



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

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41 17 Short title: MATERNAL SEPARATION AFFECTS PREPULSE INHIBITION
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3 25 The current study examined the effect of early-life stress in C57BL/6 offspring reared
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6 26 under four conditions: typical animal facility rearing (AFR, Control), early handling (EH, daily
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8 27 15 min. separation from dam), maternal separation (MS, daily 4 hr. separation from dam), and
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10 28 maternal and peer separation (MPS, daily 4 hr. separation from dam and from littermates). After
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12
13 29 weaning, mice across these four conditions were either housed socially (2 - 3/cage) or in
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15 30 isolation (1/cage) and then tested for prepulse inhibition in adulthood. Isolation-housed MPS
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17 31 subjects displayed greater deficits in prepulse inhibition relative to socially-housed MPS subjects
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20 32 while socially-housed AFR subjects displayed greater deficits in prepulse inhibition relative to
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22 33 isolation-housed AFR subjects. The results indicate that these treatment conditions represent a
23
24 34 potentially valuable model for evaluating the match/mismatch hypothesis in regards to
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27 35 neuropsychiatric dysfunction.
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32 37 **Keywords:** maternal separation, early handling, match/mismatch hypothesis, isolation-housing,
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34 38 prepulse inhibition
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6 39 Exposure to chronic stress during development has been associated with an increased risk
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9 40 for neuropsychiatric dysfunction in later life (de Kloet, Joëls, & Holsboer, 2005). Two primary
10
11 41 and seemingly contradictory hypotheses have been used in the evaluation of such risk; the
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13 42 cumulative stress hypothesis and the match/mismatch hypothesis. The more traditional of these
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15 43 hypotheses, the cumulative stress hypothesis, states that exposure to consecutive stressors across
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17 44 development increases allostatic load, vulnerability to aversive challenges, and susceptibility to
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19 45 neuropsychiatric dysfunction in later life (McEwen, 2003). Conversely, the match/mismatch
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21 46 hypothesis states that an individual who has experienced high levels of stress early in
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23 47 development is better able to cope with stressors later in life compared to an individual who has
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25 48 experienced no or low levels of early-life stress, and therefore, is at a decreased risk for
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27 49 neuropsychiatric dysfunction (Schmidt, 2011).
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32 50 Rodents have a long history of use in modeling such neuropsychiatric risks (Pryce,
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34 51 Rüedi-Bettschen, Dettling, & Feldon, 2002) and although data from such models are not always
35
36 52 consistent (Lehmann & Feldon, 2000), one model that is generally thought to be predictive of
37
38 53 vulnerable phenotypes is maternal separation (Branchi & Cirulli, 2014). Brief periods (~15
39
40 54 minutes) of dam-pup separation (i.e., early handling, EH) may lead to offspring exhibiting
41
42 55 decreased reactivity of the hypothalamic pituitary adrenal (HPA) axis to stress-inducing
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44 56 situations in later adult life (c.f., Kaffman & Meaney, 2007). However, this result is only
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46 57 observed if the comparison group is not handled until weaning (Levine, 2002). If the comparison
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48 58 group is a typical animal facility reared (AFR) group, no differences in stress reactivity are
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51 59 observed between these groups (Levine, 2002).
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6 60 Longer periods of dam-pup separation (between 3 - 6 hours), conversely, produce an
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8 61 exaggerated HPA axis response to a stressor in later adult life (Meaney, 2001). The two most
9
10 62 common forms of these longer periods of separation are maternal separation (MS) of the dam
11
12 63 from the pups, and maternal and peer separation (MPS) of the dam from the pups in addition to
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14 64 the littermates from one another. The effects of MS/MPS on HPA axis reactivity to stressors are
15
16 65 not consistent throughout the literature. Such inconsistencies are thought to be a consequence of
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18 66 methodological differences between laboratories, including but not limited to the timing,
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20 67 duration, and number of MS/MPS episodes (for review, c.f., Millstein & Holmes, 2007).
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25 68 Both EH and MS/MPS lead to increases in maternal care for approximately 1 - 2 hours
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27 69 after reuniting the pups with the dam (Liu et al., 1997; Macrí, Mason, & Würbel, 2004). It is this
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29 70 increase in maternal behavior that has traditionally been associated with the series of
30
31 71 downstream effects that mediate the response of the pups to stressors as adults (Meaney, 2001;
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33 72 Smotherman & Bell, 1980), although some have challenged this premise (Macrí & Würbel,
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35 73 2006).
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39 74 To date, only one study has included and systematically compared the effects of these
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41 75 three most common treatment conditions (EH, MS, MPS) on maternal care and adult offspring
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43 76 behavior in a single experiment (Bailoo, Jordan, Garza, & Tyler, 2013). In this study, we
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45 77 demonstrated that one consequence of MS/MPS is an increase in maternal care in the immediate
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47 78 reunion phase relative to EH/AFR groups. Thus, while MS/MPS groups are generally thought to
48
49 79 be associated with poorer outcomes because the longer periods of separation deprive the pups of
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51 80 maternal care (Caldji, Francis, Sharma, Plotsky, & Meaney, 2000), the results of our original
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53 81 study suggested that groups receiving the highest levels of maternal care were largely comprised
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6 82 of the MS/MPS groups and displayed decreased “anxiety-like behavior” in an open field
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8 83 compared to groups that received lower levels of maternal care (largely comprised of AFR/EH
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10 84 groups).

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12
13 85 In the current study, we extended the results of our previous work by investigating the
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15 86 development of an endophenotype related to neuropsychiatric dysfunction, prepulse inhibition of
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17 87 the acoustic startle response (Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001). Prepulse
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19 88 inhibition of the startle response is a neurological phenomenon, in which a weaker sensory
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21 89 stimulus inhibits the reaction of an organism to a subsequent strong and typically startling
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23 90 stimulus (a.k.a., sensorimotor gating) (Ison & Hoffman, 1983). Disruption of prepulse inhibition
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25 91 is noted in humans with symptoms of neuropsychiatric dysfunction and has previously been
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27 92 modeled in mice using manipulations occurring at two points in development, pre-weaning
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29 93 (maternal separation) and post-weaning (isolation-housing) (for review, c.f., Braff, Geyer, &
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31 94 Swerdlow, 2001).

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36 95 Some studies have hypothesized that the application of both paradigms successively may
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38 96 lead to potentiated deficits in prepulse inhibition in later adult life (Matsumoto et al., 2011;
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40 97 Weiss, Domeney, Moreau, Russig, & Feldon, 2001). However, no study to date has evaluated the
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42 98 additive or interactive effects of the most common dam-pup separation paradigms (EH, MS,
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44 99 MPS) in conjunction with post-weaning housing (social- vs. isolation-housing); a gap in the
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46 100 literature which this study addresses. Additionally, an AFR control group was included, as this is
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48 101 the most common reference group used in these investigations.
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53 102 The successive application of these manipulations permits for the evaluation of both the
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55 103 cumulative stress and the match/mismatch hypotheses. Specifically, if the match/mismatch
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6 104 hypothesis is supported, then based on the existing maternal behavior data (Bailoo et al., 2013),
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8 105 it was predicted that MS/MPS subjects housed in social isolation post-weaning would show an
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10 106 increased susceptibility for neuropsychiatric dysfunction (operationally defined here as
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12 107 disruption of the startle response and prepulse inhibition of the startle response) while AFR/EH
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14 108 subjects housed in social isolation post-weaning would show a decreased susceptibility for
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16 109 neuropsychiatric dysfunction. Conversely, if the cumulative stress hypothesis is supported,
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18 110 AFR/EH subjects housed in social isolation post-weaning would show the greatest susceptibility
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20 111 for neuropsychiatric dysfunction.
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27 113 **Method**

28 114 ***General Husbandry Procedures***

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32 115 Subjects were housed in 29 x 19 x 12 cm polypropylene cages on a 14:10 light/dark cycle
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34 116 with lights on at 14:00. Temperature was maintained at 21°C and humidity at 50 %. Subjects
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36 117 were provided with food and water *ad libitum*, nesting material, and Harlan Aspen Sani-Chips
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38 118 bedding approximately 1.3 cm deep. Weekly cage changes occurred between 14:00 and 15:00.

39 119 ***Breeding Subjects***

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44 120 Ten female and five male C57BL/6 mice were purchased from Harlan Laboratories,
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46 121 Frederick, MD, USA. Using a common breeding strategy, these ten females each produced three
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48 122 litters. The first two litters were used for training purposes with students and for piloting a
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50 123 behavioral test battery. Experimental subjects were produced by breeding different pairs of
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52 124 animals from the third litter of animals and their offspring onwards. Breeding for at least three
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54 125 generations was performed to reduce or remove experimental artifacts which may have arisen as
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6 126 a consequence of differential rearing, husbandry, and the laboratory environment at Harlan
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8 127 Laboratories.

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10 128 ***Experimental Subjects***

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13 129 Forty-four litters were bred across 11 cohorts and assigned via a pseudo-random manner
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15 130 to one of the four groups described below. Forty-one of these litters were primiparous.
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17 131 Assignment was such that there was always a cohort of litters representing each of the four
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19 132 groups at any given time. The average litter size was six, with a minimum of four and a
20
21 133 maximum of eight offspring. One randomly selected male and female from each of the 44 litters
22
23 134 was used in another study (Bailoo et al., 2013). The *remaining* offspring of these litters were
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25 135 used in this project (c.f., Table 1).
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30 136 **Insert Table 1 Here**

31
32 137 ***Maternal Separation Procedures***

33
34 138 Dam-offspring separations occurred from post-natal day (PND) 2 to 14 (day of birth was
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36 139 PND 0) and were performed by the same two experimenters. First, the dam was removed from
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38 140 the home-cage and placed into a clean cage with bedding. Then, pups were individually removed
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40 141 from the home-cage and placed into a clean cage with bedding. After pup removal, the dam was
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42 142 replaced into the home-cage for the duration of the separation.
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46 143 MS pups were separated from the dam for 240 minutes (between 0900 and 1300), and
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48 144 MPS pups were separated from both the dam and their littermates for 240 minutes (between
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50 145 0900 and 1300). Both MS and MPS pups were placed into a standard (29 x 19 x 12 cm)
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52 146 polypropylene cage. For the MPS group, frosted Plexiglas[®] partitions were placed within the
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55 147 cage to create eight separate compartments, one for each pup (Millstein, Ralph, Yang, &
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6 148 Holmes, 2006). This partition eliminated tactile and visual interactions between littermates.
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8 149 Using infrared heating lamps, pup cages were maintained at 31°C (\pm 2°C) for the 240 minute
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10 150 separation groups (MS and MPS) in order to prevent thermoregulatory distress. Pups in the EH
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12 151 group were separated from the dam for 15 minutes in the same manner as the MS group
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15 152 (between 12:45 and 13:00) but were not placed under heating lamps. All separation procedures
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17 153 ended at 13:00, one hour before lights on.
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20 154 For reunion, the dam was removed from the home-cage and temporarily placed into a
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22 155 clean cage with bedding (the same cage used previously). Then, the pups followed by the dam
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24 156 were replaced into the home-cage. An AFR control group was not separated from the dam but
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26 157 received the same weekly cage changes as the other three groups.
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29 158 Weekly cage changes began when the pups were seven days old (PND 7). The dam was
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31 159 removed and placed in a clean cage with bedding. Some soiled bedding from the home-cage was
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33 160 sprinkled into a new cage and the nest from the home-cage was relocated (same side/area) to this
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35 161 new cage. Pups were then individually placed in the relocated nest. The dam was then placed in
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37 162 the new home-cage. This process took less than one minute. Regular cage changes occurred on
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39 163 PND 7 and 14 between 1400 and 1500 hours.
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44 164 ***Maternal Behavior***

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46 165 The maternal behavior of the dams in each treatment group was characterized and has
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48 166 been detailed elsewhere (Bailoo et al., 2013). Briefly, maternal behaviors were recorded for one
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50 167 hour both before and after each separation period every other day from PND 2 to 14 during the
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52 168 dark phase under a 50 W infrared lamp using a closed-circuit camera connected to a high
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54 169 definition video recorder. All recordings for all groups occurred at the same time during the
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6 170 light/dark cycle. Maternal behaviors were scored using Noldus Observer 5.1 on an ethogram of
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8 171 nursing postures and parental care behaviors adapted from Stern & Johnson (1989) and Shoji &
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10 172 Kato (2006).

13 173 *Post-weaning Housing*

15 174 Subjects were weaned on PND 21. Animals that were not used in the original study
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17 175 (Bailoo et al., 2013) were randomly allocated to either social-housing (2 - 3 subjects/cage with
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19 176 their same sex, same group siblings) or isolation-housing (1 subject/cage) for the duration of the
20
21 177 experiment. When fewer than three extra animals per litter per sex were present, assignment to
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23 178 social-housing was given priority. Cage changes continued to occur once per week thereafter.

27 179 *Sensorimotor Gating Procedures*

29 180 Startle response was measured using the SR-LAB (San Diego Instruments, San Diego,
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31 181 CA, USA) startle response measurement system, including software (Paylor & Crawley, 1997).
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33 182 In this system, an acrylic cylinder (inner diameter 4 cm, length 13 cm) for holding the mouse
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35 183 was mounted on a Plexiglas[®] platform with a piezoelectric accelerometer unit attached below the
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37 184 acrylic cylinder. The piezoelectric unit transduced vibrations created by mouse body movements
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39 185 into signals that were rectified and stored by a microcomputer and then converted into a signal
40
41 186 proportional to response amplitude. The acrylic cylinder and platform were located in a sound-
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43 187 attenuated chamber with a loudspeaker located 33 cm above the cylinder and house-light.
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45 188 Baseline values of startle between the two SR-LAB chambers used in this study were equated
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47 189 using the SR-LAB Standardization Unit at the onset of the experiment.

51 190 Subjects were tested individually by one of the original experimenters in one of the two
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53 191 chambers in a predefined pseudo-random manner between PND 60 - 70. Following a 5 minute
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6 192 acclimation period in the cylinder, individual subjects were presented 50 trials over a 12.39
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8 193 minute session. Each session consisted of five different trial types presented in pseudo-random
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10 194 order in 10 blocks. Three of the five trial types consisted of a 20 ms prepulse stimulus (72-, 76-,
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12 195 84-dB white noise) presented so that the onset of the prepulse stimulus occurred 100 ms before
13
14 196 the onset of the 40 ms, 120-dB white-noise startle stimulus. The fourth of the five trial types
15
16 197 involved the presentation of the startle stimulus alone, and the fifth trial type was background
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18 198 only (65-dB) to establish baseline movement in the test chamber. The average inter-trial interval
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20 199 was 15 s (9 - 23 s range). The amplitude of the startle response was measured every 1 ms for 65
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22 200 ms starting with the onset of the startle stimulus. When a startle stimulus (120-dB white noise on
23
24 201 a 65-dB white noise background) follows a prepulse, the amplitude of the startle response is
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26 202 reduced, compared with its amplitude when the startle stimulus is presented without a prepulse.
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28 203 This amplitude reduction is called prepulse inhibition (our primary outcome variable) and is the
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30 204 percentage reduction of the mean startle amplitude for the prepulse trial expressed as the
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32 205 percentage reduction of the mean startle amplitude for startle-alone trial:

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

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42 207 included baseline startle response amplitude at 68-dB and acoustic startle response amplitude at
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44 208 120-dB.

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48 49 210 *Statistical Analyses*

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52 211 All statistical analyses were performed with IBM SPSS Statistics (version 23) using the
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54 212 MIXED procedure. Assumptions of normality of error distribution, homogeneity of variance, and
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56 213 parameter linearity were examined graphically. No transformation of data was required based on
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6 214 these inspections. Predictors used in all models were sex (male, female), pre-weaning group
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8 215 (AFR, EH, MS, MPS), post-weaning housing (socially-housed, isolation-housed), and decibel
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10 216 level (respectively 68-, 76-, 80-, 84-, 120-dB, at level 1 to account for repeated measurement).
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12 217 For all models built, 1) individual animals nested within litter, and 2) chambers were included as
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14 218 random effects to accommodate for dependencies in the experimental design. Inclusion of these
15
16 219 random effects allowed us to partition the variation associated with each of these variables, and
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18 220 to obtain a treatment effect estimate that was independent of these variables. Subject weight was
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20 221 also included as a covariate (control factor) in the model, as the intensity of the startle response is
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22 222 affected by body weight (Blażczyk & Tajchert, 1996). In all analyses, the full factorial model
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24 223 was the best model (based on ΔAIC and ΔBIC). P-values below 0.05 were considered
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26 224 statistically significant, and significant main effects and interactions were probed with
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28 225 Bonferroni corrected *post hoc* comparisons.
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227 **Results**

228 ***Prepulse Inhibition of the Startle Response***

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

232 **Insert Figure 1 here.**

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

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6 236 **Insert Figure 2 here.**
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8 237 Lastly, an interaction between pre-weaning treatment condition and post-weaning
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10 238 housing condition was observed, ($F_{1,39}$) = 8.378, p = 0.005 (Figure 3). *Post hoc* analyses
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12 239 comparing post-weaning housing condition within pre-weaning group indicated that socially-
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14 240 housed AFR subjects (M = 24.21, SE = 3.80) displayed lower levels of prepulse inhibition
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16 241 compared to isolation-housed AFR subjects (M = 39.72, SE = 3.08). The inverse pattern of
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18 242 results was observed between isolation-housed (M = 25.73, SE = 3.19) and socially-housed (M =
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20 243 37.793, SE = 3.205) MPS subjects.
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25 244 **Insert Figure 3 here.**
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27 245 ***Baseline Startle Response Amplitude (68 dB)***
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30 246 A main effect of pre-weaning group, ($F_{3,225}$) = 4.750, p = 0.004, and an interaction
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32 247 between pre-weaning group and post-weaning housing was observed, ($F_{3,225}$) = 3.101, p = 0.031.
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34 248 *Post hoc* analyses comparing post-weaning housing conditions within pre-weaning group yielded
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36 249 no significant differences between any of our groups. However, *post hoc* analyses comparing
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38 250 pre-weaning group within post-weaning housing condition indicated that isolation-housed MPS
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40 251 subjects (M = 32.25, SE = 2.86) displayed a significantly higher baseline startle response
41
42 252 amplitude compared to isolation-housed AFR subjects (M = 15.73, SE = 3.24) (Figure 4). While
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44 253 isolation-housed MS subjects also displayed higher levels of baseline startle response amplitude
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46 254 relative to isolation-housed AFR subjects (*Mean Difference* = 10.70), this difference failed to
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48 255 reach statistical significance (p = 0.055).
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53 256 **Insert Figure 4 here.**
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55 257 ***Startle Response Amplitude (120 dB)***
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6 258 A main effect of sex, ($F_{1,133}$) = 12.20, p = 0.001, was observed, indicating that male mice
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8 259 displayed a higher acoustic startle response amplitude, (M = 382.13, SE = 20.46), than female
9
10 260 mice, (M = 285.44, SE = 18.64) (Figure 5).

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13 261 **Insert Figure 5 here.**

14
15 262 A main effect of post-weaning housing was also observed, ($F_{1,133}$) = 3.926, p = 0.049,
16
17 263 indicating that isolation-housed subjects displayed a lower amplitude of the acoustic startle
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19 264 response, (M = 306.36, SE = 2.49), relative to socially-housed animals, (M = 361.21, SE =
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21 265 16.14) (Figure 6).

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24 266 **Insert Figure 6 here.**

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29 268 **Discussion**

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32 269 The overall aim of this study was to assess the additive (cumulative stress hypothesis) or
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34 270 interactive effects (match/mismatch hypothesis) of early-life experiences in the form of dam-
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36 271 offspring separation and subsequent post-weaning social housing on the manifestation of adult
37
38 272 prepulse inhibition using the C57BL/6 inbred mouse. Analysis of the primary outcome variable,
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40 273 prepulse inhibition of the acoustic startle response, provided direct evidence for the
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42 274 match/mismatch hypothesis. Specifically, isolation-housed MPS subjects displayed a deficit in
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44 275 prepulse inhibition relative to socially-housed MPS subjects while socially-housed AFR subjects
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46 276 displayed a deficit in prepulse inhibition relative to isolation-housed AFR subjects.

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49 277 ***Maternal Behavior during the pre-weaning phase***

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52 278 In the earlier experiment (Bailoo et al., 2013), we reported that the effects of the pre-
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54 279 weaning manipulations were restricted to the reunion phase with the dam, with an overall
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6 280 increase in maternal behavior for the longer separated groups (MS & MPS). Moreover, for the
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8 281 MS/MPS groups, the homeostatic balance of the pups was maintained using heat lamps ($31 \pm$
9
10 282 2°C), no indication of food deprivation was present, and correspondingly, higher weaning
11
12 283 weights were observed. Thus, subjects in the MS/MPS groups experienced a better outcome, at
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14 284 least in regards to levels of maternal care, as a consequence of these manipulations relative to
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16 285 AFR/EH groups.

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20 286 ***Effects of pre-weaning group and post-weaning housing on prepulse inhibition***

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22 287 Isolation-housed AFR subjects displayed higher levels of prepulse inhibition relative to
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24 288 socially-housed AFR subjects, with the opposite relation observed for MPS subjects; a result that
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26 289 is consistent with the match/mismatch hypothesis. This result is most likely because AFR
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28 290 subjects received lower levels of maternal care pre-weaning when compared to MPS groups,
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30 291 which has been associated with a stressful early environment and poorer adult outcomes in
31
32 292 rodents (Champagne et al., 2008). Thus, in this study, AFR subjects experienced a “match” when
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34 293 housed in isolation while MPS subjects experienced a “mismatch” when housed in the same
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36 294 manner.

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38
39 295 A main effect of decibel level was observed, indicating that as the intensity of the
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41 296 prepulse increased, inhibition of the startle response correspondingly increased. This result
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43 297 demonstrated that the prepulse inhibition experimental procedure used in this study was
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45 298 effective.

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48 299 A main effect of sex was also observed, with males displaying lower levels of prepulse
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50 300 inhibition relative to females, after correcting for body weight. This result was surprising given
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52 301 that the literature supports the contention of a sex difference, but in the opposite direction (Braff
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6 302 et al., 2001). However, in many of the studies investigating or reporting sex differences, prepulse
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8 303 inhibition of startle is generally confounded by body weight (Blaszczyk & Tajchert, 1996).
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10 304 Specifically, male rodents generally weigh more than females, have greater muscle mass and
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12 305 associated motor strength, and relatedly, display a greater startle response and a deficiency in the
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14 306 ability to display prepulse inhibition. In the few studies that we are aware of that statistically
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16 307 corrected for this sex/weight correlation, this difference disappears or at least is less clear in
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18 308 regards to the direction of this effect (e.g., Blaszczyk & Tajchert, 1996). Moreover, it is
19
20 309 important to note that this purported sex difference can be modulated by several other factors
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22 310 including, for example, female hormonal state (c.f., Braff et al., 2001, for review). Thus,
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24 311 explanation for this difference remains speculative at best and further work replicating this effect
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26 312 and detailing the neurobiological mechanism is needed.
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313 ***Effects of pre-weaning group and post-weaning housing on baseline startle (68-dB)***

314 A significant interaction between pre-weaning group and post-weaning housing was
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36 315 observed. However, probing this interaction in relation to our experimental question by
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38 316 comparing the effects of either social- or isolation-housing within pre-weaning treatment groups
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40 317 yielded no significant differences.
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318 ***Effects of pre-weaning group and post-weaning housing on the startle response (120 dB)***

319 An effect of post-weaning housing condition on acoustic startle response amplitude was
46
47 320 observed, with isolation-housed subjects displaying lower levels of startle relative to socially-
48
49 321 housed subjects. While this result is generally consistent with the literature, it should be noted
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51 322 that Geyer and colleagues, in a systematic review (2001), have stated that while some studies
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53 323 report an increase in acoustic startle response amplitude as a consequence of isolation-housing,
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6 324 others report no or the opposite effect. Therefore, acoustic startle response amplitude seems to be
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8 325 the least predictive of neuropsychiatric dysfunction, at least in regards to whether corresponding
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10 326 deficits in prepulse inhibition are observed (Varty, Braff, & Geyer, 1999; Varty & Geyer, 1998).
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12 327 This may also be true of the data in our study, with deficits in startle responding observed
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14 328 between the isolation- and socially-housed groups, but not in relation to pre-weaning treatment
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16 329 conditions.

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20 330 A main effect of sex on acoustic startle response amplitude was observed, with male mice
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22 331 displaying a greater startle response than females. However, as noted above, further experimental
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24 332 work is needed to replicate and delineate this effect.

25 333 *Limitations*

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29 334 It is important to note that this study made use of “extra” animals from litters that had
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31 335 been produced for use in a different study (Bailoo et al., 2013), and thus a fully balanced design
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33 336 was not achieved. However, with the exception of the isolation-housed EH group, and given the
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35 337 relatively large observed effect sizes, it may be argued that this experiment was sufficiently
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37 338 powered and that these data are reliable. Moreover, given that the literature suggests that the
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39 339 AFR condition is generally similar in phenotype to the EH condition, and that the maternal care
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41 340 data recorded in this study supports this “homology”, it can be speculated that the observed
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43 341 differences with the isolation-housed AFR group are also applicable to the isolation-housed EH
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45 342 group (Levine, 2002).

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49 343 The pre-weaning manipulations and their effects on maternal care were described
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51 344 previously (Bailoo et al., 2013). In those data, many aspects of maternal care were elevated but
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53 345 those differences were restricted to the reunion phase in the longer separated groups (MS and
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6 346 MPS). It was hypothesized that since maternal care has been shown to mediate the relation
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8 347 between these early experience paradigms and later offspring outcome, these groups would
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10 348 exhibit the smallest deficits in prepulse inhibition. However, in this study, we observed this
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12 349 relation only in the MPS and not in the MS group.

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15 350 Several factors may account for the lack of an observed effect in our MS group. While it
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17 351 is presumed that maternal care mediates the relation of pre-weaning separations to adult
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19 352 phenotypes, including prepulse inhibition, perhaps other unmeasured factors might also affect
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21 353 this relation (c.f., Macri & Würbel, 2006). For example, systematic work evaluating food
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23 354 deprivation, thermoregulation, ultrasonic vocalization production, and behavioral changes and
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25 355 adaptations *by the pups* (and their influence on the dam) as a consequence of these pre-weaning
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27 356 manipulations remains largely unexamined. Without systematically acquiring such information,
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29 357 it is unknown whether MS and MPS groups are equivalent. Only the levels of maternal care
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31 358 exhibited to the pups upon reunion are similar. Future studies characterizing the differences
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33 359 between the MS and MPS groups are therefore needed.

34 360 **Conclusion**

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37 361 Deficits in prepulse inhibition are noted in humans with symptoms of neuropsychiatric
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39 362 dysfunction such as schizophrenia, obsessive compulsive disorder, and attention deficit
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41 363 hyperactivity disorder (Braff et al., 2001). Considerable evidence supports a high degree of
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43 364 similarity between measures of prepulse inhibition in rodents and humans (e.g., Braff et al.,
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45 365 2001; Ellenbroek, Geyer, & Cools, 1995). Moreover, prepulse inhibition appears to be highly
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47 366 conserved among vertebrates and is one of the few paradigms in which humans and animals are
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49 367 tested in a similar fashion. Thus, investigation into the disruption of prepulse inhibition as a
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6 368 consequence of early experiences associated with an increased susceptibility for neuropsychiatric
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8 369 dysfunction is well suited to rodent models (Swerdlow, Weber, Qu, Light, & Braff, 2008).
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10 370 This study was designed to investigate the additive effects of typical stress-related
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12 371 manipulations applied at two different developmental periods, and extends previous work
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14 372 employing these manipulations. Analysis of the primary outcome variable of this study, prepulse
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16 373 inhibition, provides support for the match/mismatch hypothesis. Generally speaking, isolation-
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18 374 housing should lead to deficits in prepulse inhibition. However, in this study, AFR subjects that
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20 375 experienced the lowest levels of maternal care displayed deficits in prepulse inhibition when
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22 376 housed socially compared to isolation. Conversely, MPS subjects that experienced high levels of
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24 377 maternal care and were later housed in isolation displayed greater deficits in prepulse inhibition
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26 378 compared to MPS subjects housed socially.
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32 379 Future studies employing these early experience paradigms consecutively in the
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34 380 evaluation of adult prepulse inhibition should benefit from our results. Specifically, if isolation-
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36 381 housing is used, then robust differences can be observed simply between the AFR control
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38 382 groups, with the noteworthy difference being that social housing leads to deficits in prepulse
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40 383 inhibition relative to isolation-housing, at least in C57BL/6 mice. If the intention is to
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42 384 analogously model the match/mismatch hypothesis (also termed “differential susceptibility” in
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44 385 human research), then both the AFR and the MPS groups in social- and isolation-housing,
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46 386 respectively, can be used to model this relation.
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Notes

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For Peer Review

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504 **Tables**

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506 Table 1

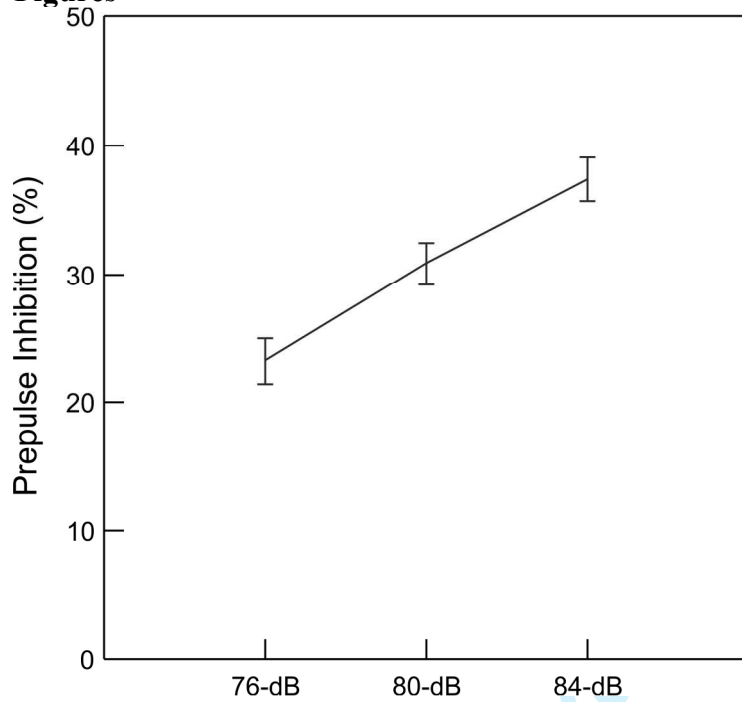
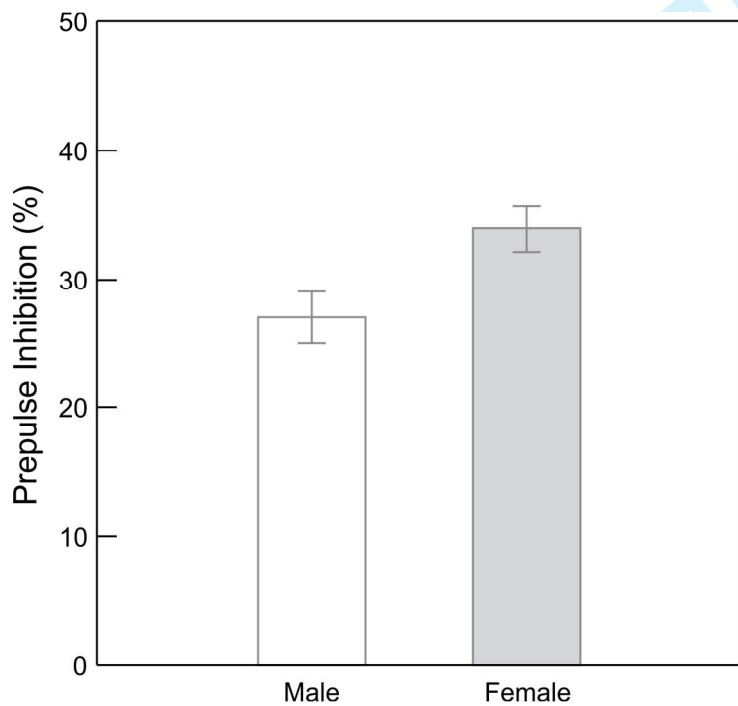
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508 *Total number of subjects used divided by pre-weaning group and post-weaning housing*
509 *condition.*

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	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

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512 **Figures**513
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515 *Figure 1.* Differences in prepulse inhibition (%) as a consequence of prepulse level (dB).
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519 *Figure 2.* Differences in prepulse inhibition (%) as a consequence of sex.
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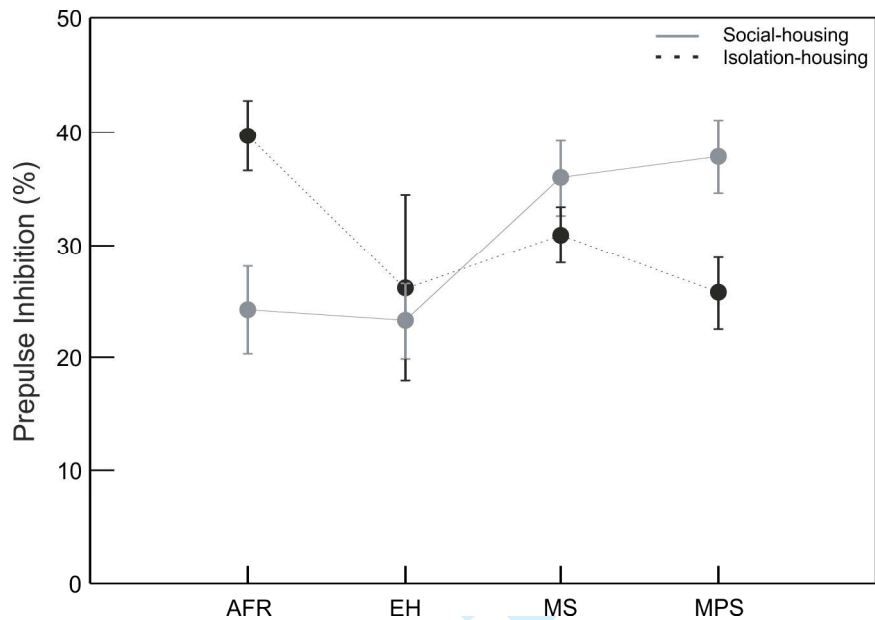


Figure 3. Differences in prepulse inhibition (%) as a consequence of pre-weaning group and post-weaning housing condition.

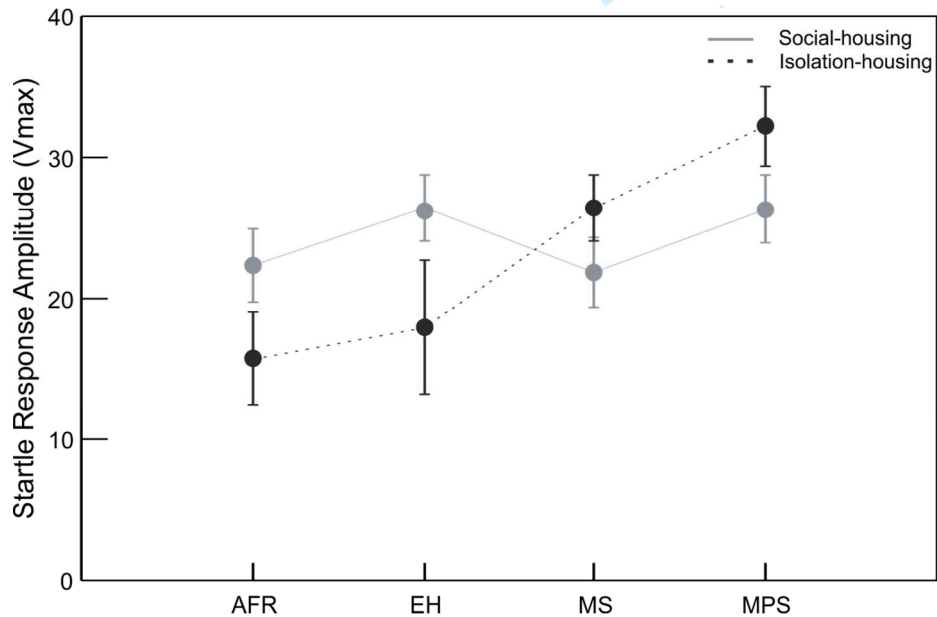
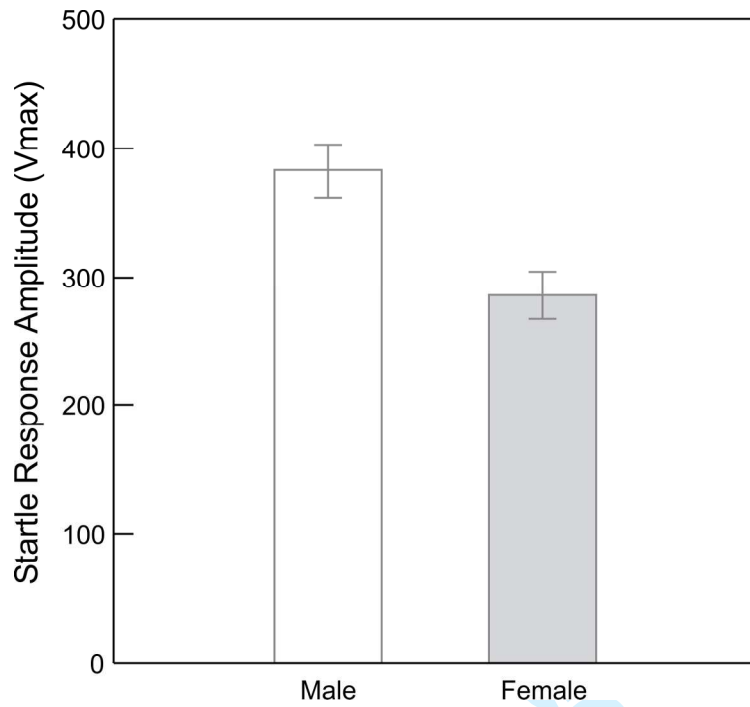
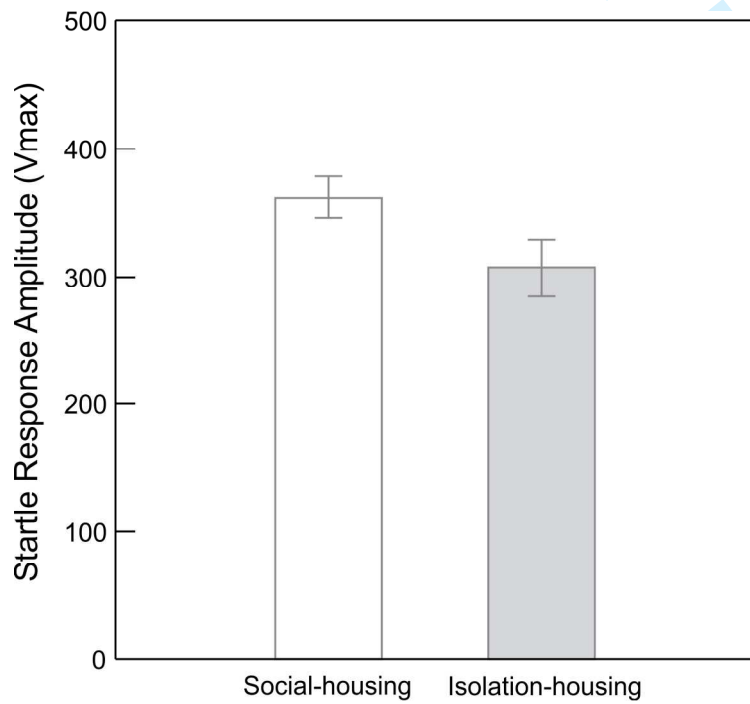


Figure 4. Differences in baseline startle amplitude (68-dB) as a consequence of pre-weaning group and post-weaning housing condition.



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



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