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Research paper

Comparison of the sensitivity of prepulse inhibition of the startle reflex and operant conditioning in an auditory intensity difference limen paradigm

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ABSTRACT

Reward-based operant conditioning (OC) procedures and reflex-based prepulse inhibition (PPI) procedures are used in mouse psychoacoustics. Therefore it is important to know whether both procedures provide comparable results for perceptual measurements. Here we evaluate the sensitivity of the C57BL/6N mouse in both procedures by testing the same individuals in the same Intensity Difference Limen (IDL) task. Level increments of a 10 kHz tone were presented in a train of 10 kHz reference tones. Objective analysis based on signal-detection theory was applied to compare the results of OC and PPI procedures. In both procedures the sensitivity increased with level increment. In agreement with the near miss to Weber's law, sensitivity increased with sound level of the reference stimuli. The sensitivity observed in the OC procedure was considerably larger than the sensitivity in the PPI procedure. Applying a sensitivity of 1.0 as the threshold criterion, mean IDLs in the OC procedure were 5.0, 4.0 and 3.5 dB at reference levels of 30, 50 and 75 dB SPL respectively. In the PPI procedure, mean IDLs of 18.9 and 17.0 dB at reference levels of 50 and 75 dB SPL respectively were observed. Due to the low sensitivity, IDLs could not be determined in the PPI procedure at a reference level of 30 dB SPL. Possible causes for the low sensitivity in the PPI procedure are discussed. These results challenge the idea that both procedures can be used as simple substitutes of one another and the experimenter must be aware of the limitations of the respective procedure.

1. Introduction

During the last few decades, the mouse has become the most frequently studied species in auditory research (Ohlemiller, 2006). This is due to the availability of numerous inbred mutant and knockout strains that allow identification of the cause of a genetically-related hearing deficit. Many of these strains show different rates of age-related hearing loss (ARHL; Willott and Schacht, 2010). ARHL is the most common human neurological disorder (e.g., Huang, 2007) and it is desirable to develop mouse models that show similar hearing disorders to those observed in human subjects. These will make it possible to unravel the physiological basis of the disorders. To make comparisons between human and mouse subjects, it is necessary to investigate different mouse strains using behavioural tests. These tests should rely on similar measures to those in psychoacoustic tests in human subjects, e.g. *d'*.

Both operant conditioning (OC) reward-based procedures and reflex-based prepulse inhibition (PPI) procedures are used in mouse psychoacoustics (for a general review of procedures for studying mouse audition, see Heffner and Heffner, 2001). OC procedures result in both hit and false alarm rates. Combined with an objective analysis based on signal-detection theory (SDT), they have the advantage of providing measures of sensitivity for the animal model that are directly comparable to those obtained in human psychophysics. PPI procedures are considered to be a suitable alternative to operant conditioning procedures. As subject

Abbreviations: IDL, intensity difference limen; OC, operant conditioning; PPI, prepulse inhibition; PPF, prepulse facilitation; ARHL, age-related hearing loss; SDT, signal-detection theory; ROC, receiver operating characteristic; ASR, acoustic startle response; SD, standard deviation; SE, standard error; GLMM, generalised linear mixed model

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training is not needed, PPI procedures are assumed to allow fast data acquisition and to be suitable for testing very young subjects (e.g., Clause et al., 2011). The percentage of inhibition of the startle response by the prepulse has usually been taken as a measure of the perception of the stimuli.

Thus, OC and PPI procedures evaluate the sensitivity on the basis of different measures. One consequence is that different threshold criteria are applied to the results from both procedures. In addition, attentive responses of the subjects are not required for PPI paradigms. In contrast, in operant conditioning experiments the subjects generally attend to the stimuli, indicating their perception by their behavioural response. Using sensitivity for level increments as a common measure of the OC and PPI procedures would allow for a better comparison of the results, as similar threshold criteria can be applied in both procedures (as well as in human studies).

In the present study, we determined the intensity difference limen (IDL) in the C57BL/6N mouse applying PPI and OC procedures in the same individuals. The discrimination of sound intensity is one of the basic tasks of the auditory system and IDL paradigms are used as standard audiological tests (e.g., Buus et al., 1982). We applied an objective analysis based on SDT to the measurement values of both procedures to allow for a direct comparison of the sensitivity. This revealed whether both procedures lead to similar IDLs if the same threshold criteria are applied. Furthermore, the effort needed for collecting the data with each of the procedures was evaluated.

2. Materials and methods

2.1. Subjects

In total, 17 mice of the C57BL/6N strain bred at the University of Oldenburg were used in the experiments. Twelve of these subjects (6 males, 6 females) participated in both the PPI and OC IDL experiments of the present study. The subjects' age during the experiments ranged from 3 to 7 months. Eight of these subjects were tested at two different ages and four of the subjects were tested at one age. This resulted in two age groups with a mean age of 116 (SD ± 15, $n = 8$) and 175 (SD ± 18, $n = 12$) days respectively. The other five subjects (3 males, 2 females) were used to find the optimal time offset for the PPI experiments.

The animals were housed in groups of 2–3 mice per cage (1284L Eurostandard Type II L, 365 × 207 × 140 mm; Tecniplast) and had ad libitum access to water. During the operant experiments the food was restricted to ensure motivation of the subjects to perform the task. The subjects were fed in the evening and the food had been eaten by the morning. The mean minimum weight of the food restricted subjects, i.e., directly before an experimental session, was 18.8 g (females) and 23.4 g (males). The mean weight of the food restricted subjects in the evening was 20.9 g (females) and 26.1 g (males) which is less than 10% below the mean weight of the strain with food ad libitum. A small transfer cage was used to transport the animals from their housing to the experimental cage. The care and treatment of the animals were in accordance with the procedures of animal experimentation approved by the Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Germany and in accordance with EU Directive 2010/63/EU.

2.2. Apparatus/setup

The animals were tested in a custom-built sound-attenuating chamber (inside dimensions: 51 cm W × 69 cm H × 100 cm D) equipped with two layers of sound-absorbing foam (one layer of Pinta PYRAMIDE 100/50 mounted on one layer of Pinta PLANO 50/0; Illbruck). To allow testing with the two different procedures in the same acoustic chamber we used removable platforms that were made of sound-absorbing foam (Pinta PLANO 50/0) that carried the respective experimental cage. These platforms could be interchanged quickly. A loudspeaker (Vifa XT25TG30-04, ASE, Germany) presenting the reference and test stimuli was positioned at a distance of 50 cm directly above the mouse. An LED light (405 lumen, XQ 9909, Ranex, Germany) was used to illuminate the chamber during the experiments (For a scheme of the setup see Fig. 1). The tone stimuli which differed in level were generated by an RX6 Multifunction Processor (Tucker Davis Technologies, USA). In the PPI procedure, the additional startle stimulus was generated by a TDT RP2.1 processor. Tone and startle stimuli were passed through two separate manual attenuators (Hewlett Packard 350D) to adjust the overall signal levels and then amplified with an RMB-1048 (Rotel, Japan) amplifier. Once a week, a ¼″ microphone (40BE, G.R.A.S., Denmark) with a G.R.A.S. pre-amplifier (Model 26CS) and power supply (Model 12A) were used to calibrate the sound-pressure levels at the position where the animal's head would be during the experiment.

2.2.1. OC setup

The platform was equipped with a doughnut-shaped cage (outer diameter 21 cm, inner diameter 7 cm; height 14 cm, made from metal wire mesh) that was mounted on a wire construction lifting the cage above the sound-absorbing foam. The cage contained a pedestal (size 5.5 cm L × 3 cm W × 3 cm H) with a light-barrier and a small feeding dish. A flexible tube connected the feeding dish with a custom-built automatic feeder that was mounted on the side panel of the chamber and dispensed custom-made reward pellets (10 mg, based on Altromin experimental diets, Lage, Germany).

2.2.2. PPI setup

The platform was equipped with an experimental cage (12 × 6 × 6 cm) with four metal legs mounted on a PVC base plate covered by sound-absorbing foam. Animal movements were measured with a piezo-electric pressure transducer (EPZ-20MS64 piezo element, EKULIT Elektrotechnik, Germany) that was integrated into the PVC plate. The output of the piezo-electric pressure transducer was amplified by a custom-built 12 V operational amplifier and recorded with an RP2.1 real-time processor (Tucker Davis Technologies, USA). In the PPI experiments, an additional piezo horn (TE 308 C, Conrad, Germany) mounted next to the stimulus loudspeaker was used to play the startle stimuli.

2.3. Stimuli

2.3.1. OC procedure

A series of 10 kHz reference tone stimuli was used as a repeated background that played continuously from the beginning to the end of the experimental session (Fig. 1, B). Each tone of the series had a duration of 100 ms (including 5 ms cosine ramps, pause length 0 ms). The single test stimulus with a level increment (1, 2, 4, 6, 8, 10 or 15 dB) replaced one of the reference stimuli in the background. With respect to the other parameters it was identical to the reference stimuli.

2.3.2. PPI procedure

The same stimuli as in the OC procedure were used. In addition, a 2–50 kHz broadband noise (duration 35 ms including 5 ms ramps) at a level of 105 dB SPL was used to elicit the startle response. The test stimulus served as the prepulse.