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Effect of acoustic stimulus characteristics on the startle response in hooded rats

Janusz W. Błaszczyk and **Katarzyna Tajchert**

Department of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland, Email: janusbla@nencki.gov.pl

Abstract. The acoustic startle response (ASR) depends on stimulus parameters such as duration, intensity and particularly on the stimulus rise time. The aim of our study was to determine to what extent the ASR parameters are affected by the spectral characteristics of the stimulus. Therefore, in this experiment the amplitude and the latency of the acoustic startle reflex were assessed for a fixed pulse duration and for a variety of stimulus frequencies ranging between 3 and 23 kHz. The ASRs were studied in 11 adult hooded rats exposed to 2-ms (120 dB SPL) tone pulses of different frequencies presented in random order, with or without 70 dB white noise background. Statistical analysis of the data revealed significant differences between ASR amplitudes for different frequencies. In our experimental situation the rats responded more readily to a low frequency stimulus. The startle amplitude decreased with tonal frequencies and distinguishable difference were seen for 3, 7, and 10 kHz pulses. However, such differences were not readily observed for higher frequencies i.e. 15, 20, 23 kHz. The same pattern of differences was observed when the acoustic stimulus was presented with the white noise background. The observed differences may be attributed, firstly, to a spectral characteristic of the stimulus and thus to an audibility in rats and secondly to a behavioral meaning of a stimulus of a different frequency.

Key words: acoustic startle, stimulus characteristics, noise, rat

INTRODUCTION

The acoustic startle response (ASR) in rats is a reaction with an evident motor component that can be elicited by a certain class of stimuli which are commonly classified as intensive signals or in a behavioral sense as very strong stimuli. However, these definitions are very crude and generally refer to experimental situations. Everyday experiences show that even weak but unexpected stimuli can initiate the startle reaction. Thus the question of the specifity of the signal in acoustic startle should not concern its physical parameters only but somehow address the behavioral and emotional context in which the stimulus appears. So far, laboratory studies have shown the main determinants to be the amplitude and rise time of the acoustic stimulus (Fleshler 1965, Marsh et al. 1973). The majority of recent studies have documented that the probability of occurrence of the ASR and its amplitude depend on acoustic stimulus duration in a very limited range and are also strongly dependent on the stimulus rise time. Lengthening the acoustic stimulus above 6 ms does not affect the amplitude of the startle reaction (Fleshler 1965, Marsh et al. 1973). On the other hand, manipulation of the stimulus rise time results in a very pronounced change of the ASR amplitude (Fleshler 1965, Chabot and Taylor 1992). Also, the probability of the startle reaction decreases with a prolongation of stimulus rise time. Chabot and Taylor (1992) showed that in 65% of rats, startle occurred in response to an 80 dB tone pulse with a rise time shorter than 12 ms. For a greater rise time even a 140 dB sound stimulus did not provoke a startle. It should be mentioned, however, that only about 50% of laboratory animals are good startlers (Plappert et al. 1993, Blaszczyk and Tajchert 1996). Some rats may have a tendency to startle while others may freeze in response to the same unexpected stimulus.

As mentioned above, the startle response is strongly dependent on the emotional state of the animal. Every new signal to which the animal is exposed, provokes a complex emotional reaction that has been named the orienting response (Pavlov 1927, Sokolov 1963, Graham 1979). Emotional components of the reaction disappear when the stimulus is repeated but in case of very strong and unexpected stimuli, the motor component of the orienting response could remain in the form of a startle (Thompson et al. 1979). It has been well documented so far that by the manipulation of an animal's emotional state, one can change the threshold of the startle reaction (Brown et al. 1951, Davis and Sollberger 1971, Davis and Astrachan 1978, Davis 1986). The paradigm of a so called 'fear potentiated startle' is widely used in pharmacological studies. In this case, the startle measurements follow a training procedure during which an arbitrary conditioned stimulus is paired with a nociceptive stimulus (e.g. electrical shock). Next, the same conditioned stimuli are used as warning signals in ASR studies. Thus one can evaluate the influence of the emotional state (in this case anxiety) on startle response.

There is no doubt that animals should exhibit greater sensitivity to some groups of signals, which are specific to their species (Hoffman and Ison 1980). In terms of information theory, these signals are described by a specific set of parameters (modality, duration, frequency, modulation, etc). For example, birds respond more readily to a visual than to an acoustic stimulus in startle tests (Stitt et al. 1976). The signal specificity is determined by some behavioral factors. These are the different warning signals as well as signals used for communication between animals. It might be expected that some types of acoustic signals that are used by rodents as warning signals (Sales and Pye 1974) may be more effective in producing the ASR. To answer this question the experiment was designed to study stimuli with different spectral characteristics.

In many experimental procedures which employ acoustic signals, changes in the acoustic background (e.g. by increasing a background noise) are used to preset an animal arousal. It has been proven so far that medium level noise (70 dB SPL) improves animal's performance during different learning procedures (Zieliński 1966, 1971, Jakubowska and Zieliński 1975). Therefore, it should be expected that the threshold of the acoustic startle may also be modified by acoustic background. 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 1974a,b, Ison and Hammond 1971, Gerrard and Ison 1990). One possible explanation for this phenomenon is that the inverted U-shaped function is the result of two separate and independent processes, arousal and sensory masking (Gerrard and Ison 1990). The initial and increasing part of the function results from a facilitating effect, while the depressing effect is caused by signal masking.

In this context it would be interesting to find how the different frequencies of an acoustic stimulus of a constant pulse duration and a fixed rise time affect the ASR, depending upon the presence of an acoustic background. Thus, the research reported here was designed to determine how changes in the characteristics of the acoustic stimulus (including the presence of a 70 dB background noise) affect the startle response in hooded rats.

METHODS

Subjects

Eleven adult male hooded rats (3-4 months old) whose body weight ranged between 220-240 g were used for the experiment. The animals were kept in home cages (5 and 6 rats per cage) and maintained on ad libitum food and water. Before testing, the rats were habituated to the experimental conditions, especially to the test cage restraints and their placement into the acoustic chamber. After five days of this habituation, the rats were tested twice a day for ten successive week days. First session was between 9:00 and 11:00 a.m. while the second one was from noon up to 2 p.m.

Apparatus

Testing was performed in a ventilated, double-walled sound-attenuating chamber (Coulbourn Instruments). The rats were tested in small cages (180 x 85 x 90 mm) constructed of plastic and aluminum rods. The cages were placed on force platforms that recorded the vertical reaction force of the animal's startle response. The signal from the platform was amplified, rectified and filtered (40 Hz low pass filter) and then sampled at a frequency of 4 kHz. Four hundred millisecond sequences of data, triggered by an acoustic stimulus, were stored on a computer for off-line analysis. The startle parameters: ASR latency-to-peak and peak amplitude were determined by the computer for each trial.

Procedure

Four animals were placed simultaneously into the acoustic chamber. The testing cages were put onto the force platforms and the chamber door was shut. An adaptation period of five minutes was allowed before testing. A sequence of acoustic pulses, separated by a fixed 30 s interstimuli interval, was presented to them. The acoustic stimuli were 6 ms in duration with 2 ms plateau and with 2 ms rise/fall times at an intensity of 120 dB SPL. The frequencies of the pulses were 3, 7, 10, 15, 20, 23 kHz and were given to the rats in random order. Each frequency was presented ten times during each experimental session. Thus, the animals received a total of 60 stimuli each session. The testing was then repeated with the same stimuli sequence presented on a 70 dB SPL continuous white noise background.

Data analysis

Each parameter of the startle response (amplitude, latency to peak, and number of responses) was analyzed using a three-way repeated measure ANOVA (Systat v. 5.0 software). Analyses were conducted using background condition, stimulus frequency, day of experiment, and all interactions as within-subject factors. When significant interactions were found, step-down tests were performed to analyze the main effects.

RESULTS

Of particular interest were the effects on ASR amplitude and latency, of the different testing pulse frequencies and the acoustic background - 70 dB white noise. A large effect was found with responses to low frequency stimuli. Analysis of the ASR amplitude for each testing frequency showed significant differences ($F(5,50) = 48.375$, $P<0.001$). The low-frequency stimulation clearly differed in their influences on startle behavior. Significant differences in the ASR amplitude were pronounced for the stimulus frequencies of 3, 7, 10 kHz. The rats responded more readily at lower frequencies. For the frequencies of 15, 20 and 23 kHz, however, we did not find significant changes in the ASR amplitude and latency. The outcome of these analyses are depicted in Fig. 1. The ASR amplitude for different stimulus frequencies was changing over the time of observation. A significant day of experiment x frequency interaction was found ($F(45,450) = 4.214$, $P<0.001$).

Startle amplitudes were significantly increased with the white-noise background at 70 dB. Relationships between stimulus frequency and the ASR amplitude are shown in Fig. 2. In the background noise condition, the analysis of variance also provided a significant difference with regard to the effect of stimulus frequency ($F(5,50) = 131.875$, $P<0.001$). There were also significant interactions between background, day of experiment and stimulus frequency ($F(45,450) = 3.195$, $P<0.001$). The critical outcome of the experiment was

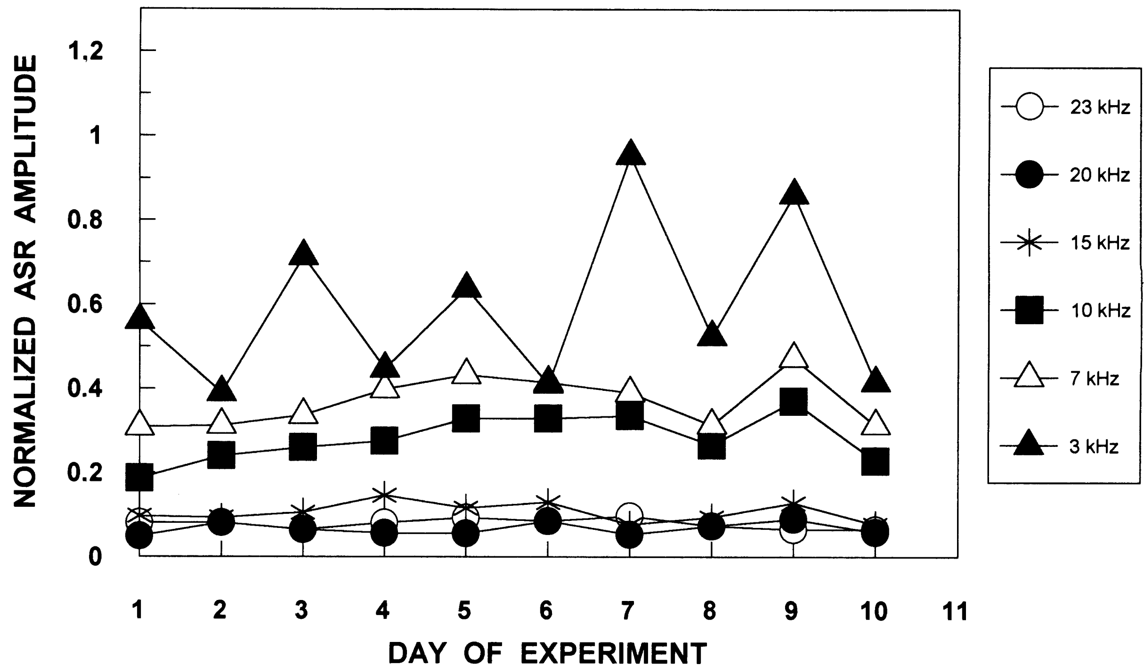

Fig. 1. Changes of the mean startle amplitudes normalized to body weight in response to 120 dB (SPL), 2 ms and of different tonal frequencies acoustic pulses during successive days of experiment.

given by this three-way interaction: the startle responsivity depends on the frequency characteristic of the stimulus and on the day of experiment.

No significant differences in the ASR latencies in both experimental conditions were found. Thus, the empirical results of this experiment may be readily summarized.

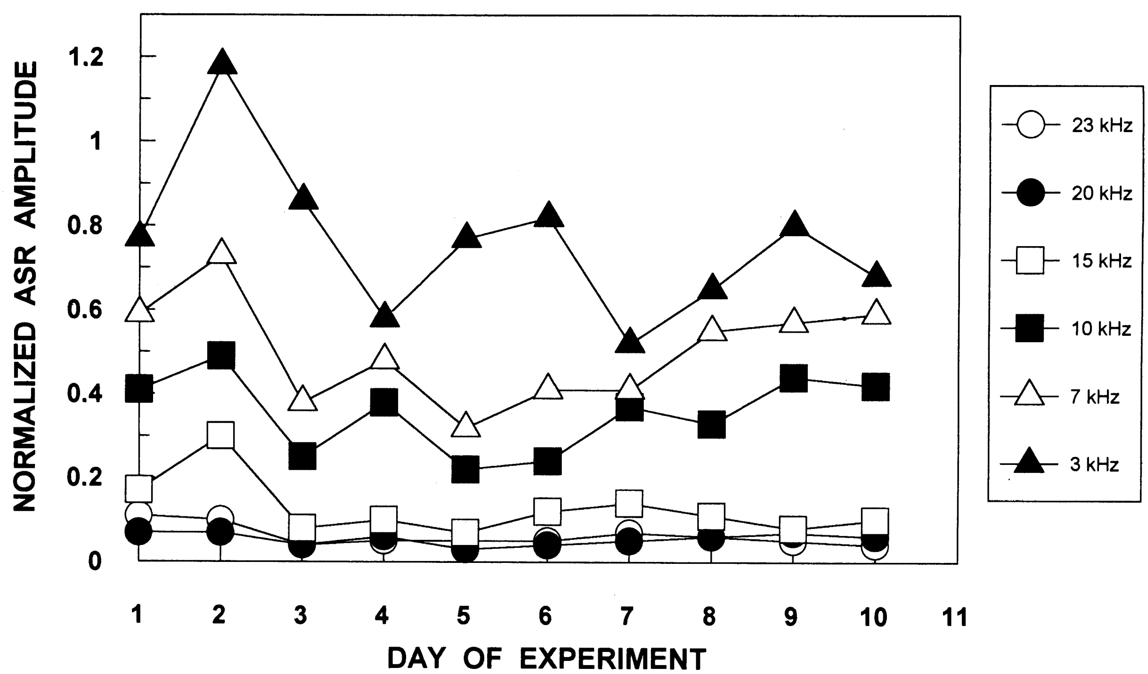

Fig. 2. Changes of the mean normalized startle amplitudes during successive days of experiment in response to the same stimuli as in Fig.1 but presented on 70 dB white noise background.

Firstly, lower stimulus frequencies enhance the acoustic startle reflex. Secondly, intense background noise enhances and modifies the acoustic startle response.

DISCUSSION

The results clearly support a difference between the intensity and probability of the ASRs elicited by a constant-intensity, and a variable-frequency acoustic stimuli. Despite the fact that the only variable manipulated during the experiment was a tonal frequency of stimulation, differences may be accounted for: (1) differences in the stimulus quality, (2) differences in the behavioral meaning of different sound frequencies and (3) differences in the perception of different stimuli.

A basic explanation of the frequency effect on rats' ASRs, observed in the experiment, arise from stimulus quality (i.e., relationships between stimulus duration and its tonal frequency) and thus in particular from its spectral characteristics. These characteristics depend upon the ratio of pulse-to-sinewave periods. As the duration of the acoustic stimulus was constant - 2 ms in our case - and the signal frequencies varied from 3 to 23 kHz, three qualities of the stimulus can be distinguished (Licklider 1963). For the lowest frequency the subject hears only a click. For the frequency of 7 and 10 kHz the sound is a click with a discernible tonal quality; and finally, for the tonal frequency, with a period about ten times shorter than the duration of the pulse, there is an on-click and an off-click with a tonal part of easily identifiable pitch in between. For the frequency of 3 kHz, when the animal hears the click the acoustic energy is spread diffusely along the frequency scale. In the second extreme, for the frequencies of 15 to 23 kHz the sound energy is concentrated within sinewave frequency and spread for onset and offset of the stimulus. Each of these three qualitively different stimuli is characterized by a different frequency spectrum. Thus the perception by the animals must be different.

The next potential factor responsible for the changes in the acoustic startle response is the difference in behavioral meaning of sounds of different frequencies. It is well documented that the rats communicate using ultrasound vocalization. It is also known that to survive in their natural environment, they must hear signals in a broader hearable frequency range. Thus it is not very surprising that the auditory system of rats is sensitive over an extraordinary range of frequencies from 0.1 to 100 kHz (Webster 1985).

The threshold behavioral data indicate that the rat is more sensitive to frequencies around 40 kHz (Gourevitch and Hack 1966). Kelly and Masterson (1977) found the second minimum threshold to be around 16 kHz. Next, in the range between 20-30 kHz the threshold decreases slowly and then a sharp minimum is observed in the vicinity of 40 kHz. All these studies indicate that rats are extremely sensitive to high frequency sounds. The hearing ability of these animals appears to be related to the frequency of the ultrasonic signals that they emit. These signals are of great importance in the life of rodents. It is suggested that ultrasonic calls in rats in the frequency range between 20 and 30 kHz might be used for communication between individuals or for echo-location (Sales and Pye 1974). It is also known that the young of a wide variety of myomorph rodents emit ultrasonic calls when they are removed from the nest (Sales and Pye 1974). These calls are mainly 4-65 ms in duration and at frequencies ranging between 40-75 kHz. The less intense calls produced by pups may be motivated by cold and hunger and initiate the retrieving response of the mother. The length of the calls, and the sound pressure level decrease as the pups grow older (Sales and Pye 1974). The authors suggested that this effects might decrease their effectiveness in eliciting maternal behavior. Handling may elicit a different type of call. A detailed investigation of both cold stress and tactile stimuli as factors motivating ultrasound emission was made by Okon in 1972 (Sales and Pye 1974).

Two types of ultrasound pulse were recorded in adult rats (Sales and Pye 1974). "Long pulses" up to 700 ms in duration and frequency of about 25 kHz were observed when animals were being removed from a cage. Short pulses of 3-60 ms duration at 45-70 kHz were produced by rats rolled onto their backs and restrained. The calls produced by these adults may be associated with aggressive or submissive responses. In this perspective, the testing signals in the present experiment must have different behavioral and emotional meanings.

The acoustic pulses presented to the rats during the experiment belong to the short pulse category with wideband spectral frequencies. These types of sounds and frequency ranges might affect the anxiety level in the animals, which could elevate the acoustic startle responses.

There is also a hypothesis (Graham 1979, Blumental and Burg 1986) that the auditory system is composed of two subsystems: one may be more sensitive to the transient features of a stimulus and the other responds better to the sustained features of a stimulus. It has been argued that the acoustic startle response is primarily an indicator of the former. As discussed above, the stimuli used in our study differed significantly in transient and tonal characteristics. For this reason, higher frequency signals having well-heard pitches were less effective in eliciting a startle.

However, an unsolved question emerged from our data. In both experimental conditions, there were reproducible periodic changes in responsiveness to the 3 kHz stimulus. The rats responded less to this frequency during every second session of the day. This effect was only observed for this particular frequency. If a possibility of a hardware error is removed, there are still several potential explanations left to explain this phenomenon. First of all, the changes may be attributed to a diurnal rhythm of the acoustic startle (Davis and Sollenberg 1971). According to these authors a magnitude of the startle response is generally higher during the night than during the day and reaches its minimum about noon. The second experimental session was perform at about this time. However the decrease of the responsiveness was only observed for one frequency (3 kHz). Since there were no such changes for the other stimulus frequencies, it may be argued that the decrease is frequency specific and can be attributed to the perception of that particular acoustic stimulus as discussed above.

The amplitude of the acoustic startle reflex in the rat is sensitive to the intensity of a surrounding background noise (Hoffman and Searle 1965, Gerrard and Ison 1990). The detailed shape of the function relating noise level to the ASR amplitude is very complex. It shows that ASR was enhanced by low levels of background noise but reduced by higher noise intensities (Davis 1974a,b, Davis and File 1984). Hoffman and Searle (1965) have suggested a sensory masking hypothesis. This hypothesis assumes that the perception of any biological signal, with the acoustic signal in particular, when presented with a noise background, requires more time because the relative strength of the signal is reduced. Thus both factors reduce behavioral effectiveness of the acoustic signal. Therefore the response of the animals is weaker. The masking hypothesis ignores the effect of arousal that is caused by the acoustic background. It has been well documented that a medium white noise level (80dB) significantly improves performance of the rats in learning tasks such as active avoidance (Zieliński 1966, 1971, Jakubowska and Zieliński 1975). There is also an important effect of white noise background - it stabilizes the acoustic background by masking most of unwanted, weak acoustic signals, which could be present during the experiment. Based upon the present results, it can be argued that this level of noise activates attention (by increasing the animal's arousal) and thus increases both the probability and the amplitude of the acoustic startle. However, further studies are needed to solve the problem of dichotomy between masking and arousal effects of background noise.

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