure 2 here.

Lastly, an interaction between pre-weaning treatment condition and post-weaning housing condition was observed, ($F_{1,39}$) = 8.378, p = 0.005 (Figure 3). *Post hoc* analyses comparing post-weaning housing condition within pre-weaning group indicated that socially-housed AFR subjects (M = 24.21, SE = 3.80) displayed lower levels of prepulse inhibition compared to isolation-housed AFR subjects (M = 39.72, SE = 3.08). The inverse pattern of results was observed between isolation-housed (M = 25.73, SE = 3.19) and socially-housed (M = 37.793, SE = 3.205) MPS subjects.

Insert Figure 3 here.

Baseline Startle Response Amplitude (68 dB)

A main effect of pre-weaning group, ($F_{3,225}$) = 4.750, p = 0.004, and an interaction between pre-weaning group and post-weaning housing was observed, ($F_{3,225}$) = 3.101, p = 0.031. *Post hoc* analyses comparing post-weaning housing conditions within pre-weaning group yielded no significant differences between any of our groups. However, *post hoc* analyses comparing pre-weaning group within post-weaning housing condition indicated that isolation-housed MPS subjects (M = 32.25, SE = 2.86) displayed a significantly higher baseline startle response amplitude compared to isolation-housed AFR subjects (M = 15.73, SE = 3.24) (Figure 4). While isolation-housed MS subjects also displayed higher levels of baseline startle response amplitude relative to isolation-housed AFR subjects (*Mean Difference* = 10.70), this difference failed to reach statistical significance (p = 0.055).

Insert Figure 4 here.

Startle Response Amplitude (120 dB)

A main effect of sex, ($F_{1,133}$) = 12.20, p = 0.001, was observed, indicating that male mice displayed a higher acoustic startle response amplitude, (M = 382.13, SE = 20.46), than female mice, (M = 285.44, SE = 18.64) (Figure 5).

Insert Figure 5 here.

A main effect of post-weaning housing was also observed, ($F_{1,133}$) = 3.926, p = 0.049, indicating that isolation-housed subjects displayed a lower amplitude of the acoustic startle response, (M = 306.36, SE = 2.49), relative to socially-housed animals, (M = 361.21, SE = 16.14) (Figure 6).

Insert Figure 6 here.

Discussion

The overall aim of this study was to assess the additive (cumulative stress hypothesis) or interactive effects (match/mismatch hypothesis) of early-life experiences in the form of dam-offspring separation and subsequent post-weaning social housing on the manifestation of adult prepulse inhibition using the C57BL/6 inbred mouse. Analysis of the primary outcome variable, prepulse inhibition of the acoustic startle response, provided direct evidence for the match/mismatch hypothesis. Specifically, isolation-housed MPS subjects displayed a deficit in prepulse inhibition relative to socially-housed MPS subjects while socially-housed AFR subjects displayed a deficit in prepulse inhibition relative to isolation-housed AFR subjects.

Maternal Behavior during the pre-weaning phase

In the earlier experiment (Bailoo et al., 2013), we reported that the effects of the pre-weaning manipulations were restricted to the reunion phase with the dam, with an overall

increase in maternal behavior for the longer separated groups (MS & MPS). Moreover, for the MS/MPS groups, the homeostatic balance of the pups was maintained using heat lamps (31 ± 2°C), no indication of food deprivation was present, and correspondingly, higher weaning weights were observed. Thus, subjects in the MS/MPS groups experienced a better outcome, at least in regards to levels of maternal care, as a consequence of these manipulations relative to AFR/EH groups.

Effects of pre-weaning group and post-weaning housing on prepulse inhibition

Isolation-housed AFR subjects displayed higher levels of prepulse inhibition relative to socially-housed AFR subjects, with the opposite relation observed for MPS subjects; a result that is consistent with the match/mismatch hypothesis. This result is most likely because AFR subjects received lower levels of maternal care pre-weaning when compared to MPS groups, which has been associated with a stressful early environment and poorer adult outcomes in rodents (Champagne et al., 2008).Thus, in this study, AFR subjects experienced a "match" when housed in isolation while MPS subjects experienced a "mismatch" when housed in the same manner.

A main effect of decibel level was observed, indicating that as the intensity of the prepulse increased, inhibition of the startle response correspondingly increased. This result demonstrated that the prepulse inhibition experimental procedure used in this study was effective.

A main effect of sex was also observed, with males displaying lower levels of prepulse inhibition relative to females, after correcting for body weight. This result was surprising given that the literature supports the contention of a sex difference, but in the opposite direction (Braff

et al., 2001). However, in many of the studies investigating or reporting sex differences, prepulse inhibition of startle is generally confounded by body weight (Blaszczyk & Tajchert, 1996). Specifically, male rodents generally weigh more than females, have greater muscle mass and associated motor strength, and relatedly, display a greater startle response and a deficiency in the ability to display prepulse inhibition. In the few studies that we are aware of that statistically corrected for this sex/weight correlation, this difference disappears or at least is less clear in regards to the direction of this effect (e.g., Blaszczyk & Tajchert, 1996). Moreover, it is important to note that this purported sex difference can be modulated by several other factors including, for example, female hormonal state (c.f., Braff et al., 2001, for review). Thus, explanation for this difference remains speculative at best and further work replicating this effect and detailing the neurobiological mechanism is needed.

Effects of pre-weaning group and post-weaning housing on baseline startle (68-dB)

A significant interaction between pre-weaning group and post-weaning housing was observed. However, probing this interaction in relation to our experimental question by comparing the effects of either social- or isolation-housing within pre-weaning treatment groups yielded no significant differences.

Effects of pre-weaning group and post-weaning housing on the startle response (120 dB)

An effect of post-weaning housing condition on acoustic startle response amplitude was observed, with isolation-housed subjects displaying lower levels of startle relative to socially-housed subjects. While this result is generally consistent with the literature, it should be noted that Geyer and colleagues, in a systematic review (2001), have stated that while some studies report an increase in acoustic startle response amplitude as a consequence of isolation-housing,

others report no or the opposite effect. Therefore, acoustic startle response amplitude seems to be the least predictive of neuropsychiatric dysfunction, at least in regards to whether corresponding deficits in prepulse inhibition are observed (Varty, Braff, & Geyer, 1999; Varty & Geyer, 1998). This may also be true of the data in our study, with deficits in startle responding observed between the isolation- and socially-housed groups, but not in relation to pre-weaning treatment conditions.

A main effect of sex on acoustic startle response amplitude was observed, with male mice displaying a greater startle response than females. However, as noted above, further experimental work is needed to replicate and delineate this effect.

Limitations

It is important to note that this study made use of "extra" animals from litters that had been produced for use in a different study (Bailoo et al., 2013), and thus a fully balanced design was not achieved. However, with the exception of the isolation-housed EH group, and given the relatively large observed effect sizes, it may be argued that this experiment was sufficiently powered and that these data are reliable. Moreover, given that the literature suggests that the AFR condition is generally similar in phenotype to the EH condition, and that the maternal care data recorded in this study supports this "homology", it can be speculated that the observed differences with the isolation-housed AFR group are also applicable to the isolation-housed EH group (Levine, 2002).

The pre-weaning manipulations and their effects on maternal care were described previously (Bailoo et al., 2013). In those data, many aspects of maternal care were elevated but those differences were restricted to the reunion phase in the longer separated groups (MS and

MPS). It was hypothesized that since maternal care has been shown to mediate the relation between these early experience paradigms and later offspring outcome, these groups would exhibit the smallest deficits in prepulse inhibition. However, in this study, we observed this relation only in the MPS and not in the MS group.

Several factors may account for the lack of an observed effect in our MS group. While it is presumed that maternal care mediates the relation of pre-weaning separations to adult phenotypes, including prepulse inhibition, perhaps other unmeasured factors might also affect this relation (c.f., Macrì & Würbel, 2006). For example, systematic work evaluating food deprivation, thermoregulation, ultrasonic vocalization production, and behavioral changes and adaptations *by the pups* (and their influence on the dam) as a consequence of these pre-weaning manipulations remains largely unexamined. Without systematically acquiring such information, it is unknown whether MS and MPS groups are equivalent. Only the levels of maternal care exhibited to the pups upon reunion are similar. Future studies characterizing the differences between the MS and MPS groups are therefore needed.

Conclusion

Deficits in prepulse inhibition are noted in humans with symptoms of neuropsychiatric dysfunction such as schizophrenia, obsessive compulsive disorder, and attention deficit hyperactivity disorder (Braff et al., 2001). Considerable evidence supports a high degree of similarity between measures of prepulse inhibition in rodents and humans (e.g., Braff et al., 2001; Ellenbroek, Geyer, & Cools, 1995). Moreover, prepulse inhibition appears to be highly conserved among vertebrates and is one of the few paradigms in which humans and animals are tested in a similar fashion. Thus, investigation into the disruption of prepulse inhibition as a

consequence of early experiences associated with an increased susceptibility for neuropsychiatric dysfunction is well suited to rodent models (Swerdlow, Weber, Qu, Light, & Braff, 2008).

This study was designed to investigate the additive effects of typical stress-related manipulations applied at two different developmental periods, and extends previous work employing these manipulations. Analysis of the primary outcome variable of this study, prepulse inhibition, provides support for the match/mismatch hypothesis. Generally speaking, isolation-housing should lead to deficits in prepulse inhibition. However, in this study, AFR subjects that experienced the lowest levels of maternal care displayed deficits in prepulse inhibition when housed socially compared to isolation. Conversely, MPS subjects that experienced high levels of maternal care and were later housed in isolation displayed greater deficits in prepulse inhibition compared to MPS subjects housed socially.

Future studies employing these early experience paradigms consecutively in the evaluation of adult prepulse inhibition should benefit from our results. Specifically, if isolation-housing is used, then robust differences can be observed simply between the AFR control groups, with the noteworthy difference being that social housing leads to deficits in prepulse inhibition relative to isolation-housing, at least in C57BL/6 mice. If the intention is to analogously model the match/mismatch hypothesis (also termed "differential susceptibility" in human research), then both the AFR and the MPS groups in social- and isolation-housing, respectively, can be used to model this relation.

Notes

The authors gratefully acknowledge the assistance of Dr. Amber N. Tyler, Dr. Douglas L. Wahlsten, Dr. Walter L. Salinger, Dr. Amrika Deonarine, Dr. Claudio L. Ferre, Dr. Emily C. Marcinowski, Dedrick Curtis, Amethyst Royal, Christina Wade-Giampanis, and Dr. Bernhard Völkl for data collection, helpful comments, and discussion in the preparation of this manuscript. Observations with and manipulations of our subjects were made in accordance with the Institutional Care and Use Committee (IACUC) guidelines at the University of North Carolina at the Greensboro, North Carolina, USA.

References

Bailoo, J. D., Jordan, R. L., Garza, X. J., & Tyler, A. N. (2013). Brief and long periods of maternal separation affect maternal behavior and offspring behavioral development in C57BL/6 mice. Developmental Psychobiology, 56(4), 674–685. http://doi.org/10.1002/dev.21135

Blaszczyk, J., & Tajchert, K. (1996). Sex and strain differences of acoustic startle reaction development in adolescent albino Wistar and hooded rats. Acta Neurobiologiae Experimentalis, 56(4), 919–925. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9033127

Braff, D. L., Geyer, M. A., & Swerdlow, N. R. (2001). Human studies of prepulse inhibition of startle: Normal subjects, patient groups, and pharmacological studies. Psychopharmacology, 156(2-3), 234–258. http://doi.org/10.1007/s002130100810

Branchi, I., & Cirulli, F. (2014). Early experiences: building up the tools to face the challenges of adult life. Developmental Psychobiology, 56(8), 1661–1674. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24986379

Caldji, C., Francis, D., Sharma, S., Plotsky, P. M., & Meaney, M. J. (2000). The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology, 22(3), 219–29. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10693149

Champagne, D. L., Bagot, R. C., van Hasselt, F., Ramakers, G., Meaney, M. J., de Kloet, E. R., … Krugers, H. (2008). Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 28(23), 6037–45. http://doi.org/10.1523/JNEUROSCI.0526-08.2008

de Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. Nature Reviews. Neuroscience, 6(6), 463–75. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15891777

Ellenbroek, B. A., Geyer, M. A., & Cools, A. R. (1995). The behavior of APO-SUS rats in animal models with construct validity for schizophrenia. The Journal of Neuroscience, 15(11), 7604–7611.

Geyer, M. A., Krebs-Thomson, K., Braff, D. L., & Swerdlow, N. R. (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: A decade in review. Psychopharmacology (Vol. 156). http://doi.org/10.1007/s002130100811

Ison, J. R., & Hoffman, H. S. (1983). Reflex modification in the domain of startle: II. The anomalous history of a robust and ubiquitous phenomenon. Psychological Bulletin, 94(1), 3–17. http://doi.org/10.1037/0033-2909.94.1.3

Kaffman, A., & Meaney, M. J. (2007). Neurodevelopmental sequelae of postnatal maternal care in rodents: Clinical and research implications of molecular insights. Journal of Child Psychology and Psychiatry and Allied Disciplines, 48(3-4), 224–244. http://doi.org/10.1111/j.1469-7610.2007.01730.x

Lehmann, J., & Feldon, J. (2000). Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? Reviews in the Neurosciences, 11(4), 383–408. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11065281

Levine, S. (2002). Enduring Effects of Early Experience on Adult Behavior. In D. W. Plaff, A. P. Arnold, A. M. Etgen, S. E. Fahrbach, & R. T. Rubin (Eds.), Hormones, Brain and Behavior, Volume 4 (pp. 535–542). New York, NY: Academic Press. Retrieved from https://books.google.com/books?id=6GgHpQdk8vYC&pgis=1

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., … Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science (New York, N.Y.), 277(5332), 1659–62. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9287218

Macrí, S., Mason, G. J., & Würbel, H. (2004). Dissociation in the effects of neonatal maternal separations on maternal care and the offspring's HPA and fear responses in rats. The European Journal of Neuroscience, 20(4), 1017–1024. http://doi.org/10.1111/j.1460-9568.2004.03541.x

Macrì, S., & Würbel, H. (2006). Developmental plasticity of HPA and fear responses in rats: a critical review of the maternal mediation hypothesis. Hormones and Behavior, 50(5), 667–680. http://doi.org/10.1016/j.yhbeh.2006.06.015

Matsumoto, Y., Niwa, M., Mouri, A., Ozaki, N., Nabeshima, T., Matsumoto, Y., … Nabeshima, T. (2011). Vulnerability in early life to changes in the rearing environment plays a crucial role in the aetiopathology of psychiatric disorders. Japanese Journal of Neuropsychopharmacology, 14(4), 459–477. http://doi.org/10.1017/S1461145710001239

McEwen, B. S. (2003). Mood disorders and allostatic load. Biological Psychiatry, 54(3), 200–207. Retrieved from http://www.sciencedirect.com/science/article/pii/S000632230300177X

Meaney, M. J. (2001). Maternal Care, Gene Expression, and the Transmission of Individual Differences in Stress Reactivity across Generations. Annual Review of Neuroscience, 24, 1161–1192.

Millstein, R. A., & Holmes, A. (2007). Effects of repeated maternal separation on anxiety- and depression-related phenotypes in different mouse strains. Neuroscience and Biobehavioral Reviews, 31(1), 3–17. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16950513

Millstein, R. A., Ralph, R. J., Yang, R. J., & Holmes, A. (2006). Effects of repeated maternal separation on prepulse inhibition of startle across inbred mouse strains. Genes, Brain, and Behavior, 5(4), 346–354. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16716204

Paylor, R., & Crawley, J. N. (1997). Inbred strain differences in prepulse inhibition of the mouse startle response. Psychopharmacology, 132(2), 169–180. Retrieved from

http://www.ncbi.nlm.nih.gov/pubmed/9266614

Pryce, C. R., Rüedi-Bettschen, D., Dettling, A. C., & Feldon, J. (2002). Early life stress: long-term physiological impact in rodents and primates. News in Physiological Sciences, 17, 150–155. http://doi.org/DOI 10.1152/nips.01367.2001

Schmidt, M. V. (2011). Animal models for depression and the mismatch hypothesis of disease. Psychoneuroendocrinology, 36(3), 330–338. Retrieved from http://www.sciencedirect.com/science/article/pii/S0306453010001551

Shoji, H., & Kato, K. (2006). Maternal behavior of primiparous females in inbred strains of mice: a detailed descriptive analysis. Physiology & Behavior, 89(3), 320–328. Retrieved from http://www.sciencedirect.com/science/article/pii/S0031938406002691

Smotherman, W. P., & Bell, R. (1980). Maternal mediation of early experience. In R. W. Bell & W. P. Smotherman (Eds.), Maternal Influences and Early Behavior (First, pp. 201–210). Spectrum Publications. Retrieved from https://books.google.com/books?id=IgmpNzaT3NkC&pgis=1

Stern, J. M., & Johnson, S. K. (1989). Perioral somatosensory determinants of nursing behavior in Norway rats (Rattus norvegicus). Journal of Comparative Psychology (Washington, D.C. : 1983), 103(3), 269–80. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2776423

Swerdlow, N. R., Weber, M., Qu, Y., Light, G. A., & Braff, D. L. (2008). Realistic expectations of prepulse inhibition in translational models for schizophrenia research. Psychopharmacology (Vol. 199). http://doi.org/10.1007/s00213-008-1072-4

Varty, G. B., Braff, D. L., & Geyer, M. A. (1999). Is there a critical developmental `window' for isolation rearing-induced changes in prepulse inhibition of the acoustic startle response? Behavioural Brain Research, 100(1-2), 177–183.

Varty, G. B., & Geyer, M. A. (1998). Effects of isolation rearing on startle reactivity, habituation, and prepulse inhibition in male Lewis, Sprague-Dawley, and Fischer F344 rats. Behavioral Neuroscience, 112(6), 1450–1457. http://doi.org/10.1037/0735-7044.112.6.1450

Weiss, I. C., Domeney, A. M., Moreau, J. L., Russig, H., & Feldon, J. (2001). Dissociation between the effects of pre-weaning and/or post-weaning social isolation on prepulse inhibition and latent inhibition in adult Sprague-Dawley rats. Behavioural Brain Research, 121(1-2), 207–218. http://doi.org/10.1016/S0166-4328(01)00166-8

Tables

Table 1

Total number of subjects used divided by pre-weaning group and post-weaning housing condition.

	Socially-housed		Isolation-housed		Total
	Male	**Female**	**Male**	**Female**	
AFR	9	12	5	10	36
EH	11	13	3	3	30
MS	11	11	14	10	46
MPS	11	12	6	11	40
Total	42	48	28	34	152

Figures

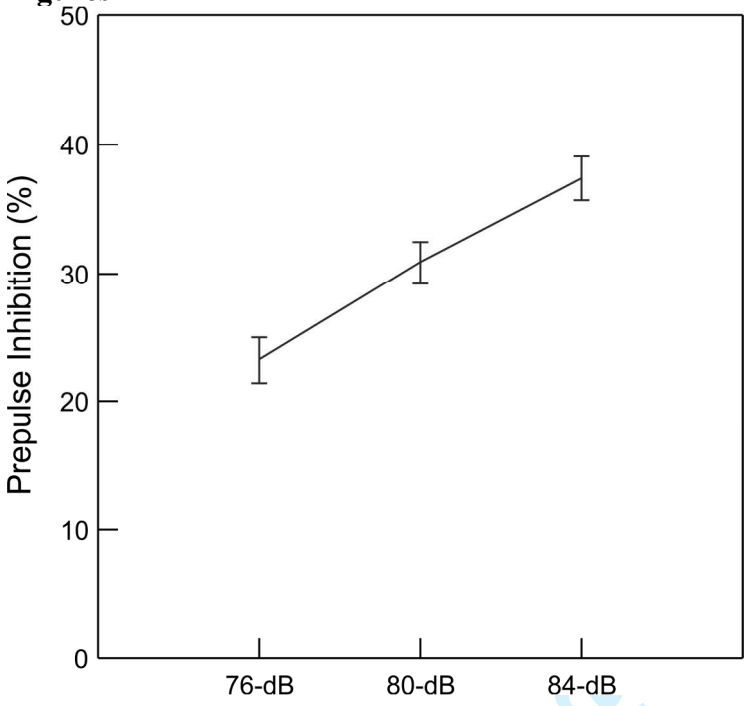

Figure 1. Differences in prepulse inhibition (%) as a consequence of prepulse level (dB).

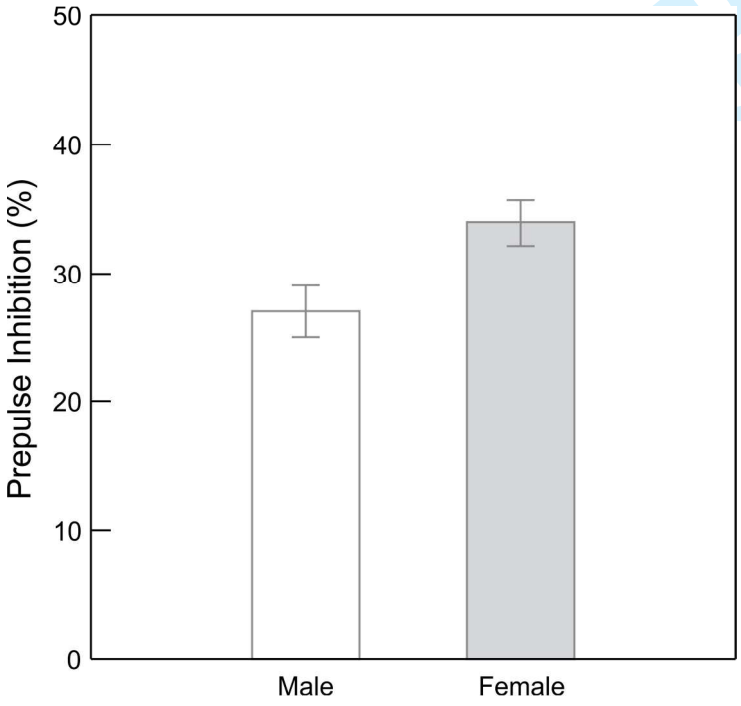

Figure 2. Differences in prepulse inhibition (%) as a consequence of sex.

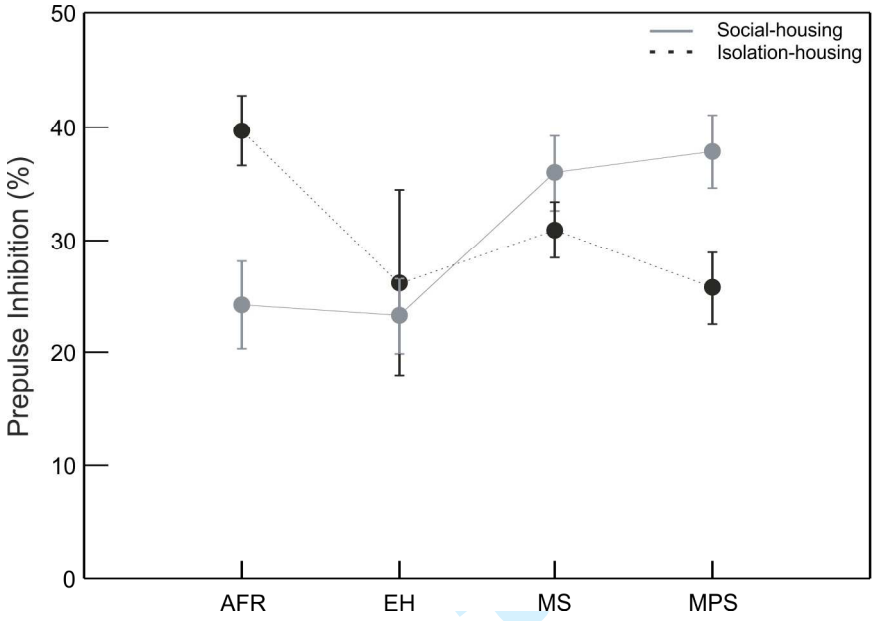

Figure 3. Differences in prepulse inhibition (%) as a consequence of pre-weaning group and post-weaning housing condition.

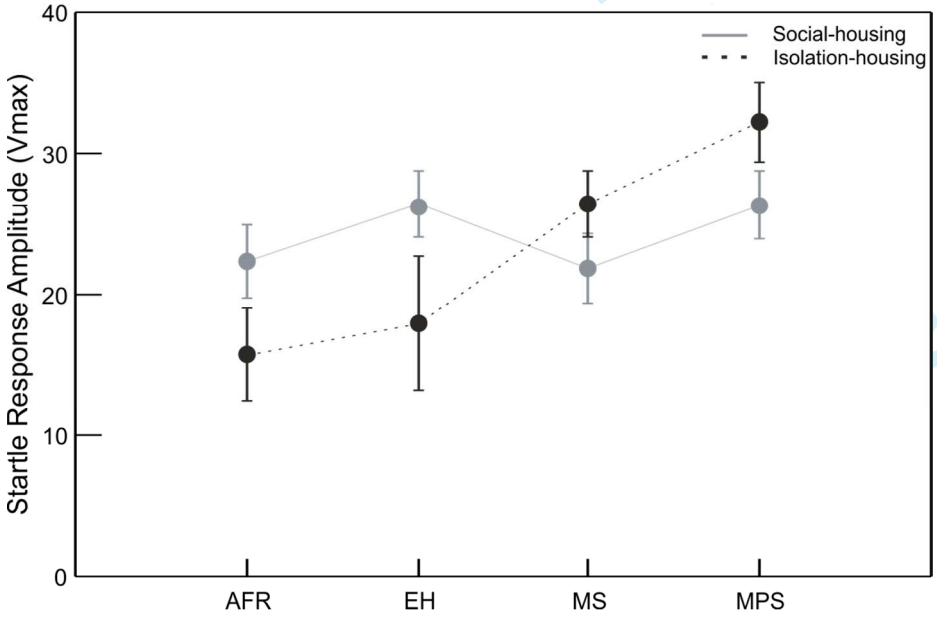

Figure 4. Differences in baseline startle amplitude (68-dB) as a consequence of pre-weaning group and post-weaning housing condition.

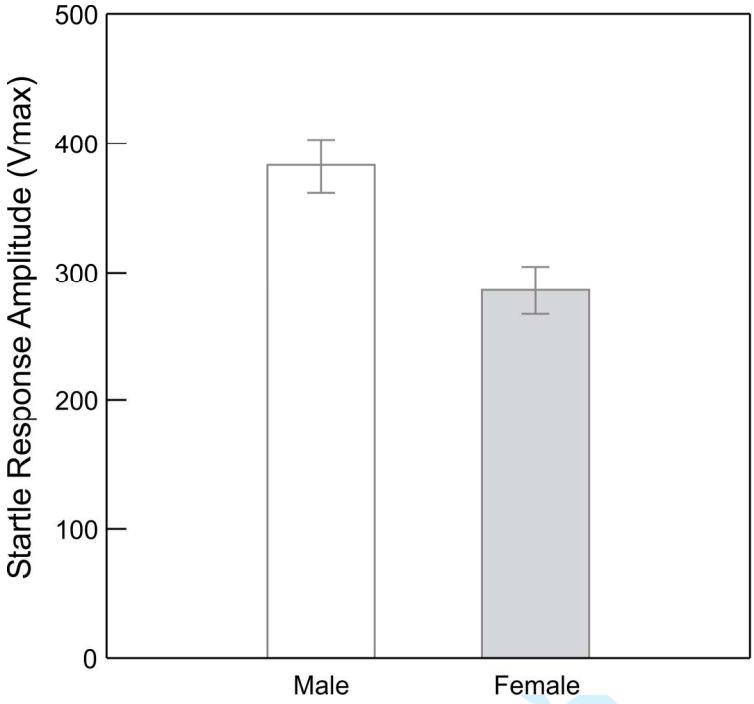

Figure 5. Differences in acoustic startle amplitude (120-dB) as a consequence of sex.

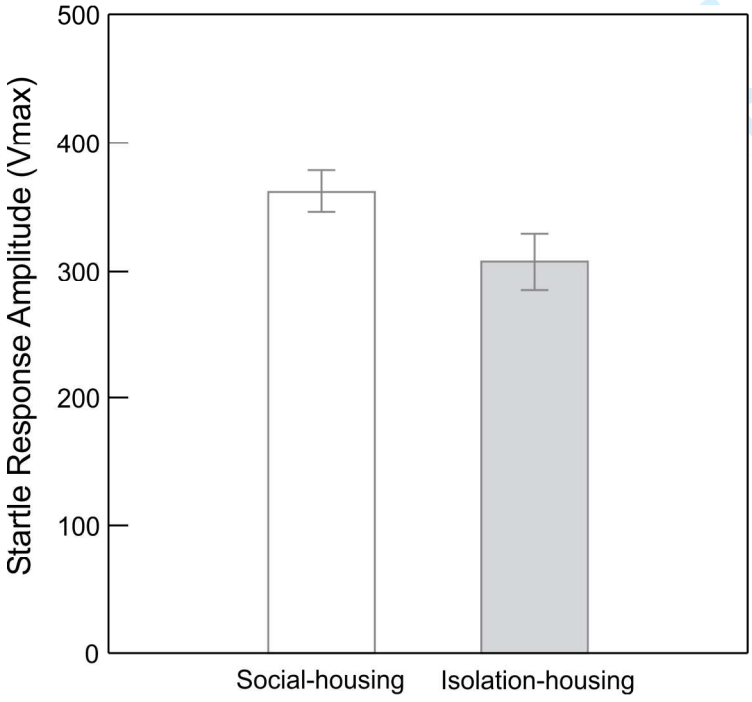

Figure 6. Differences in acoustic startle amplitude (120-dB) as a consequence of post-weaning housing condition.