Startle response to short acoustic stimuli in rats

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Abstract. The acoustic startle (ASR) is a transient motor response to an unexpected, intensive stimulus. The response is determined by stimulus parameters such as its intensity, rise time and duration. The dependence of the ASR on the stimulus duration is more complex than could be assumed from physical properties of acoustic pulse. This effect attracted the attention of few researchers. Some authors reported noticeable changes in the ASR amplitude only for very short (less than 4-6 ms) acoustic pulses. The systematic studies on the effect, however, have not been performed so far. The purpose of this study was to determine to what extent the ASR parameters are affected by the durations of the short stimulus. The amplitude of the acoustic startle reflex was assessed for a fixed tonal frequency (6.9 kHz), and for a variety of stimulus durations ranging between 2 and 10 ms. ASRs were studied in 11 adult, hooded rats exposed to a sequence of tone pulses (110 dB SPL) of different durations, presented in random order, with or without 70 dB white noise as a background. Statistical analysis revealed significant differences between ASR amplitudes for different durations. The startle amplitude increased with acoustic pulse duration and distinguishable differences were seen for stimulus duration between 2 and 8 ms. Further increase of pulse duration had no effect on ASR amplitude. The same pattern of changes was observed when the acoustic stimulus was presented with the white noise. In the tested range of stimulus duration no significant differences in the ASR latency were found. The observed differences may be attributed to changes of stimulus acoustic energy and to physiological characteristic of auditory system in the rat.

Key words: acoustic startle, stimulus characteristics, behavior, habituation, rat
INTRODUCTION

Startle response is a motor reaction to a certain class of stimuli of different modalities. Behaviorally, the startle response consists of rapid contraction of head, neck, trunk and legs muscles (Szabo 1964) in addition to the arrest of ongoing activity (Graham 1979). Auditory, visual and several types of tactile stimuli were successfully used for eliciting startle (Hoffman and Ison 1980, Ison and Russo 1990, Seaman et al. 1994, Stitt et al. 1976, Woodworth and Johnson 1988). In laboratory practice most widely used are intense auditory signals eliciting so called acoustic startle response (ASR).

Sensitivity of the ASR to a variety of experimental treatments made it an important research tool in studies of brain mechanisms of learning, memory, emotions and movement control (for review see Davis 1990, Koch 1999). Although many studies were devoted to different aspect of ASR, the properties of a reliable acoustic stimulus to elicit ASR received relatively little attention. Fleshler (1965) was the first to show that ASR can be elicited by pulses as short as 6 ms, and further elongation of the stimulus has no effect on the magnitude of the response. This work was subsequently extended by Marsh and coworkers (1973). In a systematic study they found that in the range between 80 and 125 dB, for each stimulus intensity the response magnitude increased with increasing stimulus duration, and acoustic pulse lasting 4 ms were already adequate to elicit a near maximum response. They have also computed the time constant of the neural system subserving ASR and found it to be around 3 ms, which is shorter than the time constant of the middle ear reflex (<10 ms). In numerous studies it was demonstrated that a crucial factor for the elicitation of ASR is a short rise time of the stimulus. Manipulation with stimulus rise time was found to cause pronounced changes in ASR amplitude. (Blumental 1988, Fleshler 1965, Ison 1978). Chabot and Taylor (1992) showed that in 65% of rats, startle occurred in response to 80 dB tone pulse with a short rise time. For a greater rise time even a very high sound stimulus did not elicit startle (Blumental and Berg 1986, Davis 1984, Piltz et al. 1987). These studies confirm that not the duration of the stimulus but its sudden onset is essential to elicit ASR.

In the present study I attempted to enrich the temporal ASR characteristics with a habituation/sensitization profile. One could expect that in the case of habituation the ASR to a very short stimulus (e.g. 2 ms) might disappear, in opposite to sensitization where the shortest (2 ms) stimuli might become equally effective as the longest (8-10 ms) ones.

METHODS

The research was approved by the Ethic Committee of the Nencki Institute and was conducted according to the rules of humane use of laboratory animals in experimental work.

Eleven adult male hooded rats (16 weeks old) from 3 different litters, weighing 220-240 g were used. The animals were maintained 5-6 to cage and had unlimited access to food and water. First, rats were habituated to the experimental conditions for six days by exposing them to randomly applied acoustic pulses. The habituation was performed in a testing chamber, where they were later exposed to ASR sessions. The rats’ responses were recorded during this procedure. Subsequently, rats were tested twice a day for another 6 days.

ASR testing was performed in a ventilated, double-walled sound-attenuating chamber (Coulbourn Instruments, U.S.A). The rats were tested in small plastic cages (180 x 85 x 90 mm). The cages were placed on platforms that recorded the vertical reaction force of the animal’s startle response. The signal from the platform was amplified, rectified and filtered with 40 Hz cut-off low pass filter. It was sampled then at a frequency of 400 Hz. Amplitude was computed on-line for each trial.

Four animals were tested simultaneously in the acoustic chamber. An adaptation period of five minutes was allowed before testing. In contrast to habituation procedure, during the main test, a sequence of acoustic pulses, separated by a 30 s fixed inter-trial interval, was presented to the rats. The acoustic stimuli were tone pulses (6900 Hz/110 dB, SPL) and the duration of 2, 4, 6, 8, 10 ms. The stimuli of different duration were presented to the rats in a random order. Each stimulus from the sequence was presented to the rats five times during session. Thus, the animal received a total of 25 acoustic stimuli. Then the test was repeated with the same stimuli sequence presented against a 70 dB white noise background. The order of tests (with- or without background noise) was altered every day.

Changes in the parameters of startle (the amplitude and the latency) were analyzed using a two-way repeated measure ANOVA (Systat v. 5.0). Analyses were conducted using stimulus duration and day of experiment as within-subject factors.
RESULTS

The ASR was affected by stimulus duration both when the tones were presented against the acoustic background and when the rats were tested without noise.

The ASR in naive rats was characterized by unstable responses whose amplitude changed dramatically from trial to trial. Changes of the ASR amplitude in the course of 6-day habituation are shown in Fig. 1. As seen, the ASR amplitude declined progressively, and reached the plateau on the sixth experimental day. During this period, the amplitude of the startle was dependent on the day of habituation \((F_{5,50}=18.2, P<0.0001)\) and stimulus duration \((F_{4,40}=58.72, P<0.001)\). Day x pulse duration interaction was not significant.

Changes of ASR amplitude for each pulse duration on consecutive days of experiment with and without background noise are shown in Figs. 2 and 3, respectively. Mean ASR amplitudes for particular stimulus duration during consecutive day of testing are shown in Fig. 4. Analysis of variance revealed that during habituation, the amplitude of the startle was dependent on the day of habituation \((F_{5,50}=18.2, P<0.0001)\) and stimulus duration \((F_{4,40}=58.72, P<0.001)\). Day x pulse duration interaction was not significant.

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Ductus distal ends of the ducts follow a contralateral course, anastomosing with a second duct and making a transitional gap with the anterior opening of the duct. The ducts are continuous with ducts of the vasa deferentia.
Therefore, to eliminate the effect of novelty we compared ASR magnitudes in the course of long habituation (Plappert et al. 1993). The six-day habituation has markedly reduced ASR amplitude, and importantly, the dependency of ASR amplitude on the acoustic stimulus duration had the same pattern as in naive rats.

The relatively scarce effect of background noise on ASR magnitude in the present study is a puzzle. It is known that even weak stimuli can influence startle response (Hoffman and Searle 1965). Therefore I expected that the amplitude of the ASR should be augmented by the noise. Such assumption has strong support from the literature and from our results (Blaszczyk and Tajchert 1997). Hoffman and Searle (1965) reported a simple linear increase in the ASR amplitude with background noise in rats. However, later findings described the relation between ASR amplitude and background noise as a biphasic, inverted U-shaped function (Davis 1974 a, b, Gerrard and Ison 1990, Ison and Hammond 1971). It was shown that the inverted U-shaped function is the result of two separate and independent processes, arousal and sensory masking (Gerrard and Ison 1990, Hoffman and Searle 1965, Ison and Russo 1990). The increasing part of the function results from a facilitating effect, while the decreasing part is caused by signal masking. The sensory masking hypothesis assumes that the perception of any biological signal, and acoustic signals in particular, when presented with a noise background, requires more time because the relative strength of the signal is reduced (Blaszczyk and Tajchert 1996, Davis 1974). In our experiment, when the rats were tested against white noise background, the expected augmentation of startle amplitude did not attain enough statistical significance. We can assume that for unexplained reasons the ASR magnitudes fell within the descending arm of the inverted U.

CONCLUSIONS

The startle to a short acoustic pulse is very sensitive to the energy of the stimulus within the range below 8 ms. For the stimulus duration in this range the ASR magnitude is proportional to the duration of acoustic signal indicating that the threshold response is to total energy (intensity x duration), and not just to stimulus intensity. Such stimuli are too short to activate protective internal ear reflexes and are transmitted by the short time-constant auditory subsystem. Application of pulses longer than 10 ms is not justified by physiological properties of the neural system subserving the startle response.

REFERENCES


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