Title: Stimulus quality affects expression of the acoustic startle response and prepulse inhibition in mice

Abbreviated Title: Parametric effects on startle in mice

Authors: C.W. Stoddart^{1,2}, J. Noonan² & M.T. Martin-Iverson^{1,2,3}

¹Western Australian Institute for Medical Research and UWA Centre for Medical Research, University of Western Australia, 35 Stirling Hwy, Crawley, W.A. 6009, Australia.

²Pharmacology & Anaesthesiology Unit, University of Western Australia, 35 Stirling Hwy, Crawley, W.A. 6009, Australia.

³Centre for Clinical Research in Neuropsychiatry, Graylands Hospital, Private Bag #1, Claremont W.A. 6910, Australia.

Corresponding Author:

Mr. Chris Stoddart,

Pharmacology & Anaesthesiology Unit M510

University of Western Australia

35 Stirling Hwy

Crawley, 6009, W.A. Australia.

Ph. (61-8) 9347 6426

Fax. (61-8) 9346 3469

Email: cwstodda@student.uwa.edu.au

Keywords: Startle reflex, PPI, mouse, SIRM, reflex threshold, reflex capacity

Acknowledgements: Funding for this project was gratefully received from the Western Australian Institute for Medical Research (W.A.I.M.R.) and the National Health and Medical Research Council of Australia (NHMRC).

Abstract

The relationship between stimulus intensity and startle response magnitude (SIRM) can assess the startle reflex and prepulse inhibition (PPI) with advantages over more commonly used methods. The current study used the SIRM relationships in mice to determine differences between white noise and pure tone (5 kHz) stimuli. Similarly to rats, the SIRM relationship showed a sigmoid pattern. The SIRM derived reflex capacity (R_{MAX}) and response efficacy (slope) of the white noise and pure tone stimuli in the absence of prepulses were equivalent. However the pure tone startle response threshold (D_{MIN}) was increased, while the stimulus potency ($1/ES_{50}$) was decreased when compared to white noise. Prepulses of both stimulus types inhibited R_{MAX} and increased D_{MIN} , but the white noise prepulses were more effective. Both stimulus intensity gating and motor capacity gating processes are shown to occur, dependent on prepulse intensity and stimulus onset asynchrony. Prepulse intensities greater than 10 dB below the startle threshold appear to produce PPI via stimulus intensity gating, while a motor capacity gating component appears at prepulse intensities near to the startle threshold.

Introduction

Experimentally-induced decreases of the prepulse inhibition of the startle reflex (PPI) in rodents are used to model the “deficits” in PPI observed in schizophrenics (Braff et al., 1978; Geyer and Braff, 1982; Swerdlow et al., 1986; Swerdlow et al., 2000). Since its characterisation by Graham 1975 (Graham, 1975), the model has been developed with number of major research streams. The first stream focussed on the potential of the model to screen for effective antipsychotics (Worms et al., 1983; Rigdon and Viik, 1991) and the second stream concentrated on elucidating the neurobiology underlying sensorimotor gating (Hoffman and Ison, 1980; Meloni and Davis, 1998; Pouzet et al., 1999). Another research stream has been to characterise PPI in numerous mouse strains, especially in mouse strains following transgenic or gene knockout manipulation. The methodologies used to expound these experimental aims are quite divergent, characteristic of the numerous manipulations available to the model (Geyer and Swerdlow, 1998; Flaten et al., 2005; Yee et al., 2005; Schmajuk et al., 2006). This variance in methodologies of the PPI field and the lack of inter-laboratory replication has prompted researchers to re-address certain features inherent to the model (Plappert et al., 2004; Hince and Martin-Iverson, 2005). This particular study focuses on a number of the parametric aspects of murine PPI, in particular the startle and PPI effects of pure tone (5 kHz) and white noise stimuli.

A fundamental requirement for models used in drug screening is reproducibility. The predictive validity of the PPI model for screening potential antipsychotics was reviewed in the early 1990's (Swerdlow et al., 1994). An expanding PPI literature has revealed rodent PPI models exhibit problems in reproducibility due, in part to pharmacological ligand selectivity (Seeman and Ulpian, 1988; Martin-Iverson and Else,

2000), genetic influences (Crabbe et al., 1999) and epigenetic factors, such as cross fostering (Francis et al., 2003).

Recent advances in genetic technologies have led to the frequent use of mouse models of PPI. This has revealed major differences in rat and mouse PPI (Swerdlow and Geyer, 1998). Since much of our PPI knowledge has been gathered in the rat model, researchers required new stimulus parameters to compensate for the species differences (Plappert et al., 2004). Differences in such methodological compensations have further contributed to variability between laboratories. Empirical evidence from our laboratory using two different startle packages has revealed quantitative startle differences using the same startle procedure but different calibration methods. Ison's review addresses instrumentation effects and shows some of this variance is related to differing force transducer properties of different instrumentations (Ison, 2001), such as San Diego Instruments (Swerdlow et al., 1998), Med-Associates (Hince and Martin-Iverson, 2005) and Coulbourn Instruments (Rigdon and Viik, 1991). By using both pure tone and noise stimuli with varying intensity, this study aims to characterise aspects of the murine startle and PPI phenotype and address some of these methodological issues.

The congenic strains of C3H/ mice used in this study have been selected since they share a common genetic background, but have different alleles at a locus that confers sensitivity to dopamine agonists (Stoddart et al., 2004). This experiment will aim to identify features of startle and PPI common to all three of these congenic strains, rather than analyse specific strain traits. The primary goal is to develop a standardised PPI methodology which allows appropriate comparisons across variations in stimulus type, genetic and environmental factors.

Methods

Animals

All procedures conformed to the Australian Code of Practice for the care and use of animals for scientific purposes and were conducted under approval from the Animal Experimentation Ethics Committee of the University of Western Australia. The experiments detailed in this paper used two separate colonies of mice from the Animal Resources Centre of Western Australia, where numbers were subject to breeding availability. The first experiment used experimentally naïve C3H/ mice (C3H/HeARC (n=12), C3H.PRI-*Flv*^r (n=11) and C3H.PRI-*Diht* (n=11)) aged 8-10 weeks at the time of testing (~25 g). The second experiment used experimentally naïve C3H.PRI-*Flv*^r (n=12) mice aged 12 weeks at the time of testing. Mice were housed according to the strain in groups up to 12 mice per cage and allowed to habituate to the colony room for seven days. Animals were fed ad libitum with commercial rodent pellets (Glen Forrest Animal Feeds) and had unlimited access to water. The colony room was maintained at 21°C and had a 12:12-hr light: dark cycle with light period from 0600 to 1800 hours.

Apparatus

Startle reflex measurements were carried out using two different Med Associates MED-ASR-PRO1 startle packages (MED Associates Inc, Georgia VT, USA). Both packages used load cells but the Med Associates startle reflex software upgrade (Med Associates SOF-815 v4.43 to SOF-815 v5.0) meant the chambers were calibrated differently. In both packages startle response was evaluated as the area under the curve for the largest peak amplitude. Area under the curve provided a more reliable estimate of

startle magnitude at calibration sensitivities set to evaluate the orienting response to the prepulse.

Eight startle reflex systems (chambers) were used for startle reflex measurement. Each system was placed within a sound-attenuating chamber equipped with a ventilation fan (~63 dB when operating).

In the older startle package (v 4.43) stimuli decibel levels were recorded with a calibration direction sound level meter (Type 1405C Serial No. A3747, Dawe Instruments Limited). Startle chamber load cells were calibrated using the Med-Associates solenoid loaded with a 13.55 g weight. Chambers were set such that the mean solenoid calibrator response for each platform was 140 calibration units.

The stimulus intensity of the eight startle reflex systems with the upgraded software package (v5.0) were calibrated daily using the Med Associates ANL-929A-PC USB sound pressure level measurement package (microphone contained in the acrylic mouse restraint). Startle chamber load cells were calibrated daily using calibration weights, such that 120 g equated to 240 units under the calibration setting.

Experiment 1 used the new startle reflex package while Experiment 2 was completed using the older software package. In both experiments mice were restrained in the startle chambers using Med Associates ENV-263A animal holders (3.2 cm dia.)

Procedures

Startle Experiment 1. - *White noise vs pure tones*

C3H/ mice (n=34) were used to compare the effects of white noise and pure tones (5 kHz) on startle reactivity and PPI. Each session began with a 5 min acclimation period to a 70 dB white noise background, which persisted throughout the session as background

noise. Background noise intensity was selected as the lowest 5 dB increment capable of masking the noise of the ventilation fan (67 dB). Startle stimuli were 40 ms duration pulses of either white noise or pure tones (5 kHz). The 5 kHz pure tone was selected on the basis of speaker frequency sensitivity and the fact that 5 kHz pure tones show little age related decline in C3H/ mice (Trune et al., 1996). In addition, 5 kHz pure tones show a linear stimulus intensity: response magnitude proportion not apparent at some other frequencies (Parham and Willott, 1988).

Stimulus onset asynchronies (SOA) of 10, 50 and 100 ms were used for the prepulse trials. Prepulse stimuli were either white noise or a 5 kHz pure tones of 75 dB (5 dB above background) paired with the startle stimuli of the same quality. All prepulse stimuli had durations of 8 ms with 1 ms rise and fall times. This experiment used 2 types of stimuli (white noise and pure tone), 11 startling pulse intensities (70-120 dB in 5 dB increments) and 4 prepulse conditions (no pulse, 10, 50 & 100 ms SOA) to give 88 unique trial types. Each session consisted of 3 blocks of 88 randomised trials and subjects were tested over two consecutive days, so that each mouse experienced 6 repeats for each trial type. The trial order varied across all 3 blocks and all animals received the same trial order. A variable inter-trial interval of 10-15 s was used.

Startle Experiment 2. – *Prepulse stimulus intensity and the startle reflex*

The second experiment was a prepulse intensity investigation using a separate colony of experimentally naïve C3H.PRI-*Flv^r* mice (n=12). The test session involved presentation of 180 trials divided into 3 blocks, where each block consisted of 3 sets of 20 trials. Startling stimulus (S_2 – 40 ms duration, 0 ms rise/fall time) and prepulse stimulus (S_1 – 8 ms duration, 1 ms rise/fall time) were presented as 5 kHz pure tones

while the background noise used was a 65 dB white noise (the lowest intensity capable of masking the ventilation fan noise, 63 dB). Individual trial types included: 4 startling stimulus only trials (2 with $S_2=110$ dB, and 2 with $S_2=115$ dB) and 16 prepulse + pulse trials with SOA = 50 ms (8 trials with $S_1=65-100$ dB in 5 dB increments + $S_2=110$ dB; 8 trials with $S_1=65-100$ dB in 5 dB increments + $S_2=115$ dB). All trial types within a set of 20 trials were randomized in order, but all mice followed the same order. Pure tone stimuli were used to allow for selection of a greater number of prepulse intensities below startle threshold.

On the day after the completion of the prepulse intensity experiment, the mice were tested in a startling stimulus only program to generate a SIRM function for this colony. This program consisted of 192 trials divided into 3 blocks of 8 sets of 8 trials. Each set contained 8 randomised startling stimulus only trials with intensities varying from 85-120 dB in 5 dB increments.

Calibration Comparison

This study investigated calibration differences between the Med Associates ANL-929A-PC USB sound pressure level measurement package and the calibration direction sound level meter (Type 1405C Serial No. A3747, Dawe Instruments Limited) using the newer Med Associates MED-ASR-PRO1 startle package (SOF-815 v5.0). In this study stimuli between 75-100 dB (at 5 dB increments) were delivered as determined by the ANL-929A sound pressure level measurement package and plotted against the measurements made with the Dawe sound level meter.

Data Analysis – Fitting Method

The relationship between stimulus intensity and acoustic startle response is described by a sigmoidal relationship (Martin-Iverson and Stevenson, 2005). In order to assess the effect of stimulus type and prepulse, sigmoidal curves were fitted with a non-linear regression method to individual mice across the range of stimulus intensities for each SOA condition using the logistic function:

$$(1) \quad y = \frac{1}{\frac{1}{R_{MAX}} + b_0 b_1^x}$$

Where y is defined as the ASR, b_0 and b_1 are the logistic coefficients, reflex capacity (R_{MAX}) is the mathematical asymptote and x is the stimulus intensity in dB. The Levenberg-Marquardt algorithm for iterative estimation was used to determine b_0 , b_1 , and R_{MAX} , with constraints $b_0 > 0$, $0 < b_1 < 1$, and $0.9 \times \text{observed maximum response} \leq R_{MAX} < 2 \times \text{observed maximum response}$ (see Hince and Martin-Iverson, 2005). Several parameters were estimated from this model; D_{MIN} , the response threshold and point of maximum acceleration of the function; ES_{50} , the intensity that produces a half-maximum response and the *slope* of the linear region bounded by D_{MIN} and D_{MAX} , (symmetrical with D_{MIN} reflected about ES_{50}). Where the algorithm predicted values for D_{MIN} , D_{MAX} or ES_{50} outside the range of stimulus intensities used, the value was constrained to the nearest boundary range to which it fell (i.e. 120 dB for the upper limit or 70 dB for the lower limit). This was done to constrain values to the range of intensities used, which is a conservative estimation approach. If there was 100% inhibition and a flat line relating stimulus intensity to response magnitude (i.e. when prepulse $R_{MAX} < \text{startle stimulus only } D_{MIN}$), the lowest a threshold could possibly be is the highest intensity used (120 dB). In

these cases, R_{MAX} was set to the maximum observed value, slope was set to 0 and ES_{50} and D_{MIN} were set to 120 dB.

The stimulus intensity response magnitude (SIRM) parameters (R_{MAX} , D_{MIN} , ES_{50} and *slope*) derived from the fitted logistic functions were analysed by the general linear model repeat measures analysis of variance (GLM-ANOVA) with prepulse (4 levels with the first being the absence of a prepulse) and stimulus type (2 levels) as within-subjects variables. Where the assumption of sphericity was violated as determined by Mauchley's W statistic), the Geisser-Greenhouse df correction for exact F was used for assessment of significance.

Prepulse inhibition of the reflex capacity was calculated using the following equation:

$$(2) \quad \%PPI = \frac{R_{MAX_startle} - R_{MAX_prepulse}}{R_{MAX_startle}} \times 100$$

$R_{MAX_startle}$ = R_{MAX} for the SIRM curve in the startle stimulus alone condition

$R_{MAX_prepulse}$ = R_{MAX} for the SIRM curve in the prepulse condition

In addition, the shift in threshold was calculated by subtracting the startle stimulus only threshold ($D_{MIN_startle}$) from the startle reflex threshold in the presence of the prepulse ($D_{MIN_prepulse}$), because the stimulus measure (dB) is a log scale. GLM ANOVA was then conducted on the PPI data to assess the effect of stimulus type (2 levels) and prepulse SOA (3 levels).

Pairwise comparisons were carried out using Sidak's t-test method adjusted for post hoc multiple comparisons. Alpha level of 0.05 was used for all statistical tests. The partial η^2 was used as a measure of effect size and the power was also reported in some

cases where no significant result was obtained to assist interpretation. All the logistical fitting and statistical analysis was carried out using SPSS-PC 12.0 for windows.

Results

Experiment 1 – The effect of stimulus quality on startle and PPI

Stimulus type effects on startle

The first experiment showed sigmoid relationships between stimulus intensity and the startle response in mice presented with either white noise or pure tone (5 kHz) stimuli (Fig. 1). In a proportion of mice, PPI was so large that the sigmoidal stimulus: response relationship was lost due to the near 100% inhibition of response. The frequency of individuals in each prepulse condition showing a sigmoidal stimulus: response relationship is reported in Table 1. This shows the stimulus effects on the proportion of mice showing complete inhibition. In individuals showing a sigmoidal relationship between stimulus and response, the mean goodness of fit for each condition is shown in Table 2. The mean squared correlation co-efficient (R^2) for the white noise stimuli (0.828 ± 0.024) was significantly lower ($F_{(1, 33)}=21.5$, $p<0.0005$) than the pure tone data (0.934 ± 0.008), shown in table 2. Not surprisingly, inhibition of the startle reflex by prepulses was associated with a breakdown of the SIRM relationship, and this was strongest with the most effective inhibiting stimulus (the white noise). In these cases the conventions for determining stimulus parameters for analysis are described in the methods section.

Differences in the relationships between startling stimulus intensity and the startle response as a function of stimulus type (white noise or pure tone) involved selectively the stimulus measure, threshold (D_{MIN} , which is a measure of *stimulus sensitivity*) and the

parameter most strongly dependent on threshold (but also modified by efficiency, as indicated by slope) the ES_{50} measure. Like the pharmacological dose response parameters ED_{50} or EC_{50} , the ES_{50} is the startling stimulus intensity that sustains a half-maximal response and is the inverse of *stimulus potency*. Both the reflex threshold and ES_{50} with white noise startling stimuli were significantly lower than those with pure tone (5 kHz) stimuli (threshold: $F_{(1, 33)}=121.93$, $p<0.0005$, see Fig. 2b; ES_{50} : $F_{(1, 33)}=100.73$, $p<0.0005$; see Fig. 2d). Mice were more sensitive to white noise stimuli than to 5 kHz pure tone stimuli suggesting that white noises were more potent startling stimuli than pure 5 kHz pure tones.

Interestingly, while the differences in stimulus type resulted in the curves being shifted to the right by pure tones relative to white noise tones, as shown by increased thresholds (decrease in stimulus sensitivity) and ES_{50} s (decrease in potency), there was no effect of stimulus type on either the response measure (R_{MAX}), or the sensorimotor integration measure (slope, the change in response due to a 1 dB increase in startling stimulus intensity within the dynamic range). There was no main effect of startling stimulus type on R_{MAX} on trials with the startling stimulus only ($F_{(1, 33)}=2.28$, $P=0.145$; see Fig. 2a) or on slope ($F_{(1, 33)}=0.27$, $p=0.610$, $\eta^2=0.008$ see Fig. 2c).

The MedAssociates startle package allows for multiple data collection periods within a single trial, where recording was divided into a null, prepulse and startle period. Responses to the prepulse alone were measured in trials with a prepulse period equal to the startle period (i.e. when SOA = 100ms). Analysis of the responses to the prepulse only component of the 100 ms SOA trials showed the startle response to the white noise prepulse was significantly greater than the pure tone prepulse ($F_{(1, 33)}=225.0$, $p<0.0005$;

Fig. 2a). This supports the potency finding, suggesting that white noise is a significantly more potent startle stimulus than pure tones.

Stimulus type effects on PPI

Reflex Capacity - R_{MAX}

Unlike the startling stimulus only condition, the R_{MAX} in the presence of both a prepulse and a startling pulse was dependent on stimulus type, with PPI being greater with white noise prepulses relative to pure tone prepulses (main effect of stimulus type, $F_{(1, 33)} = 177.3$, $p < 0.0005$, $\eta^2 = 0.843$). Figure 2a shows that reflex capacity was reduced by the prepulses of both stimulus types, as a function of SOA as shown by a significant main effect of the SOA ($F_{(2.7, 89.5)} = 136.7$, $p < 0.0005$, $\eta^2 = 0.806$). There was also a significant interaction between the stimulus type and the SOA ($F_{(1.8, 59.5)} = 32.8$, $p < 0.0005$). This interaction is due to two major effects as shown by pairwise comparisons. The first is that there is greater inhibition of startle R_{MAX} on prepulse + pulse trials with white noise ($p < 0.0005$ for each SOA), but no stimulus difference on the startle stimulus only trials. Secondly, the prepulse with 50 ms SOA for white noise stimuli produced greater inhibition than prepulses with either 10 or 100 ms SOAs ($p < 0.0005$ for each pairwise comparison) but effects of pure tone stimuli did not differ significantly among SOAs.

PPI can be assessed in four ways when the startle reflex is assessed by SIRM functions: decreases in reflex capacity (R_{MAX}) or efficiency (slope), or increases in thresholds or ES_{50s} . The measure closest to the conventional measure of PPI, as a prepulse-induced reduction in startle magnitude elicited by a startling stimulus of a single absolute intensity, would be the reduction in reflex capacity relative to the startling pulse only condition (Fig. 3a). The %PPI R_{MAX} data show that white noise prepulses more

effectively reduce reflex capacity relative to pure tone prepulses (main effect of stimulus type; $F_{(1, 33)}=201.4$, $p<0.0005$). Interestingly both stimulus types produced a maximum reduction in R_{MAX} with an SOA of 50 ms (Fig. 3a), characteristic of the inverted U shape of PPI observed in other human (Braff et al., 1978; Flaten et al., 2005) and rodent studies (Martin-Iverson and Else, 2000). The effect of SOA was more prominent when using the white noise stimuli, shown by the significant interaction between stimulus type and SOA ($F_{(1.5, 50.3)}=12.5$, $p<0.0005$). This indicates that SOA becomes more important when the magnitude of PPI is larger (See Fig. 3a).

Startle reflex excitability/threshold - D_{MIN}

Startle reflex excitability (D_{MIN}) differed significantly depending on the stimulus type (stimulus type main effect, $F_{(1, 33)}=135.6$, $p<0.0005$). The threshold of white noise-elicited startle was significantly lower than the threshold of the 5 kHz pure tone elicited startle, independent of the presence of prepulses.

The prepulses had an overall effect of increasing the startle reflex threshold (main effect of prepulse: $F_{(2.3, 77.4)}=15.2$, $p<0.0005$), effectively shifting the SIRM curves to the right. When a GLM ANOVA was conducted on the pure tone thresholds alone, the effect of the prepulse-induced shift was significant but with a relatively small effect size ($F_{(2.2, 73.1)}=3.12$, $p=0.045$, $\eta^2=0.087$). No significant pairwise comparisons were reported.

The magnitude of prepulse-induced shifts in the reflex threshold can be more clearly seen in figure 3b, in which the prepulse-induced shift in threshold relative to the startle stimulus only condition is shown. There was a significant interaction between stimulus type and SOA on the prepulse-induced shift in threshold ($F_{(1.9, 62.3)}=17.6$,

$p < 0.0005$), but not a significant main effect of stimulus type. The 10 ms white noise prepulse facilitated low intensity startle (Fig. 1a), shifting the SIRM curve to the left. The 100 ms white noise prepulse increased the threshold (D_{MIN}) to a higher intensity, shifting the SIRM curve to the right. The effect of the white noise prepulse at the 50 ms SOA was intermediate between the 10 and 100 ms SOAs (Fig. 3b). Pure tone prepulses at all SOAs shifted the startle threshold to a higher intensity. Thus, the effect of SOA of the prepulses on startle thresholds differed markedly between the two stimulus types.

Startle Stimulus Efficiency – Slope

The sigmoidal relationship of the SIRM algorithm shows a dynamic range between D_{MIN} and D_{MAX} that exhibits a more-or-less linear relationship between stimulus intensity and startle response. The slope of the dynamic range bounded by D_{MAX} and D_{MIN} can be used to evaluate the efficacy with which a stimulus elicits a startle response. Under startle stimulus only conditions there was no effect of stimulus type on startle efficacy (Pairwise comparisons, $p = 0.61$). Despite the pure tone stimulus being less potent and having an increased threshold, the efficiency with which the stimulus induces startle within the dynamic range is equivalent to white noise.

Figure 2c shows that the prepulses significantly inhibited the startle stimulus efficacy regardless of the type of stimulus (main effect of prepulse $F_{(2,3, 75,9)} = 22.7$, $p < 0.0005$). The prepulse-induced reductions in efficacy were larger when white noise stimuli were used relative to pure tone stimulus (stimulus type by SOA interaction: $F_{(1,9, 63)} = 3.4$, $p = 0.045$, $\eta^2 = 0.092$). Interestingly within a stimulus type, there were no significant differences between SOAs.

Startle Stimulus Potency - ES_{50}

Similar to the ED_{50} or EC_{50} in pharmacological dose response curves, the ES_{50} represents the stimulus intensity that elicits a half maximum response. Stimuli that produce SIRM curves with a low ES_{50} value are considered more potent. From a logical viewpoint, ES_{50} depends on threshold, slope and R_{MAX} ; that is changes in either of those measures must alter the ES_{50} . In this experiment, white noise proved to be a more potent stimulus than the 5 kHz pure tone for both the startle stimulus only and prepulse conditions (main effect of stimulus type $F_{(1, 33)}=81.6$, $p<0.0005$), and this is attributable to differences between the stimulus-induced startle thresholds.

There was a significant stimulus type by SOA interaction on ES_{50} ($F_{(2.3, 75.9)}=22.7$, $p<0.0005$), due to large SOA effects in white noise trials which was not seen in the pure tone trials (Fig. 2d). Comparing the pure tone startling stimulus only condition to the average of all other pure tone prepulses failed to show a significant effect of the pure tone prepulse on stimulus potency (Helmert contrast – Startle stimulus only vs all SOAs - $F_{(1, 33)}=2.7$, $p=0.11$, $\eta^2=0.075$).

The direction and magnitude of prepulse-induced shifts in white noise potency was dependent on SOA, shown in figure 3b. In general there were two effects of prepulses on white noise trials: facilitation of stimulus potency at the shortest (10 ms) SOA and the inhibition of stimulus potency at the longest (100 ms) SOA. No prepulse effects were observed on potency at the intermediate (50 ms) SOA (see Fig. 2d). These SOA effects are similar to those seen on thresholds (Fig. 2b).

Experiment 2.

Prepulse Intensity and Startle Reflex Threshold

Experiment 2 was designed to assess the effect of increasing prepulse intensity (i.e. non-startling prepulse up to the lower end of dynamic range) on the PPI of two fixed startle stimuli, 110 and 115 dB respectively. SIRM curves were subsequently determined in the absence of prepulses and plotted over the prepulse intensity results in Figure 4. For simplicity, figure 4 is presented as PPI means collapsed over startle stimulus intensity (110 & 115 dB), since there was no significant interaction between startle stimulus intensity and prepulse intensity ($F_{(3,4, 37.6)} = 2.37$, $P = 0.078$, $\eta^2 = 0.177$), similar to findings in other labs for a narrow range of high intensity startle stimuli (Stitt et al., 1976).

There was no main effect of startle stimulus intensity, however there was a main effect of prepulse intensity ($F_{(3, 37.6)} = 27.96$, $p < 0.0005$). This effect was to increase PPI at prepulse stimulus intensities close to the startle threshold (D_{MIN}), while PPI was relatively insensitive to prepulse intensity changes at low stimulus intensities (65-80 dB).

Calibration Differences

In order to address some differences between the SIRM startle thresholds seen in Experiments 1 and 2, the calibration methods were compared in the “new” Med Associates startle systems. One of the significant differences between the two calibration methods is that the ANL software microphone is contained within the acrylic startle holders while the dB meter microphone is in an open area of the box above the platform. Figure 5 shows that there is a 13 dB difference in the stimulus intensities recorded on the DAWE dB meter compared with the ANL software calibration. Interestingly, there was only a 5 dB difference in the white noise background noise, which suggests that the acrylic holders are capable of insulating at least 5 dB of white noise. This level can be increased depending on the stimulus frequency (5 kHz = 13 dB), speaker quality and

distance from the microphone. The C3H.PRI- Flv^r threshold in Experiment 1 was 91.2 dB (data not shown) while the C3H.PRI- Flv^r threshold in Experiment 2 was 97.3 (Fig. 4), corresponding well with the background noise acoustic insulation levels of about 5 dB.

Discussion

This study demonstrates that a sigmoidal SIRM relationship is present in mice, conserved across prepulse condition, mouse substrain, experimental time and startle equipment. Summary parameters derived from the regression analysis can be used for comprehensive assessment of the startle phenotype and the reflex modifying characteristics of a prepulse. These parameters serve as a method of standardising PPI in a manner similar to the standardising of drug experiments through the use of dose-response curves. In doing so, this method of assessing PPI provides information on stimulus intensity gating (startle thresholds), motor capacity gating (R_{MAX}), sensorimotor gating (slopes) and stimulus potency (ES_{50}). While the net result of PPI is a sensorimotor process, the SIRM analysis can identify particular parametric regions where different effects of the prepulse dictate the outcome. For example stimulus intensity gating is particularly reflected in the threshold measure, because it occurs at a point where there is very little startle response and prepulse-induced changes in response capacity are not necessarily reflected in shifts in threshold (for example, Figures 2a and 2b illustrate a significant PPI of R_{MAX} with pure tone stimuli but no significant effect on thresholds). On the other hand, large changes in startle stimulus intensity from the intensity near that which produces R_{MAX} would have no observable effects on the response. R_{MAX} is a specific measure of the maximum response that can be elicited by increasing stimulus intensity under the current conditions, making prepulse-induced changes to this measure a motor capacity gating effect. Both slope and ES_{50} measures reflect processes in the dynamic range, where a small stimulus change can produce a large response change and can influence either thresholds or R_{MAX} . Therefore these measures can be interpreted as

reflecting mainly stimulus intensity gating, motor capacity gating or sensorimotor gating. In the present study, comparisons were made on these parameters between stimulus types (white noise vs 5 kHz pure tones) and among prepulse SOAs.

The major difference between the startle responses induced by white noise and pure tone stimuli (5 kHz) was the reflex threshold (D_{MIN}). White noise stimuli elicited startle at a lower intensity than pure tone (5 kHz) stimuli, demonstrating the utility of the startle threshold to assess stimulus intensity gating. In particular, observed threshold differences correspond to the frequency-specific differences of auditory thresholds in mice measured behaviourally (Ehret, 1976). Ehret (1976) evaluated auditory thresholds of pure tone and white noise stimuli measured by conditioned behaviour response in outbred NMRI mice (*mus musculus*) reporting response thresholds for 40 ms stimuli of 17 dB for white noise and 31 dB for pure tones (5 kHz). This difference is identical to the 14 dB stimulus difference in startle stimulus only threshold observed in the present study. Since there was no stimulus difference in reflex capacity (R_{MAX}) or efficacy (slope), the threshold difference translated into a potency (ES_{50}) difference. Note, however, that ES_{50} can be influenced by either R_{MAX} threshold or slope (or both), so that potency is not an independent measure.

Differences in stimulus potency could reflect differences in the central processing of these stimuli or they could be related to peripheral detection and transduction of the stimuli. Differences in peripheral detection and transduction could result from the frequency-dependent nature of acoustic transduction in the cochlea. Briefly, sound is propagated through the fluid in the cochlea (perilymph and endolymph) as mechanical waves, where hair cells on the organ of Corti are tuned to certain frequencies depending

on their longitudinal position on the basilar membrane (de Boer, 1991). The tendency for the cochlea to translate frequency into place means that only a limited group of nerve fibres are activated, where high frequency sound is translated near the oval window and low frequency sound is translated at the apex of the cochlea (de Boer, 1991). The frequency dependent displacement of the basilar membrane is controlled by the stiffness of the membrane (Ehret, 1978). Multi-component sounds (white noise) thus activate a number of distinct groups of nerve fibres distributed across the basilar membrane based on the fundamental frequencies comprising the sound (de Boer, 1991). For louder sounds more pulses are carried by the fibres involved and a larger number of fibres are activated (De Boer et al 1991). These auditory nerve fibres synapse with the cochlear nuclei, which decode these impulses and send information throughout the CNS. With respect to startle stimuli, cochlear neurons synapse to the caudal pontine reticular nucleus (PnC) (Koch, 1999).

Differences in startle thresholds for pure tone and white noise may be related to the number of auditory nerve fibres activated and the number of pulses each fibre carries. For a given pure tone stimulus intensity, a small number of frequency-dependent auditory nerve fibres will be carrying a set number of pulses associated with that intensity. For a white noise stimulus of the same intensity, the same number of pulses is carried by each nerve fibre, but a higher proportion of fibres are activated resulting in more intense stimulation of cochlear nuclei. Indeed in humans, the subjective loudness (phons) of noise increases as the bandwidth of the noise increases, even though the physical intensity (dB) remains the same (Scharf and Houtsma, 1986). Peripheral summation of

intensity only appears relevant to threshold, since the response efficacy and capacity of white noise and pure tone startle stimuli are equivalent.

Although pure tone (5 kHz) stimuli required higher intensities to reach the reflex capacity, the R_{MAX} did not differ between the two stimulus types. That is, the maximum startle magnitude elicited by the two stimuli did not differ, indicating that R_{MAX} can be dissociated from stimulus intensity gating. Furthermore, once the startle threshold was reached, the response efficacies (slope) of the two stimuli were equal. Therefore it appears the difference in startle induced by pure tone (5 kHz) stimuli is a result of a sensory difference rather than a response effect. This represents a different sensory modulation to startle than the reduction in reflex response induced by bilateral lesion of the dorsal cochlear nucleus (Meloni and Davis, 1998), analogous to a reduction in R_{MAX} using the SIRM methodology.

The prepulses modified startle in ways similar to the bilateral lesions, reducing R_{MAX} , and similar to the stimulus type effect on startle threshold, that is prepulses also shifted the position of the curves on the stimulus intensity axis to the right. The reduction in R_{MAX} produced by prepulses represents a motor capacity gating effect and is similar to the typically reported reduction in magnitude to a single, high intensity startling stimulus. Prepulses also affect stimulus intensity gating, as shown by the prepulse-induced changes to the reflex threshold (D_{MIN}), and may increase or decrease it. The discussion of prepulse effects below is somewhat limited by the experimental design, since prepulse type was paired with the corresponding startle stimulus type. It would be interesting to further examine the effect of prepulses types by independently varying prepulse and pulse stimulus type.

The prepulse-induced reductions of reflex capacity and efficacy (slope) occur irrespective of SOA or stimulus quality. However, the magnitude of reduction of the reflex capacity is dependent on both stimulus type and SOA. An effect of stimulus type on auditory threshold (perception) was apparent, where the stimulus with the lower detection limit (pure tone 5 kHz) also showed less potent PPI. This relationship of prepulse salience (perceived intensity) and magnitude of R_{MAX} reductions in Experiment 1 is consistent with both the PPI intensity results in Experiment 2 (Fig. 4) and reports in the literature (Van den Buuse and Eikelis, 2001). The results of Experiment 2 further suggest that an abrupt enhancement of PPI by higher intensity prepulses occurs when those prepulses approach the intensity of the startle threshold.

The prepulse can also facilitate the startle response under certain conditions (Martin-Iverson and Else, 2000; Plappert et al., 2004; Schmajuk et al., 2006). In the current study prepulse facilitation (PPF) was only observed at low startling stimulus intensities when the prepulse intensity was close to the response threshold. PPF of response magnitude was only recorded with a white noise prepulse at the 10 ms SOA.

One question is: why doesn't the short SOA pure tone prepulse facilitate the startle threshold? The 10 ms SOA is one at which prepulse facilitation of response magnitudes elicited by white noise bursts have been shown (Meloni and Davis, 1998), and occurs here for startle thresholds after a white noise prepulse. Other experiments in C3H/ mice have shown short SOA (12.5 ms) pure tone (14 kHz) prepulses effectively facilitate the startle response to white noise stimuli (Plappert et al., 2006). It has been argued that there is summation of both prepulse facilitation and prepulse inhibition effects at short SOAs (Valls-Sole et al., 1999; Plappert et al., 2004), which may be why

inhibition is maximal at somewhat longer SOAs than 10 ms (cf. Martin-Iverson and Else 2000). The prepulse intensity for white noise stimuli (75 dB) was near the startle threshold while the same intensity for the pure tone was on average more than 10 dB below the startle threshold. Thus, it appears that a prepulse with a very short SOA may add to the overall energy produced by the startling stimulus only if that prepulse has of a sound pressure level intensity close to the startle threshold intensity. If intense prepulses can directly depolarise cells in the reflex startle circuit, then SOA becomes relevant in the context of dynamic cellular electrophysiology in the PnC (Schicatano et al., 2000). This does not happen with the pure tone stimulus as the startle threshold is much higher than the prepulse intensity used in this experiment. However, it is also possible that the startle responses at short SOAs (fig. 2a) represent the response to the prepulse (latency ~40 ms) and not PPF of the startle stimuli.

Combining these findings with the results of Experiment 2 suggest a biphasic effect of the prepulse related to intensity. When the prepulse intensity is above acoustic threshold but below startle threshold, there appears to be a stable effect of the prepulse, consistent with the hypothesis of pedunculopontine tegmental nuclei projecting to startle reflex interneurons (Koch et al., 1993; Koch, 1999), and is referred to above as stimulus intensity gating. When the prepulse intensity approaches startle reflex threshold, the prepulse is capable of activating the startle cells directly and thus the temporal relationship between SOA and hyperpolarisation/ depolarisation of the cell become significant for temporal summation (Schicatano et al., 2000). However, when the SOA is too long for temporal summation, then the intense prepulses may activate motor capacity gating (i.e. the startle cells initially activated by the intense prepulse move into a

refractory phase in which higher stimuli are necessary to reactivate them). This summation/ inhibition of startle reactivity by prepulses appears to occur at the level of the PnC, rather than the periphery, since heritable rat strain differences in acoustic PPI correlate with inheritance of similar deficits to visual stimuli (Weber and Swerdlow, 2007). Thus, “powerful” prepulses of any modality (indexed relative to the background stimulus and reflex threshold) can facilitate startle at short SOAs but increase inhibition at longer SOAs. This may explain the often observed prepulse facilitation of startle latencies that can co-occur with magnitude inhibition (Valls-Sole et al., 1999).

There appears to be two different types of prepulse facilitation (PPF), augmentation of startle with discrete pulses of short SOA and augmentation of startle using continuous pulses of a long duration (Hsieh et al., 2006). With respect to short SOA PPF, research with the “Hybrid” strain of mice has shown that the amount of PPF is dependent on SOA, frequency and intensity of the prepulse (Plappert et al., 2004) and is more prominent in mice than in rats (Reijmers and Peeters, 1994). Short SOA PPF has also been reported in humans and was hypothesised to result from a biphasic effect of the prepulse (Valls-Sole et al., 1999). Prepulses cause sub-threshold activation of the PnC and coincidence of activation by the startle stimulus when the prepulse SOA is short, results in expression of short-latency facilitation before the inhibitory actions of the prepulse (through the pedunculopontine tegmental nucleus) can be mediated (Valls-Sole et al., 1999). The observations of short SOA PPF in this experiment support this hypothesis and further suggest sub-threshold stimulation of the PnC only occurs when the prepulse intensity is close to intensity of startle threshold. Since only white noise prepulses, close to the startle threshold, induced significant facilitation.

Currently there is no standard protocol for PPI testing (Parisi and Ison, 1979; Swerdlow et al., 2002). While most experiments tend to phenotype their subjects for startle reactivity prior to PPI testing (Swerdlow et al., 1998) many of these experiments do so using a single startle intensity, which may or may not correspond to the reflex capacity of individual subjects. Since individual subjects show variance in startle threshold and reflex capacity, selecting a single intensity does not appropriately match startle, since the stimulus intensity may be close to either D_{MAX} or ES_{50} for different subjects. In addition, single intensity experiments will not be able to identify calibration differences since there is no sensory index (threshold) to reference, only an independent quantification of startle magnitude. The SIRM methodology represents a significant improvement to the PPI model, since it can explain differences in startle threshold as a function of calibration intensity differences and show discrete startle modulations by the prepulse. The three independent parameters that together characterise the SIRM function, R_{MAX} (response capacity), D_{MIN} (reflex threshold) and slope (efficacy) and one dependent parameter, ES_{50} (inverse of potency), allow comparisons across individuals, strains and species without the confounds produced by using a single fixed intensity where different individuals, strains and species have different sensitivities to the stimulus.

References

- Braff D, Stone C, Callaway E, Geyer M, Glick I, Bali L (1978) Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology* 15:339-343.
- Crabbe JC, Wahlsten D, Dudek BC (1999) Genetics of mouse behavior: interactions with laboratory environment. *Science* 284:1670-1672.

- de Boer E (1991) Auditory Physics. Physical principles in hearing theory III. Physics Reports 203:125-231.
- Ehret G (1976) Temporal auditory summation for pure tones and white noise in the house mouse (*Mus musculus*). Journal of the Acoustical Society of America 59:1421-1433.
- Ehret G (1978) Stiffness gradient along the basilar membrane as a basis for spatial frequency analysis within the cochlea. Journal of the Acoustical Society of America 64:1723-1726.
- Flaten MA, Nordmark E, Elden A (2005) Effects of background noise on the human startle reflex and prepulse inhibition. Psychophysiology 42:298-305.
- Francis DD, Szegda K, Campbell G, Martin WD, Insel TR (2003) Epigenetic sources of behavioral differences in mice.[see comment]. Nature Neuroscience 6:445-446.
- Geyer M, Swerdlow NR (1998) Measurement of startle response, prepulse inhibition, and habituation. In: Current Protocols in Neuroscience (Crawley JN, Skolnick P, eds), pp 8.7.1-8.7.15. New York: Wiley.
- Geyer MA, Braff DL (1982) Habituation of the Blink reflex in normals and schizophrenic patients. Psychophysiology 19:1-6.
- Graham FK (1975) The more or less startling effects of weak prestimulation. Psychophysiology 12:238-248.
- Hince DA, Martin-Iverson MT (2005) Differences in prepulse inhibition (PPI) between Wistar and Sprague-Dawley rats clarified by a new method of PPI standardisation. Behavioral Neuroscience 119:66-77.

Hoffman HS, Ison JR (1980) Reflex modification in the domain of startle: Some empirical findings and their implications for how the nervous system process sensory input. *Psychological Review* 87:175-189.

Hsieh MH, Swerdlow NR, Braff DL (2006) Effects of background and prepulse characteristics on prepulse inhibition and facilitation: implications for neuropsychiatric research. *Biological Psychiatry* 59:555-559.

Ison JR (2001) The acoustic startle response: Reflex elicitation and reflex modification by preliminary stimuli. In: *Handbook of Mouse Auditory Research: From behavior to molecular biology* (Willott JF, ed), pp 59-82. Boca Raton: CRC Press.

Koch M (1999) The neurobiology of startle. *Progress in Neurobiology* 59:107-128.

Koch M, Kungel M, Herbert H (1993) Cholinergic neurons in the pedunculopontine tegmental nucleus are involved in the mediation of prepulse inhibition of the acoustic startle response in the rat. *Experimental Brain Research* 97:71-82.

Martin-Iverson MT, Else D (2000) PHNO, a selective dopamine D2 receptor agonist, does not reduce prepulse inhibition of the startle reflex in rats. *Psychopharmacology* 151:38-48.

Martin-Iverson MT, Stevenson KN (2005) Apomorphine effects on emotional modulation of the startle reflex in rats. *Psychopharmacology* 181:60-70.

Meloni EG, Davis M (1998) The dorsal cochlear nucleus contributes to a high intensity component of the acoustic startle reflex in rats. *Hearing Research* 119:69-80.

Parham K, Willott JF (1988) Acoustic startle response in young and aging C57BL/6J and CBA/J mice. *Behavioral Neuroscience* 102:881-886.

Parisi T, Ison JR (1979) Development of the acoustic startle response in the rat: ontogenetic changes in the magnitude of inhibition by prepulse stimulation. *Developmental Psychobiology* 12:219-230.

Plappert CF, Pilz PK, Schnitzler HU (2004) Factors governing prepulse inhibition and prepulse facilitation of the acoustic startle reflex in mice. *Behavioural Brain Research* 152:403-412.

Plappert CF, Kuhn S, Schnitzler H-U, Pilz PKD (2006) Experience increases the prepulse inhibition of the acoustic startle response in mice. *Behavioral Neuroscience* 120:16-23.

Pouzet B, Feldon J, Veenman CL, Yee BK, Richmond M, Nicholas J, Rawlins P, Weiner I (1999) The effects of hippocampal and fimbria-fornix lesions on prepulse inhibition. *Behavioral Neuroscience* 113:968-981.

Reijmers LG, Peeters BW (1994) Effects of acoustic prepulses on the startle reflex in rats: a parametric analysis. *Brain Research* 667:144-150.

Rigdon GC, Viik K (1991) Prepulse inhibition as a screening test for potential antipsychotics. *Drug Development Research* 23:91-99.

Scharf B, Houtsma AJM (1986) Audition II: Loudness, pitch, localisation, aural distortion, pathology. In: *Handbook of perception and human performance. Volume I: Sensory processes and perception* (Boff KR, Kaufman L, Thomas JP, eds), pp 15_11 - 15_60. New York: Wiley.

Schicatano EJ, Peshori KR, Gopalaswamy R, Sahay E, Evinger C (2000) Reflex excitability regulates prepulse inhibition. *Journal of Neuroscience* 20:4240-4247.

Schmajuk NA, Larrauri JA, Hagenbuch N, Levin ED, Feldon J, Yee BK (2006) Startle and prepulse inhibition as a function of background noise: a computational and experimental analysis. *Behavioural Brain Research* 170:182-196.

Seeman P, Ulpian C (1988) Dopamine D1 and D2 receptor selectivities of agonists and antagonists. *Advances in Experimental Medicine & Biology* 235:55-63.

Stitt CL, Hoffmann HS, Marsh RR (1976) Interaction versus independence of startle-modification processes in the rat. *Journal of Experimental Psychology: Animal Behavior Processes* 2:260-265.

Stoddart CW, Martin-Iverson MT, Jablensky A, Urosevic N (2004) A novel mouse Chr5 locus Diht controls dopamine-induced hypothermia. *Mammalian Genome* 15:901-913.

Swerdlow NR, Geyer MA (1998) Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophrenia Bulletin* 24:285-301.

Swerdlow NR, Varty GB, Geyer MA (1998) Discrepant findings of clozapine effects on prepulse inhibition of startle: is it the route or the rat? *Neuropsychopharmacology* 18:50-56.

Swerdlow NR, Braff DL, Geyer MA (2000) Animal models of deficient sensorimotor gating: what we know, what we think we know, and what we hope to know soon.[see comment]. *Behavioural Pharmacology* 11:185-204.

Swerdlow NR, Braff DL, Geyer MA, Koob GF (1986) Central dopamine hyperactivity in rats mimics abnormal acoustic startle response in schizophrenics. *Biological Psychiatry* 21:23-33.

Swerdlow NR, Braff DL, Taaid N, Geyer MA (1994) Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. *Archives of General Psychiatry* 51:139-154.

Swerdlow NR, Shoemaker JM, Stephany N, Wasserman L, Ro HJ, Geyer MA (2002) Prestimulus effects on startle magnitude: sensory or motor? *Behavioral Neuroscience* 116:672-681.

Trune DR, Kempton JB, Mitchell C (1996) Auditory function in the C3H/HeJ and C3H/HeSnJ mouse strains. *Hearing Research* 96:41-45.

Valls-Sole J, Valldeoriola F, Molinuevo JL, Cossu G, Nobbe F (1999) Prepulse modulation of the startle reaction and the blink reflex in normal human subjects. *Experimental Brain Research* 129:49-56.

Van den Buuse M, Eikelis N (2001) Estrogen increases prepulse inhibition of acoustic startle in rats. *European Journal of Pharmacology* 425:33-41.

Weber M, Swerdlow NR (2007) Rat strain differences in startle gating-disruptive effects of apomorphine occur with both acoustic and visual prepulses. *Pharmacology, Biochemistry & Behavior* In press.

Worms P, Broekkamp CLE, Lloyd KG (1983) *Behavioral Effects of Neuroleptics*. New York: Raven Press.

Yee BK, Chang T, Pietropaolo S, Feldon J (2005) The expression of prepulse inhibition of the acoustic startle reflex as a function of three pulse stimulus intensities, three prepulse stimulus intensities, and three levels of startle responsiveness in C57BL6/J mice. *Behavioural Brain Research* 163:265-276.

Table 1. – Frequency of mice expressing sigmoidal stimulus: response relationship

Stimulus Type *	Startle Stimulus Only	10 ms	50 ms	100 ms
White Noise #	36/36	14/36	16/36	33/36
Pure Tone	36/36	36/36	36/36	36/36

* χ^2 test reported significant effect of stimulus type (p=0.004).

χ^2 test reported significant effect of startle and SOA for the white noise stimulus (p=0.001).

Table 2. – R^2 co-efficient for the SIRM relationships in each startle condition

Stimulus Type	Startle Stimulus Only	10 ms	50 ms	100 ms
White Noise	0.828 $\pm$ 0.024	0.320 $\pm$ 0.066	0.464 $\pm$ 0.074	0.521 $\pm$ 0.048
Pure Tone	0.934 $\pm$ 0.008	0.929 $\pm$ 0.008	0.908 $\pm$ 0.014	0.926 $\pm$ 0.010

* R^2 values only included when the sigmoidal relationship present (see table 1 for frequencies).

Figure 1. Stimulus Intensity Response Magnitude (SIRM) curves for the mouse startle response with and without a 75 dB prepulse. The SIRM curves above show the mean ($\pm$ SEM) of the individually fitted non-linear regression (lines) plotted over the mean observed startle magnitude (symbols); R^2 values are presented in table 1. The amount of PPI is dependent on the stimulus type, shown here using **A**, white noise and **B**, pure tone (5 kHz) stimuli. White noise stimuli show a more potent startle response and greater PPI than the corresponding intensity of pure tone stimuli.

A

B

Figure 2. Summary values from the regression analysis (mean $\pm$ SEM) show that white noise is a more potent startle stimulus than pure tone (5 kHz) stimuli and can also induce a greater magnitude of PPI. **A**, The reflex capacity (R_{MAX}) of the startle response is significantly reduced in the presence of a prepulse and this effect is greater with white noise stimulus. Note the reflex capacities for startle stimulus only (S2 only) are equivalent while the prepulse stimulus only (S1 only) responses are significantly different suggesting a sensory difference. **B**, The startle reflex thresholds (D_{MIN}) confirm this

difference showing reflex excitability is dependent on the stimulus type. **C**, Under startle stimulus only conditions there is no difference in the efficacy of the stimulus induced response (slope) but a prepulse significantly reduces the efficacy with which a startle stimulus elicits a response. **D**, Overall these above three measures contribute to a measure of stimulus potency (ES_{50}) which shows white noise to be a more potent startle stimulus than pure tones (5 kHz). In this case, the difference in potency was largely a reflection of threshold differences thought to be related to stimulus detection thresholds. # represents a significant effect of the stimulus type, * represents a significant effect of the prepulse compared to startle stimulus only condition of the same stimulus type and ** represents a significant stimulus type effect on the prepulse only trial. All pairwise comparisons were made using Sidak's t-test method adjusted for post hoc multiple comparisons ($\alpha = 0.05$).

Figure 3. Panel **A** depicts the prepulse-induced reduction in the magnitude of startle response as a reduction in the reflex capacity (mean % inhibition $R_{MAX} \pm SEM$). The prepulse also served to change the reflex excitability, shown in panel **B** as a change in threshold (shift of D_{MIN}). The shift in the curve was plotted as the mean change in threshold, relative to the startle stimulus only condition (startle + prepulse D_{MIN} – startle D_{MIN}). Both facilitation and inhibition of startle thresholds were produced by white noise prepulses as a function of SOA. All prepulse-induced reductions to R_{MAX} and threshold shifts at each SOA in the white noise condition were significantly different from each

other. Elsewhere, # represents a significant effect of stimulus type, * represents a significant difference between the response at 50 ms and 100 ms SOA within the same stimulus type. All pairwise comparisons were made using Sidak's t-test method adjusted for post hoc multiple comparisons ($\alpha = 0.05$).

Figure 4. The bar plot (left abscissa) shows the PPI in mice (n=12) that results from using variable intensity prepulse stimuli to inhibit the startle response to a fixed intensity startle stimulus (5 kHz pure tone, 115 dB). PPI shows a significant dependence on the intensity of the prepulse, where increasing the prepulse intensity increases the magnitude of startle reductions. When these data are plotted over the fitted SIRM curve for mean response to startle stimulus only stimuli, 5 kHz pure tones (line plot – right abscissa), the intensity dependent increase is shown to occur with prepulse intensities at or above the startle stimulus only threshold. The levels of PPI are relatively stable with prepulse intensities below the SIRM curve threshold. * represents a significant difference of PPI when compared to the PPI response to the 65 dB prepulse with pairwise comparisons using Sidak’s t-test method adjusted for post-hoc multiple comparisons ($\alpha = 0.05$).

Figure 5. A comparison of the two calibration methods shows a difference of 12 dB between the ANL Med-Associates microphone calibration software and the dB meter used in the previous experiments. The symbols represent the mean DAWE dB meter measurement of a 5 kHz pure tone when each startle chamber was calibrated to the corresponding x-value using the ANL software calibration. The error bars represent the standard error of the mean DAWE dB meter measurement. Note calibration was performed in the presence of a 70 dB white noise background which showed a mean measurement on the DAWE dB meter of 75.19 ± 0.21 dB.