Acoustic Startle Responses and Prepulse Inhibition of Acoustic Startle Responses in Warsaw Alcohol High-Preferring (WHP) and Warsaw Alcohol Low-Preferring (WLP) Rats

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Abstract — Aims: An assessment of the acoustic startle response (ASR) and prepulse inhibition (PPI) of ASR in laboratory animals is used to model human anxiety and psychotic states, respectively. The aim of the study was to evaluate ASR and PPI in alcohol-naive male and female Warsaw alcohol high-preferring (WHP) and Warsaw alcohol low-preferring (WLP) rats. Methods: ASR and PPI were assessed in two separate experiments by using the SR-LAB apparatus (San Diego Instruments, San Diego, CA, USA). In the ASR session, animals (n = 13–16 rats per group) were exposed to startling stimuli of different intensities (72, 84, 98, 112 and 124 dB) in a random order. In the PPI session, prepulse stimuli (78, 81, 84 and 90 dB) preceded a pulse startling stimulus (120 dB) in a random order. The background white noise was set at 70 dB. PPI was calculated according to the formula: [(startle amplitude in prepulse-and-pulse trials)/startle amplitude in pulse alone trials] × 100%. Results: The WHP males exhibited higher startle amplitudes in response to 112 dB stimuli when compared with their WLP counterparts. The WHP females showed higher startle reactivity to 112 and 124 dB stimuli when compared with the WLP females. There were no differences between the WHPs and WLPs in PPI of ASR. Conclusion: The results of the present study suggest that exaggerated startle responses can be a physiological/behavioral marker of a propensity to abuse alcohol.

INTRODUCTION

Assessment of the acoustic startle response (ASR) and prepulse inhibition (PPI) of ASR in rodents is used to model human anxiety and psychotic states, respectively (Koch and Schnitzler, 1997; Swerdlow et al., 2000; Braff et al., 2001). In laboratory rats, the ASR is a constellation of responses to sudden, intense acoustic stimuli, including the whole-body flinch measured by a piezoelectric transducer (Yeomans and Frankland, 1995; Koch and Schnitzler, 1997). PPI refers to a reduction in startle responses to the startling stimulus if it is preceded shortly by a weaker stimulus (a prepulse) (Swerdlow et al., 2000). The ASR and PPI can be assessed in the same apparatus.

It has been suggested that the ASR correlates with innate levels of anxiety in human and laboratory subjects (Plappert et al., 1993; Krystal et al., 1997; Ray et al., 2009). Elevated acoustic startle magnitudes were found in alcohol-dependent patients who had been sober for 12–26 days prior to testing (Krystal et al., 1997). Elevated startle magnitudes can be associated with a family history of alcohol dependence. Grillon et al. (1997, 2000) have shown that responses to acoustic startle stimuli in 10- to 17-year-old sons of alcoholics were higher when compared with sons of non-alcoholic parents.

PPI of ASR is an experimental measure of sensorimotor gating, and disruptions in PPI are thought to mimic schizophrenia-like states in animal and human subjects (Braff and Geyer, 1990; Swerdlow et al., 2000). PPI deficits have been observed in schizophrenic and schizotypal patients (Cadenhead et al., 1993; Braff et al., 2001). PPI is also reduced in individuals with a high genetic loading for alcoholism (Grillon et al., 1997, 2000; Schellekens et al., 2012).

Several sets of alcohol preferring and alcohol non-preferring lines of rats have been described, including the ALKO alcohol/non-alcohol (AA/ANA) lines (Sommer et al., 2006), the Sardinian alcohol preferring/alcohol non-preferring (sP/sNP) lines (Colombo et al., 1995), the Indiana University lines (the alcohol preferring/alcohol non-preferring (P/NP) and high alcohol-drinking/low alcohol-drinking (HAD/LAD) rats) (Murphy et al., 2002) and the Warsaw alcohol high-preferring/Warsaw alcohol low-preferring (WHP/WLP) lines (Dyr and Kostowski, 2008). The alcohol preferring and non-preferring lines can be useful not only for studying mechanisms of alcohol drinking, but also for delineating mechanisms of well-known co-occurrence of alcohol use and psychiatric disorders (Helzer and Pryzbeck, 1988; Colombo et al., 1995; McKinzie et al., 2000). To the best of our knowledge, only the Indiana University lines of rats (P/NP, HAD/LAD) have been tested in the ASR and PPI procedure. Jones et al. (2000) have investigated ASR and PPI magnitudes in female P and NP rats. Alcohol-naive female P rats exhibited higher startle amplitudes than their NP counterparts. In another study, male P and NP rats were tested for the ASR in response to alcohol withdrawal after a single intragastric infusion of 4.0 g/kg alcohol (Chester et al., 2003). A statistical analysis of control groups included in the latter study confirmed increased basal startle responses in alcohol-naive P rats when compared with alcohol-naive NP subjects. In addition, alcohol-treated P, but not NP, rats showed increased startle amplitudes during alcohol withdrawal (Chester et al., 2003). In line with the above, McKinzie et al. (2000) have reported that basal acoustic startle and fear-potentiated startle responses were greater in male P than in male NP animals. Female P rats were more sensitive to amphetamine-induced potentiation of the ASR than their NP counterparts (Bell et al., 2003). Thus, it seems that an elevated ASR is associated with alcohol preference in the P/NP lines of rats.

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Several studies have addressed possible differences in the PPI of ASR between alcohol preferring and alcohol non-prefering lines of rats. Alcohol-naive P and NP rats exhibited comparable levels of PPI. Alcohol- or amphetamine-induced disruptions of PPI tended to be greater in P rats when compared with NP subjects (Jones et al., 2000; Bell et al., 2003).

Summing up, both clinical and preclinical studies suggest that heightened ASR and lowered PPI levels can be considered as a potential marker of alcohol use disorders (Krystal et al., 1997; Grillon et al., 1997, 2000; Jones et al., 2000; Bell et al., 2003). Given the above, in the present study, we decided to evaluate ASR and PPI magnitudes in alcohol-naive WHP and WLP rats.

MATERIALS AND METHODS

Subjects

Sixteen alcohol-naive male WHP rats, 14 alcohol-naive male WLP rats (330–400 g), 13 alcohol-naive female WHP rats and 14 alcohol-naive female WLP rats (230–300 g) were kept four per standard plastic cage. All the animals were around 3 months old. Males and females were housed in separate rooms with constant environmental conditions (temperature of 22 ± 1°C, 60% humidity, a 12-h light-dark cycle with lights on at 6:00 a.m.) for 3–4 weeks before the onset of experimental procedures. Standard lab chow (Labofeed H, WPiK, Kcynia, Poland) and tap water were available ad libitum.

Treatment of rats in the present study was in full accordance with the ethical standards laid down within respective European (Directive no. 86/609/EEC) and Polish regulations. All the procedures were reviewed and approved by the 2nd Warsaw Ethics Committee (authorization no. 10/2010).

Apparatus

ASR and PPI magnitudes were assessed in the same apparatus. The ASR/PPI apparatus consisted of eight identical chambers (SR-LAB, San Diego Instruments, San Diego, CA, USA). Each chamber contained a Plexiglas cylinder (inside diameter: 8.8 cm, inside length: 18.4 cm) resting on a Plexiglas frame located in a sound-attenuated and ventilated enclosure (Bell et al., 2003). Background noise and acoustic stimuli were presented via a loudspeaker mounted 24 cm above the cylinder. Startle responses, reflecting the motion of animals in the cylinder following the acoustic stimulus, were detected by a piezoelectric transducer mounted below the frame.

The administration of stimuli and response recording were controlled by the SR-LAB software. Sound levels in the chambers were measured and calibrated with a sound meter. Response sensitivities were calibrated using the SR-LAB startle calibration system.

Experiment 1: ASRs

All experiments were performed between 10:00 a.m. and 3:00 p.m. Male and female WHP and WLP rats were placed individually in the Plexiglas cylinder. Throughout the whole session, a chamber light was on, and the background white noise was set at 70 dB. Test sessions started with a 5-min. acclimatization period to accustom the rat to the experimental procedure (Bell et al., 2003). Three startling stimuli (120 dB, duration: 40 ms) were given during the acclimatization period with an average inter-trial interval (ITI) of 22.5 s (15–30 s). The ITI was randomized by the SR-LAB software.

Following the acclimatization period, the rats received 50 stimuli of different intensities (72 dB, 84 dB, 98 dB, 124 dB, stimulus duration: 40 ms) presented in a random order with the mean ITI of 22.5 s. Startle responses were measured for 100 ms after the onset of the acoustic stimulus. For each level of decibel stimulus, startle amplitudes were averaged across 10 trials. Startle responses to the three initial stimuli were excluded from statistical analyses.

Experiment 2: PPI

PPI of ASR was examined 2 days after the ASR assessment in the same groups of WHP and WLP rats. The apparatus and basic experimental conditions were identical to that described above. Test sessions started with the acclimatization period which included three startling stimuli (120 dB) to accustom the rat to the experimental procedure. The initial stimuli were followed by 100 trials (10 × 10 trials) presented in a random order. The PPI session involved: 10 trials with a sham stimulus (70 dB, 40 ms), four types (4 × 10) of prepulse trials (PP) which included only 20-ms PP stimuli (78, 81, 84 and 90 dB), 10 pulse trials (P) which included only a pulse (startling) stimulus (120 dB, 40 ms), four types (4 × 10) of prepulse-and-pulse trials (PP-P) which included a 20-ms PP (78, 81, 84 or 90 dB) followed 100 ms later by a 120-dB P stimulus. The mean ITI was 22.5 s. Startle responses were measured for 100 ms after the onset of the last stimulus within each trial. For each type of trial, startle amplitudes were averaged across 10 trials. The magnitude of PPI was calculated as a percent inhibition of the startle amplitude in the P trial (treated as 100%) according to the formula: [(startle amplitude in P trials – startle amplitude in PP-P trials)/startle amplitude in P trials] × 100%. Startle responses to the three initial stimuli were excluded from statistical analyses.

Statistics

Differences in startle responses were assessed with the aid of a mixed-factor three-way (line × sex × stimulus intensity) analysis of variance (ANOVA) with Line (WHP vs. WLP) and sex (males vs. females) serving as between-subject factors and stimulus intensity serving as a within-subject factor. PPI data were analyzed using the mixed factor three-way (line × sex × prepulse intensity) ANOVA with Line and Sex serving as between-subject factors and stimulus intensity serving as a within-subject factor. Post hoc analyses were conducted using the Newman–Keuls test. The Statistica 5.0 software package (StatSoft, Inc., Tulsa, OK, USA) was employed to analyze all data. P < 0.05 was considered significant.

RESULTS

Experiment 1: ASR

There were no differences in body weights between the WHPs and WLPs for each sex (P > 0.05).

The three-way ANOVA revealed a significant effect of line [F(1,265) = 27.5, P < 0.01], sex [F(1,265) = 4.4, P < 0.05] and stimulus intensity [F(4,265) = 85.6, P < 0.01].
Experiment 2: PPI

As expected, the three-way ANOVA confirmed a significant effect of prepulse intensity \( F(4,265) = 19.1, \ P < 0.01 \). The other effects and interactions did not reach statistical significance \( P > 0.05 \); Fig. 2).

DISCUSSION

The results of our study on the WHP and WLP rats add to a growing body of evidence indicating that alcohol preference can be associated with higher amplitudes of startle responses. Alcohol preferring P rats presented higher startle magnitudes than their alcohol non-preferring NP counterparts across a range of stimulus intensities and experimental protocols (Jones et al., 2000; McKinzie et al., 2000; Bell et al., 2003; Chester et al., 2003). Elevated acoustic startle magnitudes were observed in alcohol-dependent patients (Krystal et al., 1997) and in sons of alcoholics (Grillon et al., 1997, 2000). Recently, increased startle responses have been found in detoxified early-onset alcoholics when compared with detoxified late-onset alcoholics and control subjects (Schellekens et al., 2012). Interestingly, Chester et al. (2003) have found higher startle amplitudes in alcohol non-preferring LAD rats, when compared with alcohol preferring HAD rats. The latter findings would still indicate that the ASR is associated with alcohol preference only in the opposite direction.

It has been reported that the ASR can correlate with innate levels of anxiety in human and laboratory subjects (Plappert et al., 1993; Ray et al., 2009). Given the above, our results may be in line with the self-medication hypothesis of alcohol addiction linking alcohol drinking with high anxiety levels (Colombo et al., 1995; Schuckit et al., 1997). Alcohol-preferring P rats displayed enhanced anxiogenic-like responses in the elevated plus maze test, approach-avoidance conflict test and step-down shock test, when compared with NP rats (Stewart et al., 1993). AA rats demonstrated enhanced audiogenic immobility reactions (freezing) when compared with their ANA counterparts (Fahlke et al., 1993). sP rats were more anxious than sNP rats in the elevated plus maze (Colombo et al., 1995). Given the above, further studies are needed to examine whether WHPs and WLPs would present different levels of anxiety-like behaviors.

Different amplitudes of the ASR in alcohol preferring and non-preferring rats may result from innate differences in specific neurochemical circuits. GABAergic transmission has been implicated in the etiology of alcoholism and anxiety disorders and has been proposed to be responsible for individual differences in the ASR (Bast et al., 2001; Saba et al., 2011). The density of \( \gamma \)-aminobutyric acid receptors was greater in the cingulate cortex obtained from WHP rats when compared with WLP subjects (Dyr et al., 1999). Studies on P/NP and HAD/LAD lines have demonstrated higher densities of GABAergic terminals in the nucleus accumbens of the alcohol preferring lines (Hwang et al., 1990). Further studies are needed to evaluate whether other neurotransmitters (e.g. dopamine, serotonin and glutamate) thought to regulate startle responses could be involved in differences in the ASR between alcohol-preferring and non-preferring lines.

PPI magnitudes were similar in the WHP and WLP rats across a range of prepulse intensities. Our findings are in partial agreement with the previous studies on PPI in alcohol preferring and non-preferring lines of rats. Bell et al. (2003) and Jones et al. (2000) have found similar PPI levels in drug- and alcohol-naive P and NP subjects. On the other
hand, P rats exhibited higher sensitivity than NP rats to disruptive effects of amphetamine (4.0 mg/kg; Bell et al., 2003) and ethanol (0.5 g/kg; Jones et al., 2000) on PPI. It remains to be established whether the disruptive effects of amphetamine or ethanol on PPI would differ between WHP and WLP subjects.

Our study had some limitations. Adolescent WHP/WLP rats were not used and possible age differences were not addressed. Another limitation was that the same animals were used in Experiment 1 (ASR) and Experiment 2 (PPI). One may wish to verify the present results in PPI experiments with experimentally naive WHP and WLP animals. One may also wish to test wider ranges of pulse and prepulse intensities. Although relatively large groups of animals were tested (n = 13–16), it is possible that even larger groups are necessary to detect subtle between-line differences. Last but not least, the phase of the estrus cycle was not controlled in the present study. It has been suggested that ovarian hormones may alter the ASR in female rats (Reilly et al., 2009).

In conclusion, our findings indicate that alcohol-prefering WHP rats may be more sensitive to startle-evoking acoustic stimuli than their non-prefering WLP counterparts. On balance, the present and previous results (Grillon et al., 1997, 2000; Bell et al., 2003; Chester et al., 2003; Schellekens et al., 2012) suggest that partially overlapping neural and genetic mechanisms are involved in the regulation of startle responses and alcohol drinking behavior.

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