Optimization of a Contextual Conditioning Protocol for Rats Using Combined Measurements of Startle and Freezing

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ABSTRACT

Contextual conditioning in rats is typically quantified using startle amplitude or freezing time. Our goal was to create a robust contextual conditioning protocol combining both anxiety measures. Comparison of ten 0.8 mA – 250 ms shocks with an established shock configuration (0.3 mA – 1 s) favoured the first parameters. Next, we systematically investigated the effect of shock intensity (0.6 mA, 0.8 mA or 1.0 mA) and concurrently compared two conditioning procedures (shocks alone versus explicitly unpaired shock-tone presentations). The 0.8 mA shocks produced significant contextual freezing and startle potentiation, whereas the 0.6 mA and 1.0 mA shocks only led to a significant increase of freezing time. We found no major differences between the two types of conditioning, implying that these procedures might be equivalent. In conclusion, training with ten 0.8 mA – 250 ms shocks produced reliable contextual conditioning as measured with both startle amplitude and freezing time.

Keywords

contextual conditioning, startle amplitude, freezing, shock intensity, conditioning protocol, rat.

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INTRODUCTION

Background

In classical cued fear conditioning, a neutral cue (e.g. a tone) is repeatedly paired with an aversive stimulus (usually footshock). The tone acquires aversive properties and consequently produces fear responses. Conditioned emotional responses are also elicited by reintroducing the rat in the context (e.g. the experimental cage) in which it has previously experienced an aversive event. Contextual conditioning is enhanced when using unpredictable shocks, and can thus be obtained by training the animal with shocks alone or with explicitly unpaired shocks and tones [1,3]. In future experiments, this second type of conditioning may form the optimal contrasting condition for a cued fear conditioning group, trained with explicit cue-shock pairings.

The expression of contextual anxiety in rats is typically quantified by measuring startle amplitude (of the whole-body startle reflex elicited by a loud noise) or freezing time (total immobility of the rat except for respiratory movements) [2].

To date, a whole range of protocols has been used to impose contextual conditioning, but systematic investigations of the different aspects of these protocols are largely lacking in the literature. Moreover, in most studies, anxiety is quantified with one single behavioural measure.

Aim

Our goal was to create a robust contextual conditioning protocol for rats, combining both startle amplitude and freezing time as measures of contextual anxiety. Therefore, we compared different shock parameters and training protocols.

MATERIALS & METHODS

Subjects

The experiments were conducted on 80 male Wistar rats (24 rats in experiment 1, 56 rats in experiment 2) weighing 200–250 g at arrival. They were housed in groups of 3 with food and water ad libitum available. The rats were maintained on a 12-h light–dark cycle (lights on at 6:00 a.m.) with a room temperature of ±21°C. All experiments were carried out in accordance with protocols approved by the animal ethics committee of the Katholieke Universiteit Leuven.

Experimental Setup

To record the startle amplitude, a stabilimeter device was used. The rats were placed into an acrylic cylindrical rat holder (7.6 cm inner diameter, 14.3 cm length) with a grid floor, which was firmly placed on the response platform by four thumb screws. The grid floor consisted of nine 3-mm-diameter stainless steel bars spaced 9 mm apart, through which footshocks could be delivered (ENV-414SA-SR + ENV-262B-GF, Med Associates, St. Albans, VT, USA). The stabilimeter and platform were located inside a ventilated sound-attenuating chamber (65 x 52 x 52 cm) (MED-ASR-PRO1, Med Associates). A red light bulb (3.8 W) in this 'startle box' was continuously on. The behaviour of the animals was recorded by a video camera (DCR-SR55E Super NightShot Plus, Sony, Tokyo, Japan)

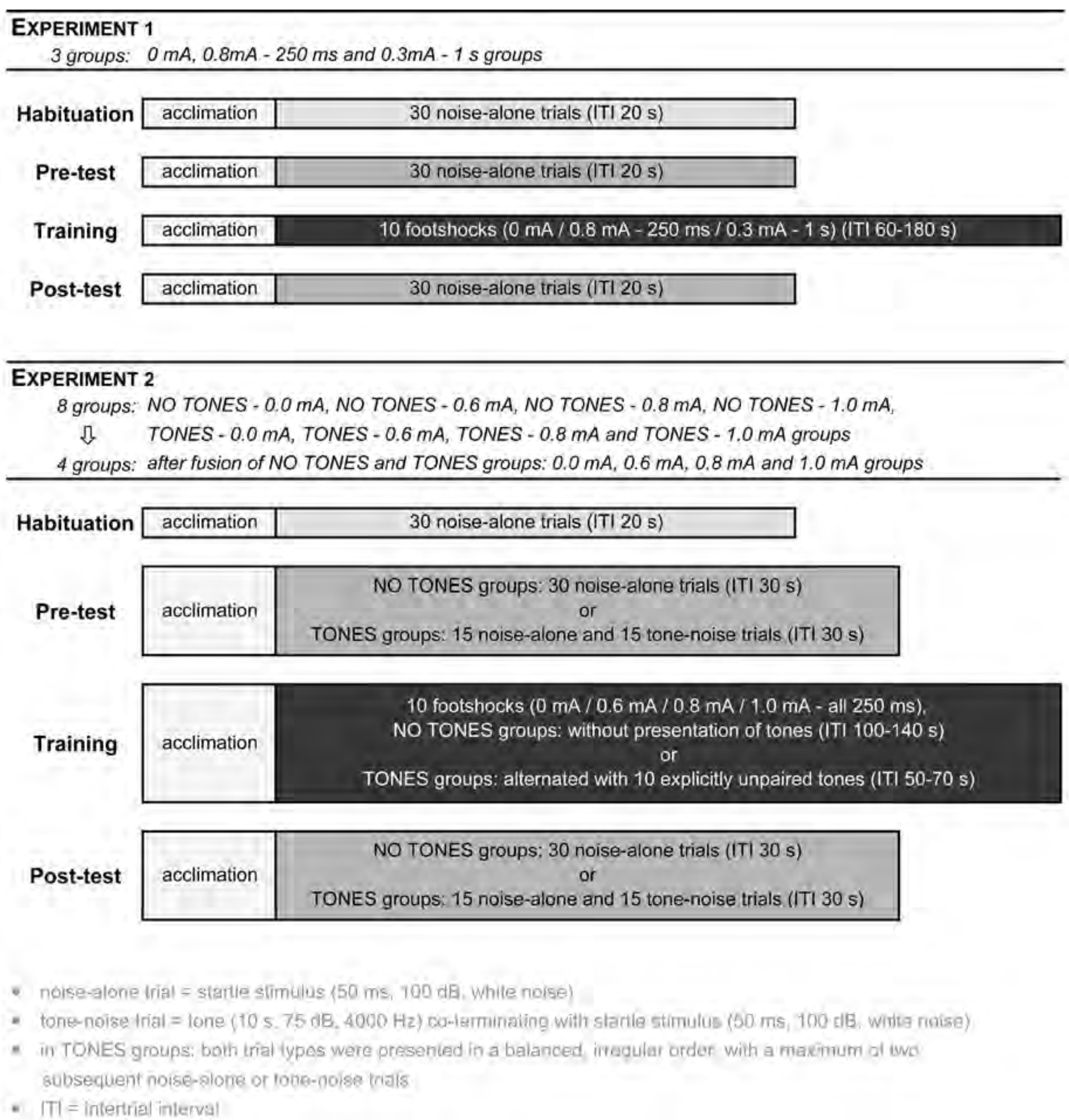

Figure 1. Experimental designs.

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Eds. A.J. Spink, F. Grieco, O.E. Krips, L.W.S. Loijens, L.P.J.J. Noldus, and P.H. Zimmerman

positioned in front of the rat holder. Afterwards, the freezing behaviour during the first 5 min of the test sessions was analysed from videos by a blinded observer.

The startle reaction of the rats generated a pressure on the response platform and analogue signals were amplified, digitized, and processed by software (Startle reflex, version 5.95, Med Associates) provided by the manufacturer of the equipment. The presentation and sequencing of the acoustic stimuli and footshocks were controlled by the same software (which does not allow to administer shocks longer than 250 ms in a protocol with unpaired tones). One of two loudspeakers, both located 7 cm behind the rat holder, was used to deliver a continuous white background noise (55 dB), the other speaker delivered the startle and tone stimuli. The amplitude of the startle response was defined as the first peak accelerometer voltage that occurred during the 200 ms after onset of the startle stimulus. The stabilimeter and loudspeakers were calibrated before each experiment.

Figure 1 shows the designs of experiments 1 and 2. On 4 consecutive days, the rats were placed in the startle box and after 5 min. of acclimation (background noise only), the session started. Freezing during acclimation and startle amplitude on noise-alone trials were measured on pre-test (baseline) and post-test (expression of anxiety). Repeated measures ANOVAs were used to compare the contextual conditioning potential of the different shock parameters and conditioning protocols. Since we found no significant effects of the presence or absence of tones during testing and training, we redid the ANOVAs for experiment 2 and omitted the factor 'presence of tones'. Tukey's post-hoc tests were carried out, with the significance level set at $p < 0.05$.

RESULTS

In experiment 1, we compared the contextual conditioning potential of 0.8 mA – 250 ms shocks with an established shock configuration (0.3 mA – 1 s) [4] and a non-shocked control group. With the 0.8 mA – 250 ms configuration, significant contextual conditioning was achieved, as measured with both startle amplitude and time of freezing. On the contrary, the 0.3 mA – 1 s shocks only produced a significant increase of freezing time, not of startle amplitude.

In experiment 2, we wanted to investigate whether we could still improve the protocol, to obtain even more robust results. We therefore investigated the effect of shock intensity, comparing 0.6 mA, 0.8 mA and 1.0 mA shocks with a non-shocked control. Since the 0.8 mA shocks produced both significant contextual fear-potentiated startle and freezing, this was the optimal configuration compared with the lower or higher shock intensities (0.6 mA and 1.0 mA) which only had a significant effect on time of freezing, but not on startle amplitude. In addition, we compared two different conditioning procedures (shocks alone versus explicitly unpaired shock-tone presentations) and found no major differences between them, implying that these procedures might be equivalent.

CONCLUSION

The aim of this study was to produce reliable contextual conditioning as measured with both startle amplitude and freezing. This goal was achieved using a protocol with 10 unsignalled 0.8 mA - 250 ms shocks.

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