

# Sex and strain differences of acoustic startle reaction development in adolescent albino Wistar and hooded rats

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**Abstract.** Acoustic startle responses (ASR) were studied in 12 young Wistar albino and in 15 hooded rats of both genders. The six week old animals were first exposed to a 6.9 kHz tone pair of 2 ms pulses of 120 db intensity with the inter-stimulus interval (ISI) between 2 and 11 ms. ASR amplitudes and latencies as a function of the ISI, animal strain and gender were recorded and analyzed for ten consecutive weeks. No differences in the ASR amplitude between Wistar and hooded rats were found. ASR amplitude increased during the experimental period and followed body weight increase. Significant differences were also observed between male and female rats in their startle responses to acoustic stimuli. Generally, male subjects responded with a greater ASR amplitude than females, and the changes may be attributed to the difference in neuromuscular development between genders. This experiment sets a background for further developmental studies.

**Key words:** acoustic startle, development, rat

## INTRODUCTION

The mammalian startle reflex can be activated by a sudden intense stimulus, e.g., sharp touch, sound pulse, or light flash. This response consists of a strong muscle contraction of the whole body (Szabo 1965). This short-latency response begins in the head and spreads down the body producing transient flexor contractions that can be recorded as changes in the limb-ground reaction forces. The primary neural pathway controlling this neuromuscular response has been well identified (Davis et al. 1982, Hitchcock and Davis 1986).

The startle reflex is a part of the complex reaction of animals to the changes in its environment. Exposure to a new stimulus of an arbitrary modality that appeared suddenly in the environment results in the cessation of an animal's current activity and in its characteristic reaction - commonly called the orienting response. Many somatic and vegetative symptoms accompany this reaction. Increased blood pressure, muscle contractions, looking in the direction of the stimulus, are the most pronounced symptoms of this response (Pavlov 1927, Sokolov 1963).

The transient muscle contraction evoked by strong sensory stimulation seems to be preparation for postural and/or locomotor behavior. Depending on the behavioral context of the stimulus, different animals respond with either "freezing" or escape responses. The startle reflex initiates these responses that are crucial for the animal's survival. The startle reflex can be modified by the emotional state of the animal or by a weaker stimulus that does not elicit the startle reaction by itself (Peak 1931, Pinckney 1976). This discovery resulted in studies of fear-potentiated startle, a technique that benefits both neurophysiology and pharmacology (Brown et al. 1951, Casella and Davis 1985, Casella et al. 1986, Hitchcock and Davis 1986).

The muscle contractions that are observed in startle reaction are readily measurable as changes in the limb ground reaction forces, the method of ground reaction monitoring is commonly used to study the startle reflex. Another easily accessible

measure of the startle response is an eye blink which can be used to study the reactions to weaker stimuli (Peak 1931).

Techniques involving reflex modification that utilized pairs of pure tone pulses or white noise pulses have been successfully used in a variety of experiments to assess changes in auditory functioning (Leitner et al. 1993) as well as to evaluate the emotional state of the animal (Marsh et al. 1973). Temporal parameters of such stimulation determine the characteristics of the startle response (Ison et al. 1973, Marsh et al. 1973) which allows for the evaluation of the neuromuscular system involved in the reaction. Based upon this research direction we tried to establish whether the temporal characteristics of the acoustic prepulses, and the ISI in particular, in adolescent rats are similar to those in adults. We hypothesized that in an early stage of development, due to the immature motor and emotional systems, the inter-stimulus interval corresponding to the maximal startle response may be relatively longer as compared with the 3 ms interval reported for adult rats (Marsh et al. 1973).

While the acoustic startle response is being studied in many laboratories, none of the studies have examined changes of the reflex in adolescent rats. Also few studies have compared different strains for differences in their startle responses. Very recently, however, such comparative studies were published (Glova and Hansen 1994). The purpose of the research presented here was to compare changes in the acoustic startle response in development of young male and female rats of the two most commonly studied rat strains i.e., Wistar albino and hooded rats. We proposed that the morphological changes of the body in the two genders and strains that appeared during the development might also influence the ASR amplitude and latency. This paper presents the results that constitute the base for our current studies of animals emotional development.

## METHODS

Changes in the acoustic startle reflex were studied in the two strains of rats: 12 Wistar albino, from

two litters (5 males and 7 females) and 15 hooded, from two litters (6 males and 9 females). The litter sizes at birth were 6-8 pups. Four week old rats were separated from their mothers. The rats were housed in groups of seven or fewer siblings and maintained under normal light/dark daily condition with *ad libitum* access to food and water. The rats were handled and adapted to the test situation by being placed in the cages and into an acoustic chamber. Five week old pups weighing between 33-65 grams at the beginning of the experiment were used in these studies.

The startle responses were recorded once a week for ten successive weeks. The rats were tested always at the same time i.e., between 10:00 and 1:00 to avoid the effect of the daily rhythm on the startle responses (Chabot and Taylor 1993). The study was conducted in a ventilated double wall acoustically insulated test chamber (Coulbourn Instruments). Four animals were put into the cages and then placed on the force platforms mounted in the chamber. A strain gages attached to the force platform transduced the startle response. Changes in the ground reaction forces recorded by the transducers were digitized with a sampling frequency of 4095 Hz; they were subsequently rectified and filtered (40 Hz low pass filter).

The startle reflex was produced by a pair of strong acoustic pulses (frequency 6.9 kHz; 2 ms pulse duration and 2 ms rise time; 120 db pulse intensity) presented against the background of 70 db white noise. The inter-stimulus intervals within each pair were between 2 and 11 ms. The length of the ISI was randomly assigned for the particular pair of acoustic stimuli, and the same order of ISIs (8, 10, 9, 11, 2, 6, 5, 7, 3, 4 ms) was used in consecutive testings.

Mean responses (ASR amplitudes and latencies) were determined for each animal for each week of the experiment. These means were then averaged for each strain and for each gender. A mixed design multivariate repeated measures analysis of variance (ANOVA type VI, Lindquist 1953) were then performed. Some subsequent statistical analysis of the ASR amplitude were done on normalized data. The

amplitude were normalized to the body weight (see Results for details).

## RESULTS

The data were analyzed to determine: (1) the effect of age on the ASR amplitude and latency; (2) the differences between the two strains of rats in the ASR response; (3) the differences between the genders in the ASR parameters; and (4) the effect of different interstimuli intervals on ASR.

All of the rats readily responded to the acoustic stimuli, and performance between the two rat strains in the ASR was not statistically different. However, some significant differences in the ASR pattern development in both Wistar and hooded rats were indicated by a significant strain x age x ISI interaction ( $F(88,2200)=1.29$ ;  $P<0.05$ ). Because of this lack of difference between the two strains, further analysis of the ASR amplitude and the latency data was done on the data collapsed across strain groups.

Both Wistar and hooded rats exhibit the same characteristic pattern of the acoustic startle responses changes during adolescent development period. First of all it was seen in the ASR amplitude evoked by the first pair of the acoustic stimuli (8 ms inter-stimulus interval) in each experimental session. This amplitude was significantly lower than the response to the following sound pulse pair (e.g., Fig. 1).

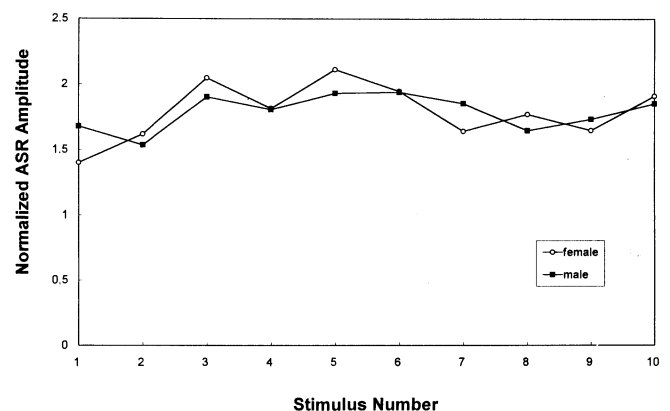


Fig. 1. The mean ASR amplitude in the order of the stimuli presentation during the first experimental session.

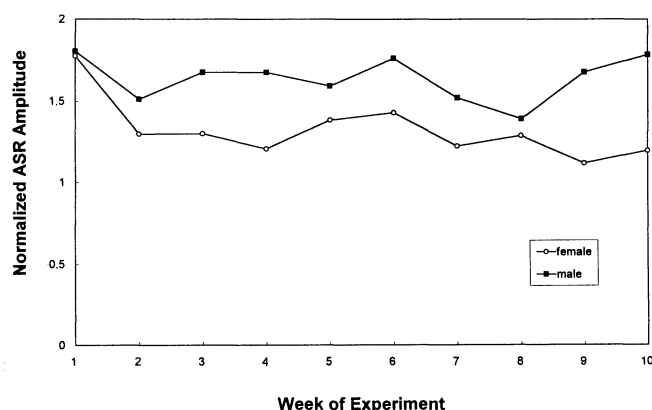


Fig. 2. Changes of the mean ASR amplitude during successive weeks of the experiment.

We attribute this finding to the catalepsy that might appear in restrained rats in response to a strong sensory stimulus (Plappert et al. 1993). In fact, 27% of the rats did not respond at all during the first experimental session. Despite of this, the mean normalized ASR amplitude for the entire first experimental session was significantly higher as compared with the following sessions (Fig. 2). This effect was markedly pronounced in the females.

The ASR amplitude increase during the experiment followed the body weight increase. We found a strong correlation between the body weight and the ASR amplitude during successive weeks of study. This correlation was at  $r=0.95$  level in the female rats and at 0.98 for the male rats. These high correlation factors allow us to normalize the ASR amplitude to the body weight.

The effect of the first exposure to the strong acoustic stimuli was also observed in the ASR latency. Statistical analysis of the data (mixed design ANOVA, type VI) compared the distribution of the latencies and the normalized ASR amplitudes in Wistar albino and hooded rats for the different ISIs. This analysis revealed a significant effect of the ISIs for the latency of the ASR ( $F(11,275)=13.75$ ;  $P<0.001$ ). Also as shown in Fig. 3 and evidenced by the results of post hoc Duncan tests ( $P<0.05$ ), the ASR latency decreased as a function of age.

During the 10 weeks of the experiment, the pups' body weight increased steadily. These changes were different for the male and the female pups as

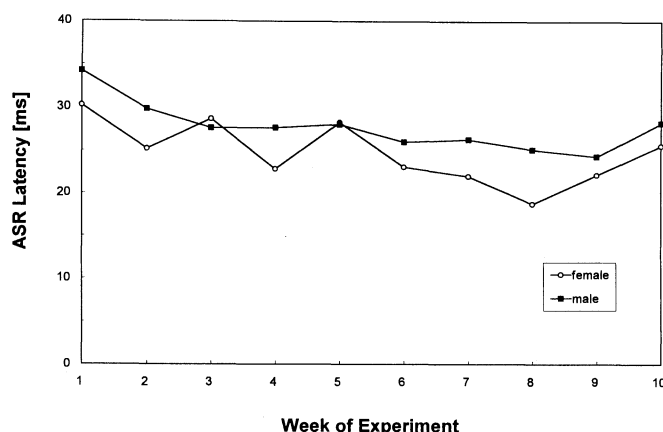


Fig. 3. Changes in the mean ASR latency during successive weeks of the experiment.

well as for the Wistar and hooded rats (Fig. 4). The most pronounced increase of the body weight was seen in the male hooded pups whereas the female Wistar rats gained weight more slowly. Multivariate repeated measures analysis revealed significant sex and strain effects on body weight increase. The body weight increase was higher in males as compared with female pups ( $F(1,23)=44.4$ ;  $P<0.001$ ). There was also a significant strain effect on body weight ( $F(1,23)=7.5$ ;  $P<0.02$ ). Hooded rats gain body weight faster than the Wistar pups.

Three-way ANOVA revealed significant gender ( $F(1,25)=20.75$ ;  $P<0.001$ ), age ( $F(9,225)=21.1$ ;  $P<0.001$ ) and the interstimulus interval ( $F(11,275)=11.6$ ;  $P<0.001$ ) effects on the acoustic startle response parameters. Generally, the male rats responded to the acoustic stimuli with greater amplitude for all

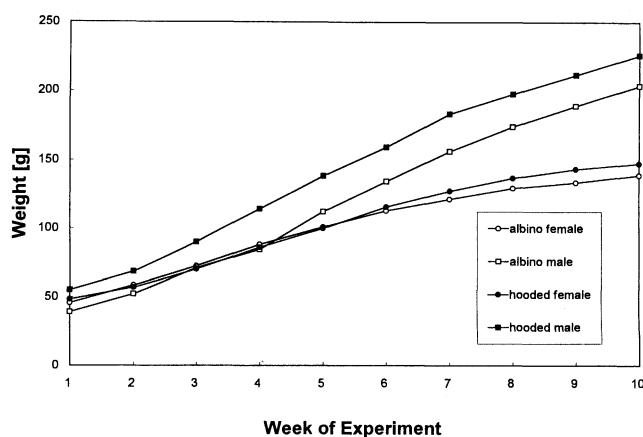


Fig. 4. The increases of mean body weight for male and female rats of the two strains during the experiment.

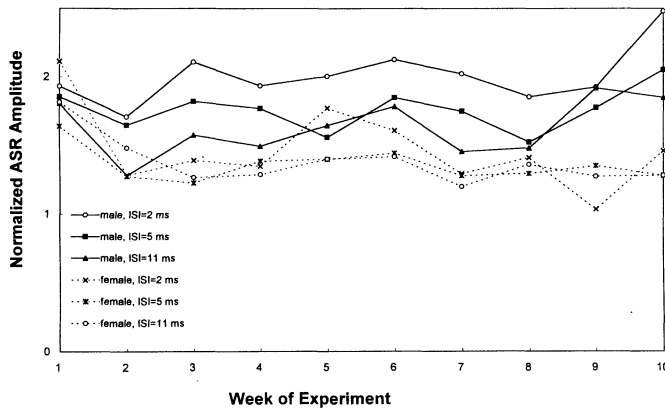


Fig. 5. Changes of the ASR amplitude during successive weeks for different interstimuli intervals (ISI).

ISIs (Fig. 5) except for the very first week of the study when the ASR amplitudes were similar in both male and female pups. Whereas the absolute ASR amplitude increased during the period of observation, the normalized amplitude of the ASR remained constant during the experiment (linear regression coefficient  $a=-0.004$ ).

Figure 6 illustrates changes in the grand means of ASR latency for ten different ISIs range between 2 and 11 ms for male and female rats. The shortest latencies of the ASR were observed for the ISI equal to 11 ms for all weeks of studies. On the other hand, for the inter-stimulus interval equal to 2 ms the latency of the startle acoustic response was significantly longer ( $P<0.001$ ).

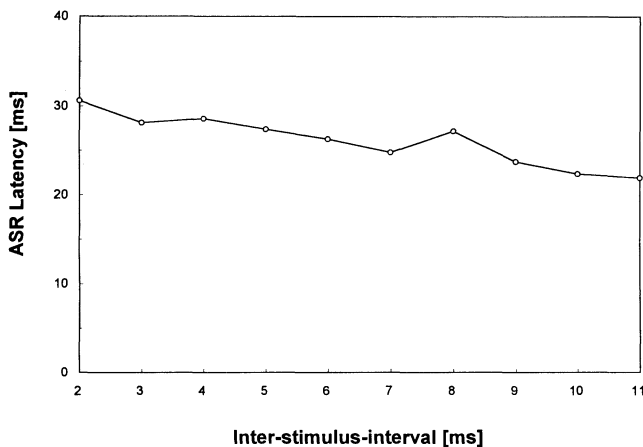


Fig. 6. The ASR latencies for different interstimuli intervals for male and female rats.

## DISCUSSION

The methodology of this study allows for an evaluation of the acoustic startle response by measuring changes in the ground reaction forces. The motor component of the ASR begins in the head muscles and spreads down the body as a transient wave of flexor contractions (Szabo 1965). Thus, the ground reaction forces recorded in the experiment involved the net torque produced by muscle contractions and transmitted to the support surface through the animal's limbs. The magnitude of the ASR measured in this way depends, therefore, upon both biomechanical and neural factors. Two biomechanical parameters seem to be of major significance: the total power of the motor system and body weight. These parameters change during the entire period of observation.

Neural factors such as the maturation of the acoustic and neuromuscular systems as well as the emotional development of the pups are involved. It is well established that maturation of the rat nervous system is largely completed within first four weeks of life (Buelke-Sam et al. 1984, Geisler et al. 1993) so that the changes in the startle response as evidenced by our findings cannot be related to this maturation process. However, rapid skeleto-muscular and emotional development of the animal has been observed during the period of this study i.e., up to four months of life.

The findings of this study demonstrate an interesting developmental effect, namely a close relationship between the animal's body weight and the ASR amplitude. Our results show that the sizes and body weights of the male and female rats were not different at the very beginning of the study. At the same time no difference was apparent between both genders in their ASR parameters. However, such differences have gradually developed during successive weeks of the experiment. These changes in body weight were positively correlated with the ASR amplitude. In this period of life, the increase of the body weight is mainly caused by an increase of the total muscle mass which determines the strength of the animal's motor system. The effect of

muscular strength increase during the period of rapid growth and, especially, the increase of the force produced by antigravitational postural muscles, apparently contribute to the augmentation of the startle response. During this early period of life we observed a gradual differentiation of the development between the male and female rats. Male rats' muscles grew heavier and stronger.

The major findings of this study were the difference in the ASR in the male and female rats and the lack of the differences between Wistar and hooded rats. The body weight and its inertia might affect both the amplitude and the latency of the ASR. The same rate of the muscles contraction evokes a higher postural force reaction in heavier animals. In addition, the increase of the body weight affects the body's inertia and thus might result in the increase of the ASR latency. This increase is due to an increase of electromechanical delay i.e. the delay between onset of EMG activity and the beginning of force development in the muscles (Norman and Komi 1979).

We found a strong effect of the ISI on the ASR parameters. It is well known that changes in the sensory environment correlate with an acoustic stimulus producing ASR can modify the startle reaction (Ison et al. 1973, Marsh et al. 1973, Pinckney 1976, Stitt et al. 1976, Leitner et al. 1993, Reijmers and Peeters 1994, Seaman et al. 1994). It is well documented that ASR inhibition can be observed in rats, when an acoustic prepulse precedes the startle stimulus by 10-500 ms (Hoffman and Searle 1965, 1968, Ison and Krauter 1974, Ison 1978). At intervals of 0-10 ms, a prepulse has been reported, however, to facilitate the startle reaction. The startle reaction inhibition by acoustic prepulses is mostly considered as a hard-wired phenomenon not being influenced by experience. However, when the ISI was very short (3-13 ms) during successive experiments a change from facilitation to inhibition was observed (Reijmers and Peeters 1994).

Our results evidenced the shortening of the ASR latency. This phenomenon could be related to the maturation of the motor system. It seems, however, that the contribution of the learning effect and emo-

tional development cannot be presently excluded. This hypothesis is supported by the results of an analysis of the normalized data. Such normalization minimizes the effects due to morphological heterogeneity between subjects. The mean normalized ASR amplitude for the first experimental session was significantly elevated and gradually decreased during successive sessions. This effect was markedly pronounced in the female pups.

The effect of the first exposure to the strong acoustic stimuli was also observed in the ASR latency. Thus, our results clearly indicate a difference between male and female responses to the acoustic startle stimulus. In conclusion, it appears that physiological and morphological changes that are observed during the development of rats have a profound effect on the ASR amplitudes and latencies. The increase of the ASR during ontogenesis might be related to the rapid development of the neural and the musculo-skeletal systems.

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