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Age-associated improvements in cross-modal prepulse inhibition in mice

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Abstract

Prepulse inhibition (PPI) is an operational measure of sensorimotor gating that is thought to probe pre-attentional filtering mechanisms. PPI is deficient in several neuropsychiatric disorders, possibly reflecting abnormalities in frontal-cortical-striatal circuitry. Several studies support the predictive validity of animal PPI to model human sensorimotor gating phenomena but only limited studies have addressed the effects of aging. Studies in humans suggest that PPI is improved or unaffected as humans age (>60 years) and does not correlate with cognitive decline in aged populations. Rodent studies to date, however, suggest that PPI declines with age. Here we tested the hypothesis that PPI measures in rodents are sensitive to stimulus modality, with the prediction that intact sensory modalities in aged animals would be predictive of aging-induced increases in PPI. To test our hypothesis, we assessed PPI using acoustic, tactile, and visual prepulses in young (4 month) and old (23 month) C57BL/6N mice. Consistent with data across species, we observed reduced startle reactivity in older mice. Aging effects on PPI interacted significantly with prepulse modality, with deficient acoustic PPI but increased visual and tactile PPI in aged animals. These data are therefore consistent with PPI studies in older humans when controlling for hearing impairments. The results are discussed in terms of 1) cross-species translational validity for mouse PPI testing, 2) the need for startle reactivity differences to be accounted for in PPI analyses, and 3) the utility of cross-modal PPI testing in subjects where hearing loss has been documented.

Keywords

Prepulse Inhibition; aging; cross-modal; translational validity

Introduction

The startle response is an unconditioned reflexive response to a sudden intense tactile or acoustic stimulus. The magnitude of the startle response is inhibited when the startling stimulus is preceded by a non-startling prepulse. This phenomenon is termed Prepulse Inhibition (PPI) (Graham, Putnam, & Leavitt, 1975). PPI is used to measure an organism's ability to filter out extraneous sensory information during stimulus processing. It is considered a bottom-up pre-attentional process that contrasts with effortful top-down control of attentional performance (D. L. Braff & Light, 2004; Young, Powell, Risbrough, Marston, & Geyer, 2009b).

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Many neuropsychiatric populations that report difficulty ignoring or inhibiting external or internal stimuli exhibit deficits in PPI. Patient groups such as schizophrenia (D. Braff *et al.*, 1978; K. Ludewig, Geyer, & Vollenweider, 2003; Swerdlow, Weber, Qu, Light, & Braff, 2008), Tourette's syndrome (Swerdlow, Karban *et al.*, 2001), Panic Disorder (S. Ludewig *et al.*, 2005), and Bipolar Disorder (Perry, Minassian, Feifel, & Braff, 2001), all exhibit deficient PPI compared to normal control subjects. PPI has therefore been widely used in the preclinical literature in attempts to model the gating deficits observed in these patients (Geyer, 1999, 2006; Geyer, McIlwain, & Paylor, 2002; O'Tuathaigh *et al.*, 2007; A. Ouagazzal, Grottick, Moreau, & Higgins, 2001; Powell, Young, Ong, Caron, & Geyer, 2008; Swerdlow *et al.*, 2008). Preclinical testing of PPI is possible because this phenomenon is observed across species, from mice (Dulawa & Geyer, 1996), to rats (Swerdlow, Braff, Geyer, & Koob, 1986), and non-human primates (Winslow, Parr, & Davis, 2002). PPI is one of the first and most widely described cross-species translational tests available (D. L. Braff & Geyer, 1990; Swerdlow *et al.*, 2008) enabling the elucidation of the neurocircuitry and genetic regulation of this phenomenon (Swerdlow, Geyer, & Braff, 2001; Geyer *et al.*, 2002). Thus PPI represents a useful task for investigating behavioral sequelae of altered neural processing relevant to inhibition and sensory processing.

Given the link between aging and a global loss of inhibitory functions (Healey, Campbell, & Hasher, 2008; Woodruff-Pak, 1997), it is surprising that the role of aging in PPI has not been well defined. To date, the studies of PPI and aging in humans, rats, and mice, have exhibited limited consistency. Contrary to a putative general loss of inhibition with aging (Healey *et al.*, 2008), initial human studies suggested there is no effect of age on PPI (Harbin & Berg, 1983; K. Ludewig, Ludewig *et al.*, 2003). It was suggested that some previous reports of aging effects on PPI (positive or negative) may be compromised by measurement techniques, difference scores influenced by lower startle amplitude in aged subjects (Harbin & Berg, 1983), low sample sizes (K. Ludewig, Ludewig *et al.*, 2003), or sample bias (e.g. a lack of demonstrable age-related effects on other age-sensitive cognitive tests; (Ellwanger, Geyer, & Braff, 2003). In other words, these studies might have inadvertently selected subjects that could be deemed as 'aging successfully' (Glatt, Chayavichitsilp, Depp, Schork, & Jeste, 2007), underestimating aging-related changes in PPI across the larger aging population. In a cross-sectional study with large sample sizes across multiple age groups, deficient cognitive performance in a speed of processing task, and PPI normalized for baseline startle magnitude, PPI appeared to increase up to middle age (>50) and older compared to younger (<20) comparison subjects (Ellwanger *et al.*, 2003). Improved PPI with age - as opposed to impaired - does not conform to the theory of loss of inhibitory function with increasing age (Healey *et al.*, 2008). PPI may, however, be measuring a different form of inhibition (Kok, 1999) and/or may probe neural circuits that continue to develop over adulthood (Sowell *et al.*, 2003). Thus further study is required.

To use animal models to elucidate the neurobiological underpinnings of aging effects in PPI, however, consistent effects across species are required. Initial studies of PPI in CBA/J mice across ages (2, 7 and 22–27 mo), reported a drop in startle response consistent with humans, but with no effect of age on PPI (Ison, Bowen, Pak, & Gutierrez, 1997). Ouagazzal, Reiss, & Romand (2006) observed poorer PPI with increased age in C57BL/6 mice when utilizing acoustic prepulses (10 vs. 22 months). However, given the hearing loss of mice with increased age (A. M. Ouagazzal *et al.*, 2006), assessing PPI across multiple prepulse modalities (e.g. acoustic, tactile, visual) may provide a more accurate picture of inhibitory mechanisms underlying PPI that are independent of specific sensory mechanisms, as demonstrated previously (Barr, Fish, & Markou, 2007). The addition of other cognitive measures can also aid in interpretation of relative age sensitivity of the chosen population, as shown in Ellwanger and colleagues, (2003).

In the present studies, we sought to examine the role of aging on PPI in mice while controlling for the possible confounds described above. Given the wide use of C57BL/6 mice and their common use as a background strain in genetic studies, we examined PPI performance in young (5 months) and old (22 months) C57BL/6 mice. Testing included intra- and cross-modal assessments using acoustic, visual, and tactile prepulse stimuli, large sample sizes, and statistical normalization strategies to account for low baseline startle in older mice. We also examined age effects on cognitive performance in other tasks to confirm aging-related behavioral abnormalities in the population tested (data to be presented elsewhere). We hypothesized that cross-modal testing in C57BL/6 mice exhibiting demonstrable effects of aging in other areas would result in alterations in PPI performance consistent with humans whereby older mice would exhibit better PPI than younger mice (Ellwanger *et al.*, 2003).

Methods and Materials

Subjects

Male 3 mo C57BL/6N mice were purchased from Charles River Laboratories, and 22 mo (n=32/group) mice from the National Institute of Aging stock located in Charles River Laboratories (Wilmington, MA). Mice were housed 2 per cage with food and water provided *ad libitum* in a temperature controlled room under reverse 12h/12h light cycle (lights off at 8:00 AM). All testing occurred between 10:00 AM and 6:00 PM and was conducted in accordance with the “Principles on Laboratory Animal Care” NIH guidelines and with the University of California, San Diego animal care committee approval. Experiments are presented here in the order in which they were conducted with at least 1 week between each experiment.

Apparatus

Startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) consisted of nonrestrictive Plexiglas cylinders 5 cm in diameter resting on a Plexiglas platform in a ventilated chamber. High-frequency speakers mounted 33 cm above the cylinders produced all acoustic stimuli. Scrambled, constant-current footshocks were delivered through a cradle-shaped grid (seven bars with a diameter of 1.6 mm) mounted on the floor of the cylinder. Footshocks, startle intensities, air puffs, and light presentations were controlled by SR-LAB software. Piezoelectric accelerometers mounted under the cylinders transduced movements of the animal, which were digitized and stored by an interface and computer assembly. For experiment 1 and 2, beginning at the stimulus onset, 65 consecutive 1 ms readings were recorded to obtain the peak amplitude of the animals' startle response to either acoustic (40 ms) or airpuff (30 ms) startle stimuli. For experiment 3, 2–65 ms recordings were obtained, the first starting at the prepulse onset, and the second starting at the pulse onset. This trial design enabled accurate measurement of peak reactivity to both footshock and startle stimuli. Peak responses to these stimuli are presented in arbitrary units. A dynamic calibration system was used to ensure comparable sensitivities across chambers. Sound levels were measured as described elsewhere (Mansbach and Geyer, 1988) using the A weighting scale in units of dBA SPL. Footshock levels were verified by using a 1 k Ω resistor across the bars of the shock grids and measuring the voltage drop between the bars to calculate the constant current in milliamperes (mA). Airpuff startle pulses were delivered via software-controlled solenoid at 32 PSI for 30 ms. As in previous reports (Brody, Dulawa, Conquet, & Geyer, 2004), there was very low PPI in young mice using the puff as a pulse, thus creating floor effects precluding the detection of an age effect; hence these data were not included in the analysis. The light was delivered via a bare 15 W incandescent bulb located on the ceiling of the testing chamber. A 65 dB background was presented continuously throughout the session.

Behavioral Testing

Experiment 1: Acoustic prepulse inhibition in young and aged mice—Mice were placed into the startle chambers, after which a 5 minute acclimation period preceded testing. Startle pulses were 40 ms in duration, and prepulses were 20 ms in duration. The inter-trial interval between stimulus presentations averaged 15 seconds (range 7–23 s) for all three experiments. The acoustic startle sessions included 3 blocks. The first block included acoustic startle responding only and included stimulus intensities of 80, 90, 100, 110, and 120 dB. The second block consisted of 6 each of 105 or 120 dB startle pulse intensities and 5 each of 5 different prepulse trials: 67, 69, and 81 dB prepulses preceding a 120 dB pulse, and 73 dB prepulse preceding either a 105 or 120 dB pulse. Prepulses preceded the pulse by 100 ms (i.e. interstimulus interval, onset to onset). The third block varied the inter-stimulus interval. The block consisted of 7 startle pulses at 120 dB and 5 each 73 dB prepulses preceding a 120 dB pulse by 20, 70, 120, 360, and 1080 ms (onset to onset).

Experiment 2: Visual and Tactile prepulse inhibition in young and aged mice—Mice were placed into the startle chambers, after which a 5-min acclimation period preceded testing. The session consisted of 6 trial types that varied in modality (auditory, visual, tactile), each presented 12 times in a pseudorandom order. Trial types were: 120 db pulse, 30 ms air puff pulse, 67 db prepulse preceding a 120 db pulse, 100 ms light prepulse preceding a 120 db pulse, 67 db prepulse preceding the air puff, 100 ms light prepulse preceding a 30 ms air puff.

Experiment 3: Tactile prepulse inhibition in aged mice—Cross-modal PPI of the mice was further assessed in response to various intensities of footshock as a tactile prepulse. After a 5-min acclimation period, 5 different trial types were presented in pseudorandom order. The session consisted of 7 each of 120 db startle pulse, 81 db preceding a 120 db pulse, and 0.02 mA, 0.04 mA, and 0.06 mA footshock preceding a 120 db startle pulse.

Statistical Analysis

In all experiments, the average startle magnitude over the record window (i.e., 65 msec) was used for all data analysis. The amount of PPI was calculated as a percentage score for each acoustic prepulse trial type: $\%PPI = 100 - [(startle\ magnitude\ for\ prepulse + pulse / startle\ magnitude\ for\ pulse\ alone) \times 100]$. Data from no stimulus trials were not included in the results because the scores were negligible relative to trials containing a startle stimulus. In each experiment, a two-way ANOVA was conducted to compare means, with prepulse intensity as a within subject factor and age as a between subject factor. Tukey *post hoc* analyses were performed on data that reached statistical significance ($p < 0.05$). Further assessments used ANCOVA to assess PPI with startle reactivity values being a covariate despite the violation of the independence assumption for covariate analyses. ANCOVA results were corroborated using baseline-matched subgroup comparisons. All PPI analyses were conducted using BMDP statistical software (Statistical Solutions Inc., Saugus, MA). Correlational statistics were performed using Spearman's Rank coefficient, due to small sample sizes and to reduce the potential impact of outliers. These analyses were performed using SPSS 14.0 (Chicago IL).

Results

Experiment 1: Acoustic prepulse inhibition in young and aged mice

Aged animals exhibited significantly reduced acoustic PPI in comparison to young mice (Figure 1, left panel) [Main effect of age: $F(1,59)=36.68$, $p < 0.001$]. Acoustic PPI was significantly increased at the higher prepulse intensities [Main effect of intensity:

$F(3,177)=16.43$, $p<0.001$]. Moreover, there was a large reduction in startle reactivity in the aged group (Figure 1, left inset) [Age: $F(1,59)=32.38$, $p<0.001$]. To determine whether PPI deficits in the aged group were dependent upon baseline startle reactivity (Figure 1, right panel), we also compared prepulse trials matched for startle reactivity, (8 dB above background prepulse with 120 dB pulse in aged mice compared to 8 dB above background prepulse with 105 dB pulse in young mice). Matching for baseline startle reactivity [105 in young vs. 120 dB in aged, $F(1,59)<1$, N.S.], PPI in aged mice was still significantly lower than in young mice [Age: $F(1,59)=27.31$, $p<0.001$]. When covarying for startle reactivity, aged animals still exhibited significantly reduced PPI (-2.04 ± 12.78) compared to young mice (45.37 ± 19.04) [Age: $F(1,61)=11.56$, $p<0.05$].

Experiment 2: Visual prepulse inhibition in young and aged mice

Age effects on PPI were dependent upon prepulse modalities (Figure 2, left panel) [Main effect of age: $F(1,62)=53.15$, $p<0.001$; Age $\times$ Modality: $F(1,62)=112.14$, $p<0.001$]. Post hoc analysis showed that aged mice have significant reductions in PPI with an acoustic prepulse [Age: $F(1,59)=117.24$, $p<0.001$, Tukey test], but not with a visual prepulse [$F(1,59)=1.49$, N.S., Tukey test]. As in experiment 1, aged animals showed a large reduction in overall startle reactivity (Figure 2, left inset) [Age: $F(1,59)=39.78$, $p<0.001$]. An ANOVA with baseline startle reactivity as a covariate replicated experiment 1, with aged mice showing significantly less acoustic PPI (Figure 2, center panel) [Age: $F(1,61)=101.74$, $p<0.001$]. However, aged mice showed significantly greater visual PPI (light prepulse to 120 dB pulse) PPI [$F(1,61)=5.4$, $p<0.05$] compared to young animals. To further examine startle-independent effects of aging on visual PPI, we compared PPI of aged and young mice matched for similar startle reactivity (removal of mice with startle reactivity less than 45 or greater than 120 startle units to 120 dB pulse) which replicated the previous analysis of covariance results (Figure 2, right panel) [Age $\times$ Modality: $F(1,26)=104.13$, $p<0.001$, $N=12-16/\text{group}$] Post-hoc analysis in this baseline-matched group still showed significantly reduced acoustic PPI [Age: $F(1,26)=83.87$, $p<0.001$, Tukey test] and significantly greater visual PPI [$F(1,26)=7.56$, $p<0.05$, Tukey test] in aged compared to young mice. As in previous reports (Brody *et al.*, 2004), there was very low PPI in young mice using the puff as a pulse, thus creating floor effects precluding the detection of an age effect; hence these data were not included in the above analysis.

Experiment 3: Tactile prepulse inhibition in aged mice

Because aged mice exhibited significantly greater visual PPI than young mice, we designed an experiment to examine tactile PPI using low-intensity, short-duration electrical stimulation as the prepulse. There were no significant age effects on PPI with electrical stimulation (Figure 3, left panel) [$F(1,60)<1$, N.S.], however, PPI was significantly increased at higher shock intensities [$F(2,120)=12.65$, $p<0.001$]. As in experiments 1 and 2, aged mice exhibited a large reduction in startle reactivity (Figure 3, left inset) [$F(1,60)=119$, $p<0.001$]. When data were covaried for startle reactivity, PPI was significantly increased in aged mice (Figure 3, center panel) [$F(1,59)=3.3$, $p=0.07$] compared to young mice. When animals were removed (120 dB pulse less than 90 or greater than 147 arbitrary startle units) to baseline match startle reactivity across age (Figure 3, right inset) [$F(1,13)=4.17$, N.S.], aged mice again exhibited increased PPI [$F(1,13)=5.93$, $p<0.05$] compared to young mice (Figure 3, right panel). We also measured reactivity to the tactile prepulses, with increasing intensities inducing increased reactivity ($F(1,59)=53.0$, $p<0.0001$) which were significantly lower overall in aged mice ($F(1,59)=23.8$, $p<0.001$). In the baseline matched group (Figure 3, right panel), reactivity to the shock prepulses did not differ ($F(1,13)<1$, N.S.). Data on startle reactivity to tactile pulse alone are presented in table 1.

Correlation of PPI to cognitive performance

The mice used in the present studies were also assessed on their performance in the attentional set-shifting task (ASST; Young, Powell, Geyer, Jeste, & Risbrough, in press). We performed Spearman rank correlations of the performance of these mice in the PPI studies described above and in the ASST. Trials to criterion and mean correct latency performance measures from each stage of the ASST were utilized. Startle levels exhibited a modest correlation with simple discrimination (a measure of simple learning; Young, Powell, Risbrough, Marston, & Geyer, 2009a), in both trials to criterion and mean correct latency ($\rho = 0.338, -0.43, p < 0.05$, and 0.01 respectively). PPI with an acoustic prepulse (experiment 1) also moderately correlated with compound discrimination ($\rho = 0.346, p < 0.05$). No other significant correlations were observed ($p > 0.05$; Table 2). Correlational analyses were also performed between behaviors within each age group. The only significant correlation observed was in aged mice for trials to criterion in extradimensional shifting strongly correlated with PPI when light was used as the prepulse ($\rho = 0.656, p < 0.0005$). A Bonferroni correction was used given the number of correlations made, suggesting an $\alpha < 0.0009$ was required for statistical significance however.

Discussion

Consistent with previous reports across species, mice exhibit reduced startle amplitude with increased age, likely to be as a result of impaired hearing. Mice also exhibited increased PPI with age – consistent with human testing when group differences, measurement techniques across sensory modalities, and sample size were taken into account (Ellwanger *et al.*, 2003). Thus the present data provide further support of the cross-species translational validity of PPI testing, but also highlight the need to control for startle during analyses.

Reduction in startle reactivity with aging has been observed in many species including humans (Ellwanger *et al.*, 2003; K. Ludewig, Ludewig *et al.*, 2003), rats (Ison *et al.*, 1997; Varty, Hauger, & Geyer, 1998) and mice (Ison *et al.*, 1997; A. M. Ouagazzal *et al.*, 2006). Thus our data are consistent with previous findings. In contrast however, our data indicate that non-acoustic PPI in mice improves with age (compared to Ison *et al.*, 1997). This increase in PPI is however consistent with previous reports in humans (Ellwanger *et al.*, 2003) and was only observed using cross-modal PPI testing and when taking into account differences in startle reactivity. Differences in startle reactivity were accounted for by 1) using it as a covariate in the analyses of PPI performance, or 2) baseline matching startle reactivity between the two groups.

These techniques were possible due to the utilization of large sample sizes in the present study, which may have also contributed to the different findings from previous studies using smaller sample sizes (e.g. $n=5$; Ison *et al.*, 1997). Moreover, consistent with the study in humans (Ellwanger *et al.*, 2003), in the present study we have presented PPI data on young and aged mice which exhibit demonstrably different behaviors in other paradigms. For example, the aged mice exhibited poorer performance in the attentional set-shifting task (ASST) performance (Young *et al.*, in press). We did not observe any significant correlations between PPI and any component of the ASST in these mice when both age groups were combined. We did however observe a strong correlation between extradimensional shifting and PPI when light was used as a prepulse, in aged mice only. Thus older mice with higher PPI (using light as a prepulse) exhibited poorer set-shifting performance, a measure of executive functioning (Young *et al.*, 2009a). The fact that only light PPI showed this link weakens any sweeping statements about a PPI link to executive function in aged animals, and should be viewed with caution. Further caution is necessary given that these correlations did not reach significance following a Bonferroni correction given the large number of multiple comparisons. While an initial report in humans suggested

that PPI performance may correlate with WCST performance (Filion, Kelly, & Hazlett, 1999), these reports were not followed by peer-reviewed publications nor replicated by others (Rabin, Sacco, & George, 2009; Swerdlow *et al.*, 2005; for review see Young *et al.*, 2009a). Correlations between drug-induced improvements in PPI and executive functioning have been observed after morphine (Quednow, Csomor, Chmiel, Beck, & Vollenweider, 2008) or tolcapone (Giakoumaki, Roussos, & Bitsios, 2008) treatment in healthy controls, or nicotine in schizophrenia patients (Rabin *et al.*, 2009), and may suggest that acute improvements in PPI may be linked to improvements in frontal cortex function. Correlations of baseline PPI with cognitive measures, however, have been more mixed (for review see Young *et al.*, 2009a). The present studies are consistent with Ellwanger and colleagues (2003) in that baseline PPI performance did not correlate with simple learning (simple discrimination), or executive functioning (extradimensional shifting) in young healthy animals, nor did it compellingly predict executive function in aged mice.

The primary finding of the present studies was that consistent with humans, aged mice exhibit increased PPI that was likely to be a reflection of neuronal changes accompanying aging. The source of such changes may prove difficult to identify. One speculation is that it is possible that the increased PPI observed to tactile and visual stimuli reflect a processing bias towards these stimuli that remain relatively intact in aged animals. Indeed, the weak levels at which these measures correlate with one other or with other cognitive tasks suggests that they may reflect orthogonal processes. At the mechanism level, increased PPI can be observed with a reduction in functional dopamine D2 receptors (Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001). Thus the increased PPI with age demonstrated here could have been due to the reduction in dopamine D2 receptors observed with aging (Ingram, Ikari, Umegaki, Chernak, & Roth, 1998; Morgan *et al.*, 1987). Alternatively, it is possible that with age comes a shift in the activation of cortical-striatal loop circuitry that favors stronger inhibition of startle by prepulses (Swerdlow, Geyer *et al.*, 2001).

Although previous studies suggest that startle reactivity was unrelated to PPI performance (Ison *et al.*, 1997), recent evidence suggests there may be some commonality between the two measures. While it is true that experimental effects on startle can be observed without affecting PPI and vice-versa, it has been proposed that when startle reactivity differences are observed, measures should be taken to ensure the reliability of the findings (Csomor *et al.*, 2008). Previous research has demonstrated startle reactivity impacting PPI in mouse drug studies (Yee *et al.*, 2004), across rat strains (Hince & Martin-Iverson, 2005), in neuropsychiatric groups (S. Ludewig *et al.*, 2005; Perry, Minassian, Lopez, Maron, & Lincoln, 2007), and in human drug studies (Heekeren *et al.*, 2007; Vollenweider, Csomor, Knappe, Geyer, & Quednow, 2007), where low startle resulted in high PPI scores in each case. The impact of startle on PPI was systematically assessed by Csomor *et al.* (2008). Csomor *et al.* (2008), reported that PPI can be affected by baseline startle reactivity in humans and mice, and suggest covarying analyses with startle reactivity as a second screen when investigating experimental affects in PPI. These proposals are supported by the current data and using an ANCOVA to correct for baseline startle reactivity differences may prove to be a necessary step in interpreting PPI changes across populations with different baseline startle reactivity.

The present studies also support the use of cross-modal assessment of PPI when differences in hearing may affect PPI performance (A. M. Ouagazzal *et al.*, 2006). The differences in PPI following cross-modal PPI testing are consistent with previous research whereby PPI differences were only observed with cross-modal testing in genetically modified mice (Barr *et al.*, 2007). When investigating aging effects on rats in PPI, Varty and colleagues (1998) utilized cross-modal PPI test sessions, using light as a prepulse. Consistent with this study and previous data, reduction in startle reactivity was observed in the oldest rats (Varty *et al.*,

1998). In contrast with the present findings however, aged rats exhibited reduced PPI when compared to younger rats. The discrepancy in the findings could be species specific, for as discussed above, previous research on human and mouse PPI suggest no change or increased PPI with age (Ellwanger *et al.*, 2003; Ison *et al.*, 1997; K. Ludewig, Ludewig *et al.*, 2003). Alternatively, the discrepancy between the present study and that of Varty and colleagues (1998) could have been due to the latter study analyzing PPI without using startle reactivity as a covariate. Here we also used very mild electrical footshock to act as a prepulse to assess the tactile prepulse modality. Although these prepulses did induce some reactivity on their own, it is unlikely that this mild reactivity resulted in the increase in PPI in aged mice observed. PPI across all mice was increased with shock intensity (Table 1), yet aged mice showed lower reactivity to the prepulses while exhibiting greater inhibition than young mice. This rarely used method of tactile prepulse may therefore prove useful to probe PPI in populations with visual or auditory deficits (Parisi & Ison, 1979).

The current findings corroborate the cross-species translational validity of PPI testing. Moreover, the data lend support to an increasing number of studies documenting steps to be taken when analyzing PPI data across populations with differing baseline startle. These findings may prove important when assessing experimental effects on PPI across mouse models of neuropsychiatric/aging disorders, such as genetics of aging performance (Glatt *et al.*, 2007). Finally cross-modal PPI testing may be useful in future research investigating correlative changes in mouse cognitive performance, such as assessing processing speed/attention in 5-choice serial reaction-time tasks (Young *et al.*, 2009b). Thus genetic contribution to PPI performance could be assessed despite a reduction in baseline startle reactivity or hearing loss confounds.

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Figure 1.

Aged mice exhibit acoustic prepulse inhibition deficits independent of startle.

Left panel: Acoustic PPI in aged and young mice across increasing prepulse intensities.

Left inset: Startle magnitude to 120 db pulse across young and aged mice. *Right panel:*

Acoustic PPI to 73 db prepulse preceding a 105 db and 120 db pulse in young and aged

mice, respectively. *Right inset:* Startle magnitude to 105 db and 120 db pulse in young and

aged mice, respectively. Data shown in panels are mean % PPI $\pm$ SEM. Data shown in insets

are mean startle magnitude (arbitrary units) $\pm$ SEM. ** $p < 0.01$ vs. young, $n = 29-32$.

Figure 2.

Prepulse inhibition in mice is dependent on modality.

Effects of 73 db acoustic prepulse and light prepulse were compared in young and aged mice. *Left panel:* Aged mice exhibited deficits in acoustic but not light PPI, ** $p < 0.01$ vs. young, $n = 29-32$. *Left Inset:* Startle magnitude to 120 db pulse across young and aged mice. *Center panel:* Analysis of covariance with baseline startle reactivity. *Right panel:* Baseline matching for similar startle reactivity across groups (Young = 12, Aged = 16). *Right inset:* Startle magnitude to 120 db pulse across young and aged mice, matched for similar reactivity levels. Data shown in panels are mean % PPI $\pm$ SEM. Data shown in insets are mean startle magnitude (arbitrary units) $\pm$ SEM.

Figure 3.

Effect of tactile (low intensity foot shock) prepulse was compared in young and aged mice. *Left panel:* Aged mice exhibited no deficits in tactile PPI. *Left Inset:* Startle magnitude to 120 db pulse across young and aged mice. *Center panel:* Analysis of covariance with baseline startle reactivity. Main effect of age, $p=0.07$. *Right panel:* Baseline matching for similar startle reactivity across groups (Young = 8, Aged = 7). Main effect of age, $p<0.05$. *Right inset:* Startle magnitude to 120 db pulse across young and aged mice, matched for similar reactivity levels. Data shown in panels are mean % PPI $\pm$ SEM. Data shown in insets are mean startle magnitude (arbitrary units) $\pm$ SEM.

Table 1

Reactivity to tactile prepulse trials. Data presented as mean ($\pm$ standard deviation)

		All mice				Startle matched mice			
Prepulse (mA)		0.02	0.04	0.06	0.06	0.02	0.04	0.06	0.06
Young		15.7 ($\pm$ 15)	41.5 ($\pm$ 32)	64.7 ($\pm$ 42)	64.7 ($\pm$ 42)	14.8 ($\pm$ 16)	24.1 ($\pm$ 19)	43.2 ($\pm$ 36)	43.2 ($\pm$ 36)
Aged		10.9 ($\pm$ 7)	14.9 ($\pm$ 7)	23.7 ($\pm$ 15)	23.7 ($\pm$ 15)	11.5 ($\pm$ 12)	15.5 ($\pm$ 8)	33.3 ($\pm$ 20)	33.3 ($\pm$ 20)

Table 2

Correlations between PPI and cognitive studies

ASST Stage		PPI Acoustic	PPI Light	PPI Tactile	Startle
Trials to Criterion	SD	rho	.140	.208	.338
		p	.409	.216	.041
	CD	rho	-.200	-.153	-.119
		p	.235	.367	.483
	CDR	rho	-.086	.024	-.139
		p	.613	.888	.266
	ID	rho	-.002	-.085	-.071
		p	.990	.615	.408
	IDR	rho	.209	-.020	.160
		p	.213	.906	.346
	ED	rho	-.052	.217	-.077
		p	.758	.198	.708
	EDR	rho	.157	.154	-.052
		p	.355	.363	.759
	SD	rho	-.038	-.199	-.104
		p	.825	.237	.008
Mean Correct Latency	CD	rho	.346	-.008	.126
		p	.036	.964	.499
	CDR	rho	-.030	-.161	-.019
		p	.858	.340	.910
	ID	rho	-.097	-.076	-.063
		p	.567	.656	.710
	IDR	rho	.052	-.063	-.166
		p	.760	.711	.326
	ED	rho	-.235	-.085	-.262
		p	.162	.617	.118
		rho			
		p			
		rho			
		p			
		rho			
		p			

ASST Stage		PPI Acoustic	PPI Light	PPI Tactile	Startle
	EDR				
	rho	.182	.266	-.307	.093
	p	.281	.111	.065	.584

The Spearman's Rank correlation coefficients for PPI performance in the present studies to cognitive performance measured in the attentional set-shifting task (ASST). Each stage of the ASST is presented, including simple discrimination (SD), compound discrimination (CD), CD reversal (CDR), intradimensional (ID) shifting, ID reversal (IDR), extradimensional (ED) shifting, and ED reversal (EDR). Correlational statistics are presented for both trials to criterion and mean correct latency for each stage. Shaded cells represent significant correlations ($p<0.05$).