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## Research report

# Pre- but not post-menopausal female CBA/CaJ mice show less prepulse inhibition than male mice of the same age

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**Abstract**

Prepulse inhibition (PPI) of the acoustic startle reflex (ASR) has been reported to be weaker in females than males for both humans and rats. Although there are exceptions, on balance these data suggest that PPI is sensitive to sex-specific neurosteroids; in contrast, most studies with mice have not replicated this effect. We compared PPI for noise decrement prepulses (quiet gaps) in female CBA/CaJ mice at 3–8 months (pre-menopausal:  $n=55$ ) and 17–25 months of age (post-menopausal,  $n=33$ ) with similarly aged groups of males ( $n=48, 35$ ). Both PPI and ASR levels were significantly reduced in pre-menopausal females compared to young males, but did not differ between post-menopausal females and old males. The observed PPI decrement in young female mice compared to young males agrees with one previous report in young C57BL/6J mice as well as the majority of studies with human subjects and some strains of rats. The absence of a sex difference in PPI for old mice is consistent with the hypothesis that PPI is affected by reproductive hormones present at high levels only in pre-menopausal females. We note that this effect size for PPI is small, perhaps consistent with reports that the PPI decrement in females is restricted to certain times within the menstrual cycle in women and the estrous cycle in rats. The negative findings previously reported in the mouse can be attributed to the small effect size and to procedural differences, including stimulus conditions, and the different strains and ages of mice.

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*Keywords:* Acoustic startle; Prepulse inhibition; Sex differences; Age; Mice**1. Introduction**

The amplitude of the mammalian acoustic startle reflex (ASR) can be facilitated (“prepulse facilitation”, PPF) or inhibited (“prepulse inhibition”, PPI) by modest perturbations in the sensory milieu (“prepulses”), the data typically following a biphasic pattern of an initial stage of PPF followed by PPI at longer lead times. These effects are robust across species and across stimuli [7,12], and because both PPF or PPI provide objective evidence that the prepulse has been detected they have been used to measure sensory function and sensory deficits in animals and in “difficult to test” human subjects for many years [4,7,24,36,37]. Further interest in these phenomena was generated by a report that PPI is diminished in schizophrenic

patients [2] and the many subsequent experiments that have provided evidence that this sensorimotor gating deficit extends to other psychiatric populations [see review, 3] and also to animal models [see review, 29]. These diverse applications suggest the importance of understanding the conditions which may influence reflex modification in normal humans and laboratory animals [28] to further our understanding of this ubiquitous phenomenon and to increase its utility in laboratory and clinical experiments focused on sensory and perceptual processing and sensorimotor gating.

It is in this context that gives significance to reports that women show less PPI than men [14,27,30,31], that female rodents show less PPI than male rodents [18,23], and that PPI varies according to the stage of the menstrual cycle in women [14,31] and with the estrous cycle in rats [15]; these data suggest that the neural mechanisms responsible for PPI may vary, directly or indirectly, with sex differences in the levels of circulating reproductive hormones.

Not all reports have confirmed these findings, especially those that have studied PPI in inbred strains of mice – not in 18 strains

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of mice [19]; not in 2 strains of mice [22]; not in 3 of 4 strains of mice [32]; and not in 40 different strains of mice [35]: thus one strongly positive findings in mice [23] appears as the rare exception. The present analysis was undertaken to determine if there would be a difference in PPI between young male and female inbred mice of the CBA/CaJ strain, and if this effect might vary with the age of the mouse. This strain is of particular interest to hearing scientists who work with animal models of presbycusis (the loss of hearing in old age) as it maintains its hearing into near senescence to contrast with other strains, such as the more commonly used C57BL/6J and DBA/2J mice that (like many other less well studied mouse strains) have early onset age-related cochlear degeneration [34,38].

In our presbycusis research program we have collected PPI data from many CBA/CaJ mice using different durations of brief quiet gaps in noise as prepulses. These prepulses provide a sensory gradient that has allowed us to collect PPI measures of temporal acuity as a prelude to other experiments that compare the mouse data with gap detection thresholds obtained in human listeners [e.g. 10]. For our purposes here, the gap prepulse has an advantage over the more frequently used increment prepulse because gaps in noise produce only PPI: for increments and noise pulses the combination of PPI and PPF can overlap in time so that manipulations such as, e.g., stimulus level, that alter PPF may indirectly affect measures of PPI [1,13].

The data were not collected for the explicit purpose of contrasting the behavior of male and female mice as a function of age, but the entire sample was sufficiently large that we were able to assemble PPI data on relatively large groups of young sexually-mature mice and old and near-senescent mice, at ages that were reasonably well balanced in male and female composition. Menopause occurs between about 12 and 14 months of age in the mouse [26], and the data we assembled allowed us to compare PPI levels in female versus age-matched male mice at both pre- and post-menopausal ages. The primary outcome of this analysis was that for mice between the ages of 3 and 8 months the pre-menopausal females had lower levels of both PPI and ASR than males, but for mice between 17 and 25 months of age neither the ASR nor the PPI level of these post-menopausal females was different from that of the age-matched males.

## 2. Materials and methods

### 2.1. Subjects

The subjects were 198 mice (96 female and 102 male) of the CBA/CaJ strain, all bred in the University of Rochester Vivarium from breeding stock originally obtained from The Jackson Laboratories (Bar Harbor ME), with the periodic introduction of new breeders. They were between 3 and 25 months of age, and for this analysis they were partitioned into two age groups, the sexually mature adults from 3 to 8 months of age ( $n = 103$ : 55 F, 48 M), the old and near-senescent adults from 17 to 25 months of age ( $n = 95$ : 41 F, 54 M). Within the young group, 40 were 3 months old (24 F, 16 M); 21 were 5 months old (8 F, 13 M); 13 were 6 months old (7 F, 6 M); and 29 were 8 months old (16 F, 13 M). Within the old group, 6 were 17 months old (6 M); 18 were 18 months old (13 F, 5 M); 60 were 24 months old (22 F, 38 M), and 11 were 25 months old (6 F, 5 M). The baseline ASR is reduced in old animals, and in some the "ASR" baseline is little different from the "no-stimulus" activity level (ACT) which then severely

limits the possible maximum value of PPI. As described below (see Section 2.4) mice that did not satisfy a criterion that the ASR mean score was at least twice the amplitude of the "non-stimulus" activity control score were excluded from further analysis. All young mice exceeded the criterion, but 19 old male mice were excluded (1 in the 18 month group, 15 in the 24 month group, and 3 in the 25 month group) as were 8 female mice (5 in the 24 month group and 3 in the 25 month old group): thus just 68-old mice (33 F, 35 M) were included in the PPI analyses. While a greater proportion of males were excluded by this criterion (35% versus 20% for females), the difference was not statistically significant (Fisher's Exact Test,  $p = 0.11$ ).

The mice were maintained in group cages in a constant temperature and constant humidity environment, with a 12/12 light/dark cycle (lights on at 6 a.m.). Testing was conducted usually between the hours of 9 a.m. and 6 p.m. All procedures were approved by the University of Rochester Committee on Animal Resources, and were in accord with the regulations of the Public Health Service and the Federal Animal Welfare Act.

### 2.2. Apparatus, stimuli, and response recording

The mouse was tested while confined in a small cage, 5 cm wide, 7 cm long, and 4 cm high. The test cage was initially constructed from an acrylic block with slotted sides and roof for free sound penetration, but later cohorts of mice were tested in a cage constructed of wire mesh. The cage was mounted on a suspended acrylic platform to which an accelerometer was attached, and placed in an anechoic chamber (Eckel Corp., Cambridge, MA, Model Number 555-250-3) housed within a sound attenuating room (IAC, Bronx, NY), one mouse being tested at a time. The startle stimulus was a 110 dB (peak-to-peak, SPL linear scale) 20-ms duration noise burst provided by a white noise generator, gated through an electronic switch with  $<0.2$  ms rise and fall times, then amplified and delivered through a high frequency tweeter with maximum output at 16 kHz and a 5 dB/octave roll-off. The gap carrier (70 dB SPL RMS, linear scale) was provided by a white noise generator, gated through a second electronic switch with variable rise and fall times, and then amplified and delivered through a Panasonic high frequency leaf tweeter that varied by no more than  $\pm 6$  dB over a range of 2 kHz to 100 kHz. The ambient noise level in the chamber was less than 25 dB SPL for all frequencies above 125 Hz. Sound levels were measured with a 1/4-in. Bruel and Kjaer microphone, Model 4135, connecting to a measuring amplifier, Bruel and Kjaer, Model 2610. The output of the accelerometer that detected the ASR was integrated for 100 ms following the onset of the startle stimulus, and recorded in arbitrary voltage units (aV-units) that are linearly related to the force of the downward thrust of the reflex response. Stimulus presentation and response measurement were under computer control using custom software.

### 2.3. Experimental design

Quiet gaps in noise were shaped by 0.2 ms rise/fall times. All of the gap conditions were presented in balanced blocks of trials containing in random order two control trials, one trial for each non-zero gap duration, and an additional "no-stimulus" trial for which neither a gap in the noise nor the eliciting stimulus was provided but spontaneous activity (ACT) was measured for the standard 100 ms response period. Eleven blocks of trials were presented, with the first block being used to provide a period for adaptation to the experimental conditions. The background noise was on continuously throughout the test session save for the brief gaps. Trials were separated on average by 20 s, with a range of 15–25 s. Because gap detection in the mouse improves following testing with a supra-threshold gap [9], the main gap detection test in which gap duration was varied was preceded by two test days of familiarization with a gap of 10 ms duration. This gap was presented prior to the startle stimulus with the gap-onset to startle-onset lead time ranging from 10 to 300 ms. On the gap duration test day most mice ( $n = 103$ ) in this experiment were tested at nine different gap durations: 0 ms (providing the startle control baseline, this condition presented twice as often as the others) and 2, 3, 4, 5, 6, 8, 10 and 15 ms, all gaps ending 60 ms prior to the onset of the startle stimulus. The first cohorts of mice ( $n = 18$ ) were only tested on 8 gap durations, including 0 ms and 2 to 10 ms (missing the 15 ms gaps), and the late cohorts ( $n = 47$ ) were tested at 10 different durations, adding a 1 ms gap condition.

### 2.4. Data analysis

Prepulse inhibition for every gap duration (GD) was quantified for each mouse by the formula:

$$PPI = 1 - \frac{\text{MeanASR}_{GD}}{\text{MeanASR}_C}$$

That is, prepulse inhibition for any prepulse condition is the proportion of the mean baseline control ASR that was inhibited by the gap, so that, e.g., if for any mouse the mean response amplitude when a gap was presented ( $\text{MeanASR}_{GD}$ ) equaled its control mean ( $\text{MeanASR}_C$ ) then  $PPI=0$ ; but only if there were no evident response to the startle stimulus when the gap was presented before the eliciting stimulus would  $PPI=1.0$ . One perhaps unrecognized hazard in interpreting the values for PPI provided by this commonly used equation or its mathematical equivalents [e.g., 19,24,33 and many others] is that because  $\text{MeanASR}_{GD}$  cannot fall below the mean ACT level save for random scatter in the data, the “in principle” upper limit of PPI for any subject is determined by the formula:

$$PPI_{\text{UPPER LIMIT}} = 1 - \frac{\text{MeanACT}}{\text{MeanASR}_C}$$

This constraint has little or no effect on the measure of PPI for the young CBA/CaJ mouse because the amplitude of its control ASR is so large that even strongly inhibited responses seldom approach the ACT level, as is shown in the data analyses presented below. Although the asymptotic level of PPI has been shown to be not affected by individual differences in the ASR baseline in past research [11] and confirmed in the present experiment, a very low ASR baseline in very old CBA/CaJ mice (or hearing impaired mice) necessarily limits the maximal possible level PPI because the prepulse cannot inhibit the ASR below the artificial floor provided by background activity. For this reason we excluded any mice from the PPI analysis that had an ASR baseline less than twice that of its ACT mean, as in principle they could not achieve a PPI value of at least 0.50: as described above, we excluded 27 of the 95 old mice (19 M, 8 F).

The ASR, ACT, and PPI data were subjected to mixed design ANOVA, with Age and Sex as between-S variables and Gap Duration (GD) as the within-S variable, these major analyses conducted for all mice included in this report ( $n = 171$ ). Two additional two-way between-S ANOVA were used to analyze Age and Sex effects for the 1 ms gap condition ( $n = 153$ ) and the 15 ms gap condition ( $n = 47$ ) in the smaller numbers of mice tested at these values. The Hunyh–Feldt [8] adjustments to the within-S  $p$ -values were used to correct for the effects of non-homogeneity in inter-cell correlations. Effect size measures were based on SPSS partial-Eta-squared values ( $\eta_p^2$ ) following the ANOVA, and on GraphPad Prism software (version 4.2) for  $R^2$  following  $t$ -tests and correlation analyses.

## 3. Results

### 3.1. Startle amplitudes and background activity

In young mice the mean (S.D.,  $N$ ) control ASR for males (M) was higher than that for females (F), for YM, 4335 (1834, 48); for YF, 3452 (1497, 55). This difference was reversed in the old mice, for OM, 1659 (851, 35); for OF, 1854 (995, 33). A two-way ANOVA of these data provided a significant main effect of Age,  $F(1/166)=92.83$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.36$ , and a significant interaction between Age and Sex,  $F(1/166)=5.91$ ,  $p = 0.016$ ,  $\eta_p^2 = 0.03$ . The Sex difference was significant in the young mice,  $t(101)=2.78$ ,  $p < 0.01$ ,  $R^2 = 0.07$ , but not in the old mice,  $t(66)=0.87$ ,  $p > 0.3$ ,  $R^2 = 0.01$ . The ACT mean (S.D.) level was higher in the old (O) mice than in the young (Y), for YM, 291 (89, 47), for YF, 285 (125, 55); and for OM, 364 (125, 35), for OF, 349 (154, 33). The ANOVA of these data provided a significant Age main effect,  $F(1/166)=12.80$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.07$ .

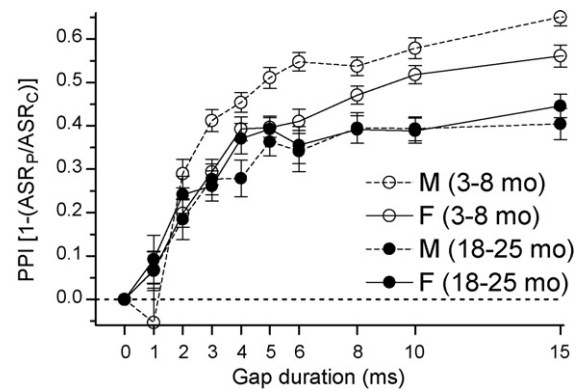


Fig. 1. Mean (S.E.M.) for PPI as a function of gap duration and age in male and female CBA/CaJ mice tested when young (48 M, 55 F) or in near-senescence (35 M, 33 F). Mean PPI was significantly reduced in young pre-menopausal female mice compared to young male mice ( $p=0.001$ ), but was not different between old post-menopausal female mice and old male mice.

### 3.2. Prepulse inhibition

Fig. 1 provides the mean (S.E.M.) PPI for the four groups across the gap-duration dimension. Increasing GD led to greater PPI in all groups, but overall PPI levels were higher for the younger mice, and, within the young groups, lower in the female mice. In contrast, the PPI levels in the OF mice were not different from those of the OM mice. The ANOVA of the PPI data between 2 and 10 ms provided a significant effect for GD,  $F(6/1002)=59.71$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.26$ , and a significant interaction between GD and Age,  $F(6/1002)=3.46$ ,  $p = 0.003$ ,  $\eta_p^2 = 0.02$ . There was also a significant main effect of Age,  $F(1/167)=17.44$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.10$ , and a significant Age  $\times$  Sex interaction,  $F(1/167)=6.18$ ,  $p = 0.014$ ,  $\eta_p^2 = 0.04$ . The overall lower level of PPI for the young female mice compared to the young male mice was significant,  $F(1/101)=11.40$ ,  $p = 0.001$ ,  $\eta_p^2 = 0.10$ , but PPI levels for the old mice did not differ between females and males,  $F(1/66)=0.357$ ,  $\eta_p^2 = 0.00$ . The ANOVA for the 15 ms data similarly provided an Age main effect,  $F(1/149)=44.41$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.23$ , and a significant Age  $\times$  Sex interaction,  $F(1/149)=5.83$ ,  $p = 0.017$ ,  $\eta_p^2 = 0.04$ . The ANOVA for the 1 ms gap duration provided no significant differences between the groups (all  $p > 0.20$ ), and overall the mean (S.E.M.) for PPI of +0.04 (0.03) was not different from the ASR control baseline. For the 2 ms gap, however, every group exhibited a significant level of PPI (all  $p < 0.01$ ). In the ANOVA of these 2-ms data neither main effect for Age or Sex was significant,  $F < 1$ , but there was a marginally significant Age  $\times$  Sex interaction,  $F(1/170)=3.76$ ,  $p = 0.054$ , reflecting a weaker level of PPI for the young female compared to the young male,  $t(101)=2.005$ ,  $p < 0.05$ ,  $R^2 = 0.04$ , and a non-significant higher level of PPI for the old female compared to the old male.

The “in principle” upper limit of PPI was reduced in the old mice, as must reflect their lower levels of ASR and higher ACT levels: for YM the mean (SD,  $N$ ) upper limit of PPI was 0.92 (0.05,47); for YF, 0.89 (0.08, 55); for OM, 0.74 (0.12, 35) and for OF, 0.75 (0.14, 33). While the ANOVA of these data showed that these upper limits of PPI varied with Age,  $F(1/166)=110.05$ ,

$p < 0.001$ ,  $\eta_p^2 = 0.40$ , in fact all of the observed PPI levels obtained at the longest GD available, 10 or 15 ms, were well below these upper limits: for YM the mean (S.D.) difference between the upper limit and the obtained PPI = 0.28(0.14); YF, 0.37 (0.18); OM, 0.34 (0.22); for OF 0.31 (0.17).

To follow up earlier studies showing that PPI is not significantly related to the ASR baseline, Pearson correlation coefficients between the control ASR baseline and observed PPI at GD of 10 or 15 ms for all groups, and none were significant: for the young mice (male,  $R^2 = 0.007$ ; female,  $R^2 = 0.001$ ) or for the old mice (male,  $R^2 = 0.08$ , female,  $R^2 = 0.04$ ). Similarly there were no significant Pearson correlation coefficients between the ACT scores and either the ASR control baseline or PPI at GD of 10 or 15 ms, for the young mice (male, PPI  $R^2 = 0.002$ , ASR  $R^2 = 0.004$ ; female, PPI  $R^2 = 0.01$ , ASR  $R^2 = 0.03$ ) or for the old mice (male, PPI  $R^2 = 0.01$ , ASR  $R^2 = 0.04$ ; female, PPI  $R^2 = 0.003$ , ASR  $R^2 = 0.003$ ).

#### 4. Discussion

The major finding of this investigation was that the strength of PPI provided by brief gaps in noise was reduced in young pre-menopausal female mice of the CBA/CAJ strain compared to that obtained in young male, but PPI levels did not significantly differ between post-menopausal female mice and similarly aged males. These data are consistent with the hypothesis that the strength of PPI is in part determined by sex-specific reproductive hormones that differ in adult male and pre-menopausal female mice, but do not differ in comparisons of old male and post-menopausal females.

The central theme of this hypothesis is that some component(s) of the hormonal complex of the pre-menopausal female reduces the level of PPI and that because this hormonal complex is diminished in the post-menopausal female mouse, then old males and females no longer differ in their PPI levels. An alternative hypothesis that must also be considered is that PPI in the young male may have been enhanced by the presence of specific male neurosteroids that decline with age, and so, similarly, the PPI advantage for the male must decline with age. There are data that more clearly support the first hypothesis rather than its alternative, in their showing that PPI varies across the menstrual cycle in women, with the PPI decrement in the female most evident in the mid-luteal stage of the cycle when both progesterone and estrogen levels are high [14,31]. Similar data show a within-subject decrement in PPI in female Sprague-Dawley rats in the proestrous phase of the estrous cycle when both estrogen and progesterone levels are high, and no significant sex differences in PPI apparent between males and females tested at other times in the cycle [15].

The finding that the age-related decrement in the strength of PPI is less in female compared to male mice similarly has two plausible alternative explanations. One is that after menopause the “normal” age decrement that occurs in both males and females is offset in the old female by the age-related decline in the levels of the neurosteroid(s) responsible for the PPI decrement in the young female. A second is that the greater age-related

decrement in PPI in old male mice compared to old females may be determined by their greater hearing loss, if these mice were to share the age effect on hearing observed in elderly men compared to women [21]. However, we have not found any sex differences in hearing thresholds for brain stem auditory evoked-potential measures across the life span in CBA mice, with both males and post-menopausal females losing about 30 dB of sensitivity across all frequencies from 14 to 30 months of age [25]. It may be noted also while there were age and sex differences in asymptotic levels of PPI, there was little evidence in the mouse for a change in gap thresholds for with either sex or with age, no group having significant PPI for the 1 ms gap, while all groups had significant PPI for the 2 ms gap. These data suggest that the pre-menopausal female deficit in asymptotic PPI can be located in sex differences in the neurosteroids that interact with the neural mechanisms responsible for PPI, rather than having their effects on afferent processes in the primary auditory system.

A second interesting sex-by-age interaction was that the baseline control ASR was more vigorous in the young male compared to the pre-menopausal female, whereas the ASR levels of the old male and the post-menopausal female did not significantly differ. There is a prior report of a more vigorous ASR in young male versus female mice in both the C57BL/6J and the C3H strains [22], which was hypothesized to result from greater anxiety in male compared to female mice. However, we might otherwise hypothesize that it is the greater muscle mass and thus strength of young male mice that is responsible for their having a more vigorous ASR, this explanation being more plausible because anxiety, as a type of stress, might have been expected to reduce rather than to enhance PPI [6,17]. The observation that the ASR difference between male and female mice is not apparent in the near-senescent mouse is worthy of further study, but of particular relevance to the focus of the present analysis, it is unlikely that these ASR differences could contribute to the sex-linked differences in PPI because of the lack of substantial correlations between individual differences in ASR and PPI values. Similarly, the higher background activity scores in old compared to young mice, both male and female, is of additional intrinsic interest, though its biological implication is presently obscure. In the present context it is important primarily because correlation analyses show that ASR and PPI scores are unrelated to individual differences in ACT scores: otherwise it could be hypothesized that changes in the ASR or PPI with age were perhaps secondary to age-related changes in motor activity that, for example, reduced the salience of the eliciting stimulus or the detectability of the gap prepulse [33].

The obtained difference in PPI between young male and female CBA/CAJ mice is consistent with data obtained in human subjects [14,27,30,31] and similar data obtained in Sprague-Dawley and Wistar rats [15,18,20] and in C57BL/6J mice [23]. But there are exceptions to these findings for both human subjects and for rats, and, especially, for mice. That greater PPI in male compared to female rats has been observed in Wistar but not in Brown Norway rats [20] points to the presence of potentially interesting strain differences in the sex effect for PPI in the rat, while it has been suggested that important methodological differences may be responsible for the failure to find consistent

PPI differences between men and women in the human literature [30]. The same may be said for the inconsistent results found in Sprague-Dawley rats, comparing the diverse outcomes of two experiments with this strain [15,27].

Part of the explanation for the apparent difference in outcome in mice must be that the effect size is relatively small, and thus finding significant differences depends on the experiment being sufficiently powerful in its use of large numbers of subjects to detect a small difference (as in the present experiment) as well as in its planned or fortuitous choice of favorable stimulus and procedural conditions and of a suitable strain of animal tested at an appropriate age. Our conclusion drawn from an exhaustive analysis of the available literature relevant to this topic of sex differences in PPI for the mouse suggests that many of the experiments tested strains that have early-onset age-related hearing loss and thus may have had a small dynamic range for PPI between the ACT and ASR levels; or tended to use more intense prepulses that may have engaged PPF or even elicited the ASR; or had insufficient numbers of subjects to show sex differences in PPI given its small effect size. In contrast, the prior positive outcome with the C57BL/6J mouse strain [23] may have resulted because testing took place after the onset of sexual differentiation and before the onset of severe hearing loss; that a range of prepulse intensities were used, some but not all providing significant differences in PPI between males and females; and perhaps most important, that a large number of mice were tested that allowed the presence of a small effect to be detected.

These behavioral experiments do not speak at all to the potential neural mechanisms by which sex-related neurosteroids may be responsible for this difference in PPI levels between young adult males and females, but the apparent phenomenological similarity of this effect in human subjects and in rodents suggests that it has a degree of generality worthy of further investigation. Given this commonality across species, and given the relatively sophisticated understanding of the neural mechanisms involved in the startle reflex and its modification in laboratory animals [5,16,29], further study may help to illuminate the mechanistic basis of these neurosteroid effects in humans and in rodents: this may advance the value of reflex modification procedures in the study of afferent processing and sensorimotor gating in the developmental and comparative laboratory and in clinical research.

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