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Effects of intensity and type of prepulse stimulus on prepulse inhibition in scopolamine treated rats

Amanda K. Andrus [a,b,*], Brian R. Marable [a,1], Gary L. Dunbar [b,c], Mark P. Reilly [b], Jacques P.J. Maurissen [a,b]

[a] Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674, USA
[b] Department of Psychology, Central Michigan University, Mt. Pleasant, Michigan 48859, USA
[c] Field Neuroscience Institute Laboratory for Restorative Neurology and Program in Neuroscience, Central Michigan University, Mt. Pleasant, Michigan 48859, USA

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Abstract

Prepulse inhibition (PPI) of the auditory startle response (ASR) is a behavioral test that has been used to measure auditory thresholds, to assess sensory-motor integration functions, and its use has been recommended in the United States Environmental Protection Agency Developmental Neurotoxicity Guideline (OPPTS 870.6300). The purpose of the present study was to determine to what extent the intensity and/or type of prepulse stimuli modulate PPI in scopolamine-treated rats. The PPI of the ASR peak amplitude was measured when the intensity of a 10-kHz prepulse tone was varied (69-, 80-, and 90 dB[A]; Experiment 1) and when both the intensity and type of auditory prepulse (a 10-kHz tone vs. a white noise burst) were varied (Experiment 2). Scopolamine treatment attenuated PPI in both experiments and interacted significantly with the prepulse stimulus intensity in Experiment 1. In Experiment 2, the percent of PPI was linearly related to prepulse stimulus intensity for trials using a tone, but was biphasic on trials using a white-noise prepulse stimulus. Prepulse stimuli of certain intensities elicited a response, and this response was greater when the prepulse stimulus was a white noise burst versus a tone of the same intensity. Further, the response to the prepulse altered the amount of inhibition and, therefore, confounded the overall measure of PPI at the higher prepulse stimulus intensity levels. Overall, these results indicate that careful consideration of the intensity and type of prepulse stimuli be taken in the context of their potential to induce a prepulse-elicited response, as well as providing the appropriate measures of such a response, when designing and interpreting PPI experiments.

Keywords: Prepulse inhibition; Scopolamine; Stimulus intensity; Frequency; Prepulse reactivity; Parameters

1. Introduction

Prepulse Inhibition (PPI) of the auditory startle response, a form of reflex modification, is a behavioral test that is used to assess the integration of sensory and motor information. Disruptions in PPI have been found in several human pathologies including schizophrenia (Braff et al., 1978) and Huntington's disease (Swerdlow et al., 1995). PPI has also been used to assess the effects of chemical exposure in regulatory toxicity studies. The developmental neurotoxicity guideline from the United States Environmental Protection Agency (USEPA) mandates that a test of auditory startle be conducted, and whereas startle habituation procedures are acceptable, it recommends PPI as the preferred test method (USEPA, 1998). The USEPA provides references for the conduct of the behavioral tests. However, while there is a rather precise method described for tests of startle habituation (Adams et al., 1985), the methods to be used for PPI (Ison, 1984) are not as specific. Differences in reflex modification techniques have been shown to affect the results of toxicity tests. For example, effects were seen in rats exposed to methyl mercury when the prestimulus was a gap in background noise but not when the prestimulus was a tone pip (Ison, 1984).

* Corresponding author. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674, USA. Tel.: +1 989 636 9298; fax: +1 989 638 9863.
E-mail address: akandrus@dow.com (A.K. Andrus).
[1] Current Address: Syngenta Crop Protection, Inc., Greensboro, North Carolina 27409, USA.

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The characteristics of the prepulse stimulus, such as stimulus duration and intensity, can also affect the result of the test. As prepulse stimulus duration increases, the level of PPI increases and typically asymptotes at prepulse stimulus durations approaching 50 ms (Blumenthal, 1999). Mansbach and Geyer (1991) showed that PPI produced by short prepulse stimulus durations was more sensitive to disruption by ketamine than when the prepulse was of longer durations.

Increasing prepulse stimulus intensity tends to increase the amount of prepulse inhibition up to some level, and this holds true across modalities (Blumenthal, 1999). Jones and Shannon (2000b) investigated the effects of varying prepulse stimulus intensity with scopolamine-treated rats and showed a greater scopolamine-induced disruption of prepulse inhibition at the lower prepulse stimulus intensities. This result provides support for claims that many drug-induced disruptions of PPI occur when there is a low signal-to-background noise ratio (Davis et al., 1990; Gewirtz and Davis, 1995). Prepulse stimulus duration and intensity, therefore, are important parameters of the prepulse stimulus that can modulate treatment effects.

Prepulse stimulus type (a white noise or a tone of a single frequency) is another aspect of the prepulse stimulus that could impact the results of PPI studies. There are few studies available comparing a white noise burst prepulse stimulus and a tone prepulse stimulus with respect to their relative effects on PPI. Braff and colleagues (2001), who compared PPI of normal and schizophrenia patients when given either a discrete or continuous presentation of a white noise versus a tone prepulse stimulus, found that higher percentages of PPI were obtained when using white noise prepulse stimuli in control subjects, and that the differences in PPI between control and schizophrenia patients was greater when discrete white noise prepulse stimuli were used. In another study, Acocella and Blumenthal (1990) found that pulse response probabilities were higher for prepulse stimuli for which the subjects were primed to attend, with this effect being larger for tone than white noise burst prepulse stimuli, but with no changes in PPI levels. These studies suggest that, under some conditions, tone prepulse stimuli may be more salient than white noise prepulse stimuli, but that, under other conditions, white noise prepulse stimuli can lead to enhanced PPI.

The specific aims of the present study were to investigate the effects of two aspects of the prepulse stimulus, intensity and type of prepulse stimulus (tone versus white noise) in scopolamine-treated rats. As mentioned above, scopolamine has been shown to attenuate PPI (Jones and Shannon, 2000a,b; Stanhope et al., 2001; Ukai et al., 2004; Wu et al., 1993). Experiment 1 was a modification of a study by Jones and Shannon (2000b) and was designed to test whether scopolamine would interact with prepulse stimulus intensity to produce decreases in PPI, and whether lower intensity prepulse stimuli would increase this effect. Experiment 2 examined the impact of prepulse stimulus type (10-kHz tone vs. white noise) on PPI. The effects of a 10-kHz tone and a white noise prepulse stimulus on PPI of scopolamine-treated rats were determined using three prepulse stimulus intensities. We hypothesized that the tone prepulse stimulus would be more detectable against a white noise background and, therefore, would produce more PPI than the white noise prepulse stimulus. If scopolamine disrupts the sensory detection of the prepulse stimulus, as previously suggested, then a greater effect of scopolamine should be seen when the prepulse stimulus is less detectable, thereby producing less PPI under conditions of white-noise versus tone prepulse stimuli.

2. Methods

2.1. Animals and husbandry

Male Sprague Dawley rats were chosen based on their use in previous PPI studies. Rats were obtained from Charles River Laboratories Inc. (Raleigh, North Carolina) and were at least 8 weeks of age prior to testing. Rats were acclimated to the laboratory for approximately one week prior to the start of the experiment. The room relative humidity and temperature were maintained within a range of 40–70% and 22±3 °C, respectively. A 12-hour light/dark photocycle was maintained with lights on at 6:00 a.m. and off at 6:00 p.m., and room air was exchanged approximately 12–15 times/hour.

Rats were housed 2 per cage in stainless steel cages that contained a feed container and a pressure activated nipple-type watering system. Rats were provided LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in pelleted form. Feed and municipal water were provided ad libitum. All rats were evaluated for general health status by a laboratory veterinarian upon arrival at the animal facility, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All procedures involving the use of animals were approved by the Institutional Animal Care and Use Committee of The Dow Chemical Company. Rats were individually identified, stratified by body weight, and randomly assigned to treatment groups.

2.2. Equipment description

A commercially available PC-based system (Med Associates, Inc., St. Albans, Vermont) was used to measure the auditory startle response. The system consisted of eight acoustically-attenuated chambers and hardware to generate the auditory stimuli and quantify the resulting responses. Each chamber contained a dual-speaker enclosure (for the generation of background noise and audio stimuli), a load-sensing platform, and an adjustable gain preamplifier. The speaker responsible for generating the pulse stimulus was an 8.9 cm piezo ceramic disc speaker (8 Ω) with a frequency response of 1.8 kHz–30 kHz. The background noise speaker was an 8.9 cm dynamic coil speaker (8 Ω) with a frequency response of 40 Hz–8 kHz. Acrylic animal holders were attached to load-sensing platforms that connected to the data acquisition system. Response data were filtered through a 5-pole lowpass Butterworth filter (13 Hz) and a Twin-T notch filter (60 Hz) and were acquired at a 12-bit resolution using a Keithley Metrabyte DAS 1402 A/D card. Each chamber was equipped with a red house light and ventilation fan, which were kept on during all experimental procedures.

Each chamber was calibrated using a standard procedure before each testing session to verify the intensities of the background noise, the prepulse stimulus and the pulse noise bursts and to standardize each load cell platform to ensure uniformity of response across all chambers. Sound intensities were measured using a Bruel and Kjær sound meter (model 2230) and microphone (type 4190). The microphone was positioned in the center of an animal holder at approximately ear level for the rat. The background noise was measured using an A-weighted dB scale with the meter set to random mode, and this measurement was used to standardize noise levels across chambers. The prepulse and pulse stimuli were also measured using an A-weighted dB scale, however, the meter was set to impulse mode to better characterize the intensity of short sound bursts.

Load cell responses were balanced using a procedure built into the system software and a spinner-type dynamic calibrator (Med Associates, Inc., St. Albans, Vermont). This calibrator applied a 10-Hz sinusoidal force to each load cell platform. The response was acquired by the software, which displayed the average positive and negative peak for each load cell. By adjusting the gain on each platform's preamplifier, the response of each load cell could be standardized to near equivalency (±2 response units). To maximize the resolving power of the analog-to-digital converter, preamplifier settings were chosen based on the expected maximum response amplitudes for rats of this strain and body weight. Auditory response amplitudes were expressed in arbitrary units (au), with each unit equal to one digital step.

2.3. Testing procedures

On the day prior to each test/dosing day, the rats were acclimated to the testing procedures without being dosed. They were brought into the laboratory and acclimated to the laboratory conditions for 30 min and then placed in the acrylic animal holders and acclimated for 5 min in the chambers in the presence of 60 dB (A) white background noise, a red house light, and ventilation fan. The background noise and the ventilation fan were of equivalent sound power (i.e., background noise + fan noise = 60 dB [A]). After the acclimation period, rats were exposed to prepulse and pulse stimuli that were presented in the same manner as the next day's test session.

On the test days, rats were brought into the testing laboratory prior to each testing session and allowed 30 min to acclimate to the laboratory conditions prior to dosing. Twenty minutes after dosing with scopolamine, rats were placed in the acrylic animal holders, and acclimated for 5 min in the chambers under identical conditions as used in the previous session. Following the 5-min acclimation period, the test session began. For each experiment, rats were counterbalanced across the eight test chambers in such a way as to maximize the number of rats/treatment group tested in each chamber across test sessions.

2.4. Dosing

Scopolamine hydrobromide was obtained from Sigma-Aldrich (St. Louis, MO). It was mixed in sterile physiologic saline at the required concentration. New solutions were prepared for each day of dosing. Rats were dosed by subcutaneous injection at a volume of 1 ml/kg.

2.5. Experiment 1 — tone prepulses and scopolamine

Eight rats per group were tested and groups consisted of a saline control and 0.3 mg/kg of scopolamine, which was the most effective dose of scopolamine previously reported (Jones and Shannon, 2000b). Rats used in this experiment also took part in a previous PPI experiment and were treated with the same doses two weeks prior. In the current experiment, a 10-kHz tone was used for the prepulse stimulus. To mask the ambient noise level in the laboratory and the noise of the ventilation fans, the white noise background was set at 60 dB (A) instead of 50 dB (A), as used by Jones and Shannon (2000b). The prepulse stimulus intensities used for this study were subsequently 10 dB higher. With this exception, all other parameters and the presentation of stimuli were the same.

After a 5-minute acclimation period, rats were presented with six counterbalanced presentations of six trial types each, for a total of 36 trials. Trial types consisted of three prepulse + pulse trials (10-kHz tone prepulses: 69-, 80-, and 90 dB[A]), pulse-alone trials (white noise: 106 dB[A]), prepulse-alone trials (10-kHz tone: 69 dB[A]) and null trials where no stimuli were presented, but the movement of the rat was recorded. Actual intensity levels (±standard deviations) were recorded at 69.3±0.39-, 80.1±0.23-, 90.54±0.42- and 105.9±0.83 dB(A), respectively.

All prepulse and pulse stimuli were of 20-ms duration with a 2-ms rise/fall time. The interstimulus interval was 120 ms from prepulse stimulus onset to pulse stimulus onset, and the intertrial interval varied randomly between 15 and 45 s. Data were collected in two sessions to accommodate 16 rats in eight test chambers, and test sessions lasted approximately 25 min. Rats were counterbalanced across chambers by dose groups.

2.6. Experiment 2 — tone vs. white noise prepulses and scopolamine

In this experiment, comparisons of the effects of either a 10-kHz tone or a white noise prepulse stimulus on PPI of scopolamine-treated rats were made at three prepulse stimulus intensities. Sixteen experimentally naïve rats/group were tested. Treatment groups consisted of a saline control (n=16) and 0.3 mg/kg of scopolamine (n=16). The tone and white noise prepulse stimuli were examined at three intensities (69-, 80-, 90 dB[A]), resulting in six different prepulse stimulus trial types. Pulse-alone trials (white noise: 106 dB[A]), prepulse stimulus alone trials (white noise or tone: 69 dB[A]) and null trials were also tested. Trials were counterbalanced and all other parameters were identical to those in Experiment 1. Calibrated intensity levels (± standard deviations) for white noise prepulse stimuli were 69.6±0.50-, 80.4±0.55-, and 90.4±0.43 dB(A) and for tone prepulse stimuli were 69.3±0.59-, 80.2±0.58-, and 90.2±0.53 dB(A), respectively. Pulse stimuli and white noise background intensity levels (±standard deviations) were recorded at 106.2±0.56- and 60.3±0.58 dB(A), respectively.

Due to a software limitation, a tone and a white noise prepulse stimulus could not be generated in the same test session. To maintain a within-subject design, all rats were tested twice, once with a tone and once with a white noise prepulse stimulus. Rats were counterbalanced across test days and sessions for order of prepulse stimulus type, by group, and across test chambers. Each test day had 4 sessions to accommodate 32 animals in 8 test chambers, and each test session had 36 trials and lasted approximately 25 min. Test days occurred one week apart to allow the rats to recover from dosing.

2.7. Statistical analyses

Peak response amplitudes from the pulse-alone trials and the prepulse with pulse trials were averaged for each animal separately. The percentage of PPI for each animal was calculated using the following equation: 100 × [(mean peak response amplitude for pulse-alone trials − mean peak response amplitude for prepulse with pulse trials) / mean peak response amplitude for pulse-alone trials].

Statistical analyses were conducted on the percent PPI with SPSS for Windows (version 11.0). The acceptable type I error rate was set to alpha = 0.05 for all analyses. All repeated-measure analyses of variance (ANOVA) were performed with the multivariate approach using the Pillai's trace statistic. To help aid in the interpretation of the results, additional analyses were performed on ancillary variables, such as response amplitude of the pulse-alone trials and prepulse response amplitude, as indicated below.

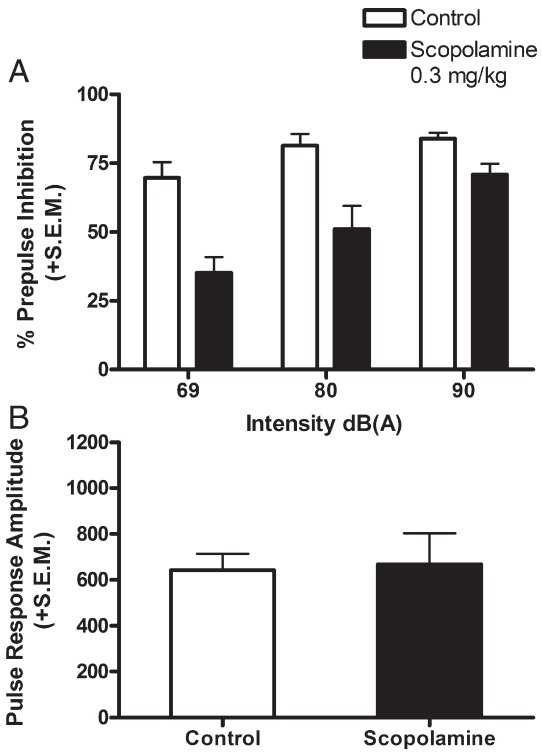

Fig. 1. Effects of tone prepulses and scopolamine treatment on PPI in rats ($n = 8$). (A) Percent PPI increased in scopolamine-treated rats with each increment in 10 kHz tone prepulse intensity. (B) No differences between controls and scopolamine-treated rats on pulse response amplitude (arbitrary units) were observed during pulse-alone trials.

3. Results

3.1. Experiment 1 — tone prepulses and scopolamine

A repeated-measure ANOVA, with the between-subjects factor of dose and the repeated factor of intensity, was used to assess the effects of scopolamine on PPI following a tone prepulse stimulus of varying intensities. Intensity of prepulse stimulus [$F(2,13) = 20.9$, $p < .001$] and scopolamine treatment [$F(1,14) = 21.2$, $p < .001$] significantly impacted PPI. Fig. 1A shows that as the prepulse stimulus intensity increased, so did PPI, and that scopolamine attenuated PPI at all three intensity levels. There was also a significant interaction of scopolamine treatment with intensity level [$F(2,13) = 5.60$, $p = .018$]. As can be seen in Fig. 1A, there was a larger decrease in PPI in scopolamine-treated rats compared to controls at lower levels of prepulse stimulus intensity. These results are similar to those reported by Jones and Shannon (2000b).

A one-way ANOVA was used to assess the effect of scopolamine on response amplitude of the pulse-alone trials. As shown in Fig. 1B, scopolamine treatment did not significantly affect response amplitude [$F(1,14) = .028$, $p = .870$].

3.2. Experiment 2 — tone vs. white noise prepulses and scopolamine

A doubly repeated-measure ANOVA was used to assess the effects of scopolamine on PPI using a tone and a white noise prepulse stimulus of varying intensities. The between-subjects factor of dose and the repeated factors of prepulse stimulus type (tone or white noise) and prepulse intensity were used in the model. As in Experiment 1, intensity [$F(2,29) = 43.1$, $p < .001$] and scopolamine [$F(1,30) = 8.87$, $p = .006$] significantly impacted PPI. However, in this experiment there was no significant intensity × dose interaction [$F(2,29) = .294$, $p = .747$], and PPI did not decrease more at lower intensities in scopolamine-treated rats (Fig. 2A) as was shown in Experiment 1. Type of prepulse stimulus (i.e., tone vs. white noise) did not significantly affect PPI [$F(1,30) = 2.91$, $p = .098$]. Similarly, the prepulse stimulus type × dose interaction [$F(1,30) = .252$, $p = .619$] and the prepulse stimulus type × intensity × dose interaction [$F(2,29) = .952$, $p = .398$] were not significant.

The effect of scopolamine on response amplitude of the pulse-alone trials was assessed by a repeated-measure ANOVA , with the between-subjects factor of dose and the repeated factor of prepulse stimulus type. As shown in Fig. 2B, scopolamine treatment significantly affected response amplitude [$F(1,30) = 5.85$, $p = .022$], but response amplitude was not affected by the type of the prepulse stimulus (i.e., tone or white noise) as these data were collected in separate test sessions [$F(1,30) = .163$, $p = .689$].

Although there were no statistically-significant differences in PPI induced by a tone versus a white noise prepulse stimulus, there was a difference in the pattern of responses of the rats

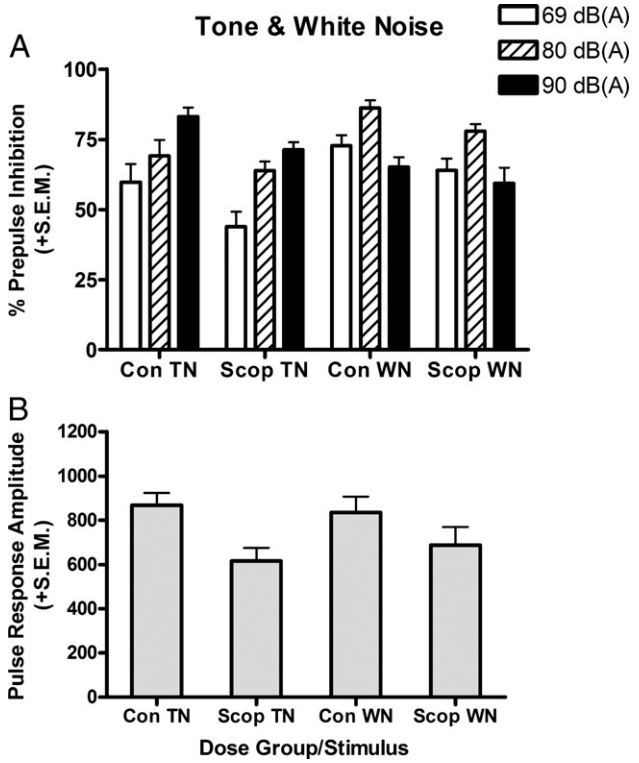

Fig. 2. Effects of tone and white noise prepulses and scopolamine treatment on PPI in rats ($n=16$). (A) Percent PPI for controls and scopolamine-treated rats as a function of increasing prepulse intensities revealed a linear response pattern when the prepulse was a 10 kHz tone (TN) and a biphasic response pattern when the prepulse was a white noise (WN) prepulse. (B) Pulse response amplitudes (arbitrary units) were reduced in scopolamine-treated rats during pulse-alone trials. Con=Saline Control, Scop=Scopolamine (0.3 mg/kg).

when they were given tone versus when they were given white-noise prepulse stimuli (Fig. 2A). Specifically, PPI induced by tone prepulse stimuli increased at each intensity level, whereas PPI induced by white noise prepulse stimuli increased as intensity increased, but then decreased at the highest intensity level.

3.3. Post hoc analyses of prepulse reactivity

To further evaluate the relationship between scopolamine treatment, prepulse stimulus type (Experiment 2 only), prepulse intensity and prepulse response amplitude in these experiments, post hoc analyses were performed. Prepulse stimulus response data of the prepulse+pulse trials, at each intensity and for each prepulse type (Experiment 2 only), were used in the analyses. In addition to examining prepulse response amplitude at the three prepulse stimulus intensities, the "prepulse" response amplitude of the null trials was also used for comparison. There were no auditory stimuli present during the null trials, so the prepulse amplitude during the null trials served as a baseline activity measurement of the rats during the test session.

3.3.1. Prepulse reactivity in Experiment 1

A repeated-measure ANOVA was conducted with dose as the between-subjects factor and prepulse intensity, including the "null" intensity, as the repeated factor. This analysis revealed a significant increase in prepulse response amplitude [$F(1,14)=38.4$, $p<.001$] in scopolamine-treated rats (Fig. 3). The dose effect was altered by the intensity of the prepulse stimulus, as evidenced by a significant dose effect×intensity interaction term [$F(3,12)=3.83$, $p=.039$].

Irrespective of dose, intensity [$F(3,12)=4.16$, $p=.031$] affected prepulse response amplitude. Simple contrasts of the prepulse stimulus intensity were evaluated by comparing the prepulse response amplitude at each prepulse stimulus intensity with the prepulse response amplitude during the null trials. The contrasts revealed a significant increase in prepulse response amplitude when the prepulse stimulus intensity was 90 dB(A) [$F(1,14)=10.3$, $p=.006$]. Fig. 3 indicates that there was a greater response to the highest prepulse stimulus intensity when compared to the response during the null trials or the other tone prepulses.

3.3.2. Prepulse reactivity in Experiment 2

A doubly repeated-measure ANOVA was conducted with dose as the between-subjects factor and the repeated factors of prepulse stimulus type and prepulse intensity, including the "null" intensity. The analysis revealed a significant increase in prepulse response amplitude in scopolamine-treated rats [$F(1,30)=15.8$, $p<.001$], but the dose effect was not altered by the type of prepulse stimulus or the intensity of the prepulse stimulus, as evidenced by the lack of a significant dose effect×prepulse type [$F(1,30)=.056$, $p=.815$] and dose effect×intensity [$F(3,28)=2.63$, $p=.070$] interaction.

Irrespective of dose, both prepulse type [$F(1,30)=29.1$, $p<.001$] and intensity [$F(3,28)=37.5$, $p<.001$] affected prepulse response amplitude. There was also a significant interaction of type of prepulse stimulus and prepulse stimulus intensity on prepulse response amplitude [$F(3,28)=12.0$, $p<.001$]. Fig. 4 shows that higher prepulse stimulus intensities increase prepulse response amplitude and that this increase was amplified when the prepulse stimulus was a white noise burst. To examine the significance of this effect, simple contrasts of

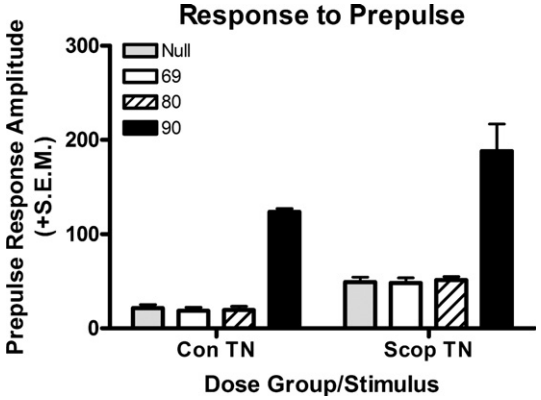

Fig. 3. Measures of prepulse response amplitude (arbitrary units) for controls and scopolamine-treated rats ($n=8$) during prepulse + pulse trials and null trials in Experiment 1 revealed significant increases to the high intensity (90 dB) 10 Hz prepulse tone in both groups of rats. Con=Saline Control, Scop=Scopolamine (0.3 mg/kg), Tn=Tone.

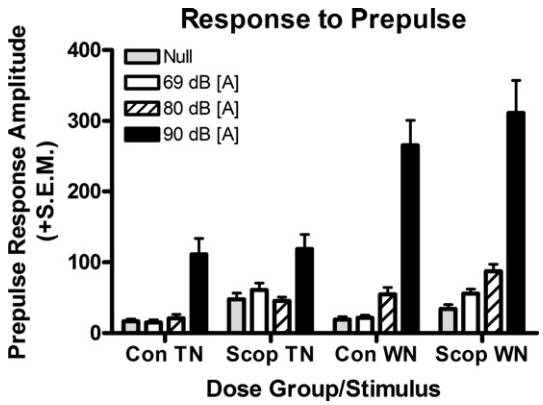

Fig. 4. Measures of prepulse response amplitude (arbitrary units) for controls and scopolamine-treated rats ($n=16$) during prepulse + pulse trials and null trials in Experiment 2 indicated that the highest prepulse stimulus intensity (90 dB) produced the largest response in both groups of rats when the prepulse was a 10 Hz tone and even more so when the prepulse was a burst of white noise. Con=Saline Control, Scop=Scopolamine (0.3 mg/kg), Tn=Tone, Wn=White Noise.

the type of prepulse stimulus and prepulse stimulus intensity interaction were evaluated by comparing the prepulse response amplitude at each prepulse stimulus intensity with the prepulse response amplitude during the null trials for tone and white noise in one analysis. The contrasts revealed a significant increase in prepulse response amplitude when the prepulse stimulus was a white noise burst and the intensity was 80 [$F(1,30)=23.9$, $p<.001$] or 90 dB(A) [$F(1,30)=23.1$, $p<.001$] when compared to the tone prepulse stimuli. While there was a significant increase in prepulse response amplitude of the higher intensity white noise compared to tone prepulse stimuli, it is also important to note that tone prepulse stimuli of 90 dB(A) also increased prepulse response amplitude.

4. Discussion

The major findings of the present study were: (1) scopolamine treatments reduced PPI; (2) scopolamine-induced PPI reductions were most apparent with a low intensity (i.e., approximately 10 dB above background) when a tone prepulse stimulus was used; (3) higher intensity prepulse stimuli produced a response, irrespective of whether the prepulse stimuli were a white-noise burst or a single-frequency (10 kHz) tone; (4) the response to the white-noise prepulse stimulus was greater than to a tone prepulse stimulus of the same intensity; and (5) prepulse-induced responses were accompanied by changes in PPI. The results from Experiment 1 replicated the findings of Jones and Shannon (2000b), including the interaction of prepulse stimulus intensity and scopolamine treatment, whereby treatment effects on PPI were greater at the lowest intensity. Experiment 2 was conducted to examine the potential difference between a white noise and a tone prepulse stimulus at increasing levels of intensity on PPI, and whether or not scopolamine treatment would alter the effects. In this experiment, as in the previous one, prepulse stimulus intensity increased PPI, and scopolamine reduced it. There was, however, no effect of prepulse stimulus type (tone or white noise) on PPI, although the results approached levels of significance. Review of the data suggested that the trend of intensity with a tone prepulse stimulus was linear, whereas the trend of intensity with a white noise prepulse stimulus was biphasic.

At the two highest prepulse stimulus intensity levels used in Experiment 2, it was noted that the rats were responding to the prepulse stimulus. An analysis of the prepulse stimulus response amplitudes during the combined prepulse+pulse trials along with the "prepulse" response amplitude of the null trials revealed that higher intensity prepulse stimuli increased the response amplitude to the prepulse, and this effect was larger when the prepulse stimulus was a white noise burst. At the middle intensity (80 dB [A]), only the white noise prepulse stimulus increased prepulse response amplitude. At the highest prepulse stimulus intensity (90 dB [A]), both tone and white noise prepulse stimuli significantly increased prepulse response amplitude, with this effect being over twice as large for the white noise prepulse stimuli. This finding is consistent with a previous report indicating that white noise stimuli elicit higher levels of response than tone stimuli of the same intensity level (Blumenthal and Berg, 1986).

The present results are also consistent with previous reports indicating that prepulse stimulus intensities that previously have been considered to be "non-startling" can, indeed, elicit a response (Dahmen and Corr, 2004). The use of what has been considered to be "non-startling" prepulse stimuli was recently assessed in a study using apomorphine treated mice by Yee and colleagues (2004). In their study, white noise prepulse stimuli at intensities of 4-, 8-, 12-, and 16 dB over a 65 dB(A) background were used with a white noise pulse stimulus of 120 dB(A) and a combination of prepulse-alone trials (of all intensities) along with the prepulse+pulse, and pulse-alone trials. Yee and collegues showed that control mice began to respond to the prepulse stimulus at higher intensities, but that the response was much greater in apomorphine-treated mice. A reduction in PPI was observed following apomorphine treatment, but the magnitude of the attenuation decreased as the intensity of the prepulse stimulus increased. Similarly, a reduction in the treatment effect of scopolamine on PPI at the high intensity prepulse stimuli was also observed in both experiments of the present study, and this also may have been due to the rats startling to the prepulse stimulus. In Experiment 2 of the present study, there was actually a reduction in PPI at the highest prepulse stimulus intensity compared to lower intensities when the prepulse stimulus was a white noise. This demonstrates the importance for not only comparing the level of prepulse stimulus intensities between studies, but also understanding that there may be a difference in PPI levels when a tone or a white noise prepulse stimulus of the same intensity is used.

Jones and Shannon (2000b) reported a significant scopolamine and prepulse stimulus intensity interaction, whereby the effects of scopolamine treatment on PPI were greater at the lower prepulse stimulus intensity levels. This interaction was observed in Experiment 1, but not in Experiment 2 of our study. This was probably due to the different patterns of PPI in the white noise (biphasic) and tone (linear) conditions. The significant interaction of scopolamine treatment and prepulse

stimulus intensity observed in Experiment 1 may have been caused by the rats startling to the highest intensity prepulse stimulus. A post hoc analysis of the prepulse stimulus response data from Experiment 1 indicated that the rats were responding to the highest intensity prepulse stimulus. In addition, prepulse response amplitude was affected by the intensity of the prepulse stimulus and whether it was a tone or a white noise burst.

The effects of scopolamine on response amplitude are less clear. It has been reported that scopolamine does not significantly affect response amplitude (Jones and Shannon, 2000a,b; Ukai et al., 2004). However, in some cases there is an apparent decrease in response amplitude with scopolamine treatment (Stanhope et al., 2001). In the present study, we found a scopolamine-induced decrease in response amplitude in Experiment 2, but not in Experiment 1. The inconsistent effects of scopolamine on response amplitude, and even to some degree on PPI, may be due to the fact that scopolamine is a broad anticholinergic drug. The strongest decreases in PPI are seen with M_3 and M_4 receptor antagonists, and since scopolamine blocks all muscarinic receptor subtypes, but with a stronger affinity for M_3 receptors, this may be the cause of the biphasic dose response seen with scopolamine on PPI measures (Ukai et al., 2004). It may be construed that antagonism of muscarinic receptor subtypes to varying degrees may also be responsible for differences seen in response amplitude.

Our results have implications for the interpretation of PPI data when high intensity prepulses are utilized. One implication is that the aspects of the prepulse stimulus that cause it to elicit a response should be considered. Stanhope and colleagues (2001) did not find an interaction of scopolamine treatment and prepulse stimulus intensity level at 3-, 6-, or 12 dB over a 70 dB background, suggesting that the level of prepulse stimulus dB over background may be more important than absolute dB levels. Although the majority of reports in the PPI literature do not indicate the use of prepulses of 20 or 30 dB over background, several recent studies have reported the use of intensities at the higher levels (Brunell and Spear 2006, Duncan et al. 2006, Moy et al. 2006).

Another implication from our results concerns the methods used to measure prepulse stimulus reactivity. Specifically, should measures include prepulse-alone trials, or is the response to the prepulse stimulus in the prepulse+pulse trials sufficient? It has been argued that measures of the prepulse response amplitude for the prepulse+pulse trials should be sufficient, since what happens after the prepulse stimulus (i.e., the pulse stimulus) should not affect the measurement of what precedes it (Dahmen and Corr, 2004). It does seem unlikely that there would be a difference between recording the prepulse response amplitude of the prepulse-alone trials verus the prepulse response amplitude of prepulse+pulse trials, although some conditioning could occur during the trials when the prepulse and pulse are paired, such that the animal may react differently to the prepulse if it has been repeatedly paired with the pulse. The current study did not test this hypothesis. While this is only speculation, this could account for the difference in prepulse response amplitudes of experiments with only prepulse stimuli (Jones and Shannon, 2000b) versus experiments that incorporate a variety of trial types, including trials with prepulse+pulse and pulse-alone trials. Further studies which systematically compare response amplitudes in rats exposed to only prepulses versus those exposed only to prepulse+pulse trials are needed to determine the extent to which previous exposures to the pulse could impact the response to the prepulse.

The significance of the prepulse response amplitude on the overall measure of PPI should not be overlooked. The relationship between the response to the prepulse and PPI appears to be that as the prepulse response amplitude increases, the level of PPI is reduced. It is unknown whether this is due to the prepulse stimulus sensitizing the pulse response or whether the pulse response amplitude being captured by the test system is actually the remnants of the prepulse response. If the latter is the case, then the measure of PPI could be directly affected such that the prepulse+pulse trials would be of similar magnitude as the pulse-alone trials.

A recent review of pharmacological studies of PPI and schizophrenia (Geyer et al., 2001) revealed that well over 150 different drugs have been tested for effects on PPI in rats alone in the last 10 years. To what extent were prepulse stimuli eliciting a response? To what extent have these studies reported response measures to the prepulse stimulus? According to Yee and colleagues (2004), studies have been conducted in which potentially startling prepulse stimulus intensities were used that could affect the conclusions, but adequate measures of responding to the prepulse were not used. The results of the current study suggest that if prepulses of 20 or 30 dB over background were used, the results of the measure of PPI could be confounded. Because the use of high intensity prepulses is sporadic, the impact that prepulse-elicited responses may have on the current literature base of psychopharmacology studies is difficult to determine, particularly since many of these studies do not report this measure, and to a lesser extent, some do not report the background noise level.

If the percentage of PPI can be influenced by the intensity of the prepulse stimulus in such a way as to prevent the detection of a treatment effect, then there is a potential for effects of toxicological significance to go unnoticed. One should also consider that a drug causing increased startle reactivity may increase the likelihood of a prepulse-elicited response while control subjects remain unaffected by the prepulse stimulus, which could influence PPI exclusively in the treated subjects and mask the true effect of the treatment. This is a hypothesis that warrants further investigation, and for this reason, prepulse stimulus reactivity should be measured and considered when interpreting the results of PPI studies. However, many drugs do not affect startle reactivity but have clear effects on PPI. Conversely, many drugs affect startle reactivity but do not alter PPI.

In summary, our results indicate that scopolamine does attenuate PPI, but the robustness of this effect is a function of the type and intensity of the prepulse stimulus that is used. In addition, our results suggest that significant scopolamine and prepulse stimulus intensity interactions occur when there are prepulse-elicited responses. This phenomenon may underlie the subtle effects of scopolamine-induced reductions in PPI that

have been reported by others (Ukai et al., 2004). As such, careful consideration should be given to the varying methods used when making comparisons as to the relative effects of pharmacological agents on the resultant measure of PPI.

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References

Acocella CM, Blumenthal TD. Directed attention influences the modification of startle reflex probability. Psychol Rep 1990;66:275–85.

Adams J, Buelke-Sam J, Kimmel CA, Nelson CJ, Reiter LW, Sobotka TJ, et al. Collaborative behavioral teratology study: protocol design and testing procedures. Neurobehav Toxicol Teratol 1985;7:579–86.

Blumenthal TD. Short lead interval startle modification. In: Dawson ME, Schell AM, Böhmelt AH, editors. Startle modification: Implications for neuroscience, cognitive science, and clinical science. New York: Cambridge University Press; 1999. p. 51–71.

Blumenthal TD, Berg WK. Stimulus rise time, intensity, and bandwidth effects on acoustic startle amplitude and probability. Psychophysiology 1986;23:635–41.

Braff DL, Stone C, Callaway E, Geyer M, Glick I, Bali L. Prestimulus effects of human startle reflex in normals and schizophrenics. Psychophysiology 1978;15:339–43.

Braff DL, Geyer MA, Light GA, Sprock J, Perry W, Cadenhead KS, et al. Impact of prepulse characteristics on the detection of sensorimotor gating deficits in schizophrenia. Schizophr Res 2001;49:171–8.

Brunell SC, Spear LP. Effects of acute ethanol or amphetamine administration on the acoustic startle response and prepulse inhibition in adolescent and adult rats. Psychopharmacology 2006;186:579–86.

Dahmen JC, Corr PJ. Prepulse-elicited startle in prepulse inhibition. Biol Psychiatry 2004;55:98–101.

Davis M, Mansbach RS, Swerdlow NR, Campeau S, Braff DL, Geyer MA. Apomorphine disrupts the inhibition of acoustic startle induced by weak prepulses in rats. Psychopharmacology 1990;102:1–4.

Duncan GE, Moy SS, Lieberman JA, Koller BH. Typical and atypical antipsychotic drug effects on locomotor hyperactivity and deficits in sensorimotor gating in a genetic model of NMDA receptor hypofunction. Pharmacol Biochem Behav 2006;85:481–91.

Gewirtz JC, Davis M. Habituation of prepulse inhibition of the startle reflex using an auditory prepulse close to background noise. Behav Neurosci 1995;109:388–95.

Geyer MA, Krebs-Thomson K, Braff DL, Swedlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology 2001;156:117–54.

Ison JR. Reflex modification as an objective test for sensory processing following toxicant exposure. Neurobehav Toxicol Teratol 1984;6:437–45.

Jones CK, Shannon HE. Muscarinic cholinergic modulation of prepulse inhibition of the acoustic startle reflex. J Pharmacol Exp Ther 2000a;294:1017–23.

Jones CK, Shannon HE. Effects of scopolamine in comparison with apomorphine and phencyclidine on prepulse inhibition in rats. Eur J Pharmacol 2000b;391:105–12.

Mansbach RS, Geyer MA. Parametric determinants in pre-stimulus modification of acoustic startle: interaction with Ketamine. Psychopharmacology 1991;105:162–8.

Moy SS, Perez A, Koller BH, Duncan GE. Amphetamine-induced disruption of prepulse inhibition in mice with reduced NMDA receptor function. Brain Res 2006;1089:186–94.

Stanhope KJ, Mirza NR, Bikerdike MJ, Bright JL, Harrington NR, Hesselink MB, et al. The muscarinic receptor agonist xanomeline has an antipsychotic-like profile in the rat. J Pharmacol Exp Ther 2001;299:782–92.

Swerdlow NR, Paulsen J, Braff DL, Butters N, Geyer MA, Swenson MR. Impaired prepulse inhibition of acoustic and tactile startle response in patients with Huntington's Disease. J Neurol Neurosurg Psychiatry 1995;58:192–200.

Ukai M, Okuda A, Mamiya T. Effects of anticholinergic drugs selective for muscarinic receptor subtypes on prepulse inhibition in mice. Eur J Pharmacol 2004;492:183–7.

USEPA. Health effects test guidelines, developmental neurotoxicity study, OPPTS 870.6300; 1998.

Wu M, Jenden DJ, Fairchild MD, Siegel JM. Cholinergic mechanisms in startle and prepulse inhibition effects of the false cholinergic precursor N-aminodeanol. Behav Neurosci 1993;107:306–16.

Yee BK, Russig H, Feldon J. Apomorphine-induced prepulse inhibition disruption is associated with a paradoxical enhancement of prepulse stimulus reactivity. Neuropsychopharmacology 2004;29:240–8.