Research Article:
There are No Sex-Specific Differences in Habituation and Prepulse Inhibition of Acoustic Startle Response in Sprague Dawley Rats

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Abstract

Habituation is characterized by a gradual decrease in response to a repeated, irrelevant stimulus. In rodents, habituation can be studied using the acoustic startle response (ASR). ASR can also be modulated by prepulse inhibition (PPI), where the presentation of a sub-threshold pre-stimulus decreases an organism’s behavioural response to a subsequent high intensity stimulus. While sex-specific differences in habituation and PPI have been observed in humans in association with several disorders, such as bipolar disorder and schizophrenia, they have not been extensively studied in a healthy model in Sprague Dawley rats. Pre-existing sex-specific differences have important implications for characterizing diseases on the basis of non-associative learning deficits. Furthermore, female subjects are often excluded from non-associative learning experiments due to potential effects of hormonal fluctuations on startle modulations. The focus of this study was to investigate any sex differences in habituation and PPI in male and female Sprague Dawley rats from postnatal day 12 to 40. Habituation was assessed through the presentation of a high intensity (105 db) acoustic stimulus for twenty trials, with stimulus duration of 20ms per trial. PPI was measured through exposure to a prepulse of three possible intensities (0 db, 75 db, 80 db) for duration of 4 ms, followed by exposure to the pulse (105 db), with an ISI of 50 ms. There was no significant difference in prepulse inhibition between sex. Similarly, there was no significant sex difference in habituation. This experiment indicates that habituation and prepulse inhibition are not significantly affected by gender differences, perhaps due in part to the adaptive nature of non-associative learning.

Introduction

The startle response is a protective reflex that prepares the organism for the fight or flight response and can be elicited from a visual, tactile or acoustic stimulus. In rats, the startle response is characterized by curled shoulders, decreased body length and accelerated heart rate. This behavioural reflex protects the vulnerable dorsal surface of the neck during a potential attack and lasts approximately 200 ms.

In rats, the advent of the acoustic startle response (ASR) corresponds with the opening of the external acoustic meatus and the onset of hearing at postnatal day (PND) 10-12. The ASR is elicited by a stimulus of 80 db or higher and there is a 5-10 ms latency period between the presentation of the acoustic startle stimulus and the bodily response. The pathway for this reaction is relatively well understood in rodents. When a rat is exposed to an auditory stimulus, spiral ganglion cells transmit this information through secondary auditory neurons to the giant neurons in the ventrocaudal region of the caudal pontine reticular nucleus (PnC) of the brainstem. The message is integrated by the giant neurons and is then transmitted to the motor neurons in the spinal cord, thus causing contraction of muscles. The ASR has a non-zero baseline and its magnitude can be altered by startle attenuating processes. Startle attenuating processes reflect important mechanisms of sensory filtering, the ability to filter extraneous stimuli and directly focus on pertinent stimuli. Sensory filtering mechanisms are important in preventing sensory flooding and cognitive fragmentation. These mechanisms can be measured in the form of habituation and prepulse inhibition (PPI) of startle responses.
Habituation is characterized by a gradual decrease in response to a repeated, irrelevant stimulus. In order to generate a startle response, the stimulus must be sudden and intense, indicative of potentially dangerous conditions. The repeated presentation of an innocuous stimulus reduces the respective startle response. Habituation has been noted in a wide variety of organisms and is thought to be the simplest form of non-associative learning.

Prepulse inhibition describes the process whereby the presentation of a non-startling prepulse 20-1000 ms before the startle eliciting pulse reduces startle magnitude. According to the “protective hypothesis” proposed by Graham, PPI reflects a sensory filter mechanism that protects ongoing processing of the prepulse against interference by the succeeding pulse. PPI allows the nervous system to temporarily adapt to the high intensity stimulus. In rats, PPI is most apparent when the prepulse in presented 20-50 ms before the pulse and can reduce the startle response by ninety percent.

The presence of PPI and habituation reflect intact information processing. Deficits in information processing result in reduced PPI and habituation and have been observed in association with disorders such as schizotypal personality disorder, obsessive-compulsive disorder, schizophrenia and post-traumatic stress disorder. Many of these neuropsychiatric disorders display age and sex-specific patterns in frequency of cognitive symptoms. In order to better characterize disorders on the basis of non-associative learning and sensory gating defects, it is important to study pre-existing age and sex differences in a healthy model.

Previous studies indicate a species- and strain-specific sex difference in ASR and its modulations. In humans, a significantly lower ASR probability has been reported in men as compared to women, while greater PPI has been reported in males when a weak prepulse is used. However, the opposite finding has been reported as in mice; C57 and C3H males exhibit higher startle amplitudes than females. The same paper reports no effect of sex on PPI but a strain-specific sex difference in habituation, where C57 males display stronger long-term habituation and C3H females display stronger short-term habituation. In Wistar rats, ASR and PPI are both greater in males than in females. In Long Evans and Roman rats, males display higher ASR but no sex effect in PPI was noted.

These findings suggest that ASR reactivity and sensory gating are dependent on the species and strain used to study this behaviour. Sprague Dawley rats are often used in sensory gating experiments due to ease of handling and robust startle responses. The purpose of this study was to investigate a difference between male and female rats in ASR reactivity and its modulations in order to determine the validity of using this strain in sensory filtering experiments. We examined sex effects on ASR reactivity, short-term habituation and PPI in male and female Sprague Dawley rats from the onset of ASR (PND 12) to PND 40. This was done in order to determine if rats displayed changes in these parameters before, during and after sexual maturity. Because Sprague Dawley rats are often used to recapitulate anxiety related features of human disorders, we hypothesized that these rats would display similar sex effects of ASR and its modulations to that seen in humans. We expected lower ASR reactivity, greater PPI and lower short-term habituation in males, in line with previously reported human data.

**Methods**

Subjects

Four male and four female Sprague Dawley rats from the same litter served as subjects for this experiment. Animals were housed in groups, maintained on a 12:12 hour light-dark cycle with food and water ad libitum. All experiments were conducted during the light cycle and at the same time of day to limit confounding effects.

Procedure

Each animal was enclosed in a sound attenuated startle box with a constant background noise of 65 dB, which reduced acoustic contamination for external sounds. Previous literature suggests that onset of hearing occurs in rats by PND 12. In order to verify that rats could hear the startling pulse and the ASR was functional, threshold testing was conducted on PND 11-12. Threshold testing was used to determine the range of intensities of the acoustic stimulus that would elicit a startle response in the organism. During the first trial, an acoustic stimulus of 76 dB (20 ms, white noise) was presented and any resulting startle was recorded. The stimulus was then increased by 2 dB for each subsequent trial. Threshold testing allowed determination of when the acoustic startle response was functional. Habituation testing began on PND 12. PPI testing began on PND 13 when rats exhibited a startle response.
response to stimulus of a slightly higher intensity (around 90 dB) than that of the prepulse and researchers were certain that the prepulse could be heard.

After handling, rats were placed in the startle boxes and allowed to acclimatize to their environment for five minutes. At this time, a background noise level of 65 dB was also presented. Prior studies indicate that the acoustic stimulus must be greater than 80 dB in order to evoke a startle response. The acoustic stimulus used in this experiment was 105 dB white noise. The duration of the stimulus was 20 ms and the interstimulus interval (ISI) was 15 ms. Habituation testing continued for 20 stimuli.

Prepulse inhibition testing began on PND 13 using acoustic stimuli of two different intensities: a low prepulse of 75 dB white noise and a high prepulse of 80 dB white noise. These prepulses were presented in a pseudorandom order, along with 0 dB prepulse. There was a 50 ms delay between the presentation of the prepulse and the presentation of the startle-eliciting pulse. The duration of the prepulse was 4 ms. Prepulse inhibition testing continued for 30 trials with ten trials each consisting of the presentation of no prepulse, low prepulse or high prepulse before the startling pulse.

**Apparatus**

ASR amplitude values were measured using the Med Associates Startle Reflex system and Med Associates Startle Reflex version 5.95 software 2007. Animals were placed in small holders mounted on species-specific load cell platform. These platforms measure vertical dislocation due to movement by the animal. Changes in vertical dislocation were transduced into voltage and analyzed by a connected computer that translates this signal into startle units.

**Data Analysis**

For habituation testing, a habituation score specific to each rat for each testing day, was calculated. ASR amplitude values were normalized to the highest value among the first three startle presentations. An average of the last five normalized scores was then used as a habituation score. SigmaStat 3.5 was used to run a two-way ANOVA to determine whether the effects of age and sex on habituation score were statistically significant.

For prepulse inhibition testing, the average of all no prepulse trials for each testing day for each rat was used as the baseline startle response. The average of startle responses evoked by low pulses and high prepulses were respectively calculated. This value was expressed as a percent of the previously calculated baseline startle response. The percent scores for each sex were then averaged specific to post natal day. A two-way ANOVA was performed to determine whether the effects of sex and high and low prepulse on prepulse inhibition were statistically significant.

**Results**

**Habituation**

ASR amplitude values were significantly higher ($t(38) = 5.54, p < 0.001$) in female rats than male rats. Figure 1 shows the average startle amplitude of male and female rats to the 20 startle stimuli. Both sex groups show a decline of startle responses over the presentation of 20 stimuli suggesting that short term habituation was intact in these rats.

As indicated on Figure 2, there was no significant difference ($F(1,6) = 1.003, p = 0.32$) in habituation between any of the different age groups. A general trend indicated that males habituated more than females. This trend reversed during PND 23-27, the age at which rats reach sexual maturity. Habituation scores are grouped into five developmental periods. These periods correspond to very young age (soon after onset of hearing, PND 13-16), before sexual maturity (PND 18-22), during sexual maturity (PND 23-27), after sexual maturity (PND 29-32), and adolescent period, where normal levels of habituation are expected (PND 33-40).

**Prepulse Inhibition**

Prepulse inhibition significantly increased when a high prepulse was used compared to the low prepulse ($F(1,62) = 5.086, p = 0.02$). However, there was no significant effect of sex on prepulse inhibition ($F(1,62) = 0.468, p = 0.50$) with a high or low prepulse.

**Discussion**

Female subjects are often excluded from sensory filtering experiments due to potential effects of hormonal fluctuations. This reduces the validity of such experiments as it restricts understanding of neural pathways to male subjects. This study demonstrates that there is no difference between sex in habituation and prepulse inhibition in Sprague Dawley rats and therefore female rats may be suitable subjects when studying startle behaviour.
Figure 1: ASR amplitude mean ± SEM for female and male Sprague Dawley rats averaged across testing days.

Figure 2: Mean ± SEM short term habituation scores for male and female Sprague Dawley rats across development.

Figure 3: Mean ± SEM of prepulse inhibition in male and female Sprague Dawley rats when a low prepulse (75 db) preceded the startling pulse.
In addition, these results correspond with previous research conducted in humans\(^{\text{13,14}}\), suggesting that Sprague Dawley rats are a good model for studying neural pathways associated with sensory gating. It is important to note that these preliminary results should be supported by a more extensive study using a greater sample size.

Contrary to previous studies with rats, which showed the opposite effect\(^{\text{15}}\), a sex difference was noted for startle reactivity whereby females exhibited greater ASR amplitude than males. This effect may be due to the large individual differences between absolute startle amplitudes in animals together with the relatively low number of animals used in this study. However, the fact that this effect was reported in human data as well suggests an effect of gender on startle reactivity\(^{\text{13}}\).

The startle response measured in this experiment is subject to modulations through both habituation and sensitization. This may be explained by the dual process theory proposed by Groves and Thompson\(^{\text{16}}\). Groves and Thompson’s ‘dual process theory’ states that sensitization and habituation interact to produce the strength of the startle response\(^{\text{18}}\). Sensitization increased when the stimulus is perceived to be aversive by the organism\(^{\text{18}}\). It is possible that the 105 dB stimulus used in this experiment was too aversive for the young rats and therefore sensitization lead to an amplification of the behavioural response. Other factors that may have influenced habituation levels include our calculation method, as there is no standard procedure for calculating habituation scores, or a genetic influence as all subjects were from one litter. Further studies should eliminate confounding litter effects by comparing male and female rats across litters.

Prepulse inhibition of the ASR increased between PND 13-PND 20 and stayed relatively stable to PND 43. This is consistent with previous research which finds that older rats (70 days of age) had significantly greater PPI than younger rats (40 days of age)\(^{\text{19}}\). All rats exhibited prepulse facilitation during the first testing days (males at PND 12 and females at PND 13). In addition, males and females exhibited decreases in prepulse inhibition around PND 16. This may suggest that the functioning of pathways underlying prepulse inhibition are dependent on experience. Indeed, past research has shown that young rats demonstrate an experience related shift from facilitation to attenuation. There was no significant sex difference in PPI with a high or low prepulse. Due to its adaptive nature, it is possible that PPI is a well conserved response, not dependent on sex-specific hormonal fluctuations.

ASR reactivity and sensory gating have been widely used as translational tools in neurological research. However these phenomena may be subject to strain-, sex- and experience-specific changes. This study highlights the importance of investigating factors that govern prepulse inhibition and habituation of the acoustic startle response. Future studies should investigate the interaction between sex and age in order to better utilize sensory filtering mechanisms as a research tool.

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**Figure 4:** Mean ± SEM prepulse inhibition in male and female Sprague Dawley rats when a high prepulse (80 db) preceded the startling pulse.
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References

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