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Acoustic startle response in the opossum *Monodelphis domestica* in comparison with the Wistar albino rat

Short communication

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Abstract. The acoustic startle response (ASR) was studied in 20 gray short-tailed opossums (*Monodelphis domestica*) and its characteristics were compared with those obtained from responses of 20 Wistar albino rats. The animals were exposed to 10 ms, 110 dB white noise acoustic pulses in the Coulbourn apparatus. Amplitude of ASR was normalized to the weight of animals and then analyzed. The results show that probability of a freezing response is much higher in the opossums that generally respond with lower startle amplitudes in comparison to rats. These differences may reflect different emotional characteristics of the two species, different reactions to fear in opossum and/or different ecological specializations of the two species.

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The startle response is an important defensive reflex that is present in all vertebrates, but its mechanisms were modified many times in the course of evolution of vertebrates (Hale et al. 2002). In mammals it is a relatively simple reflex with its center in the brainstem reticular formation (Koch and Schnitzler 1997). It's reliably elicited by intense stimuli of several sensory modalities. The most frequently studied is the acoustic startle response (ASR), a generalized muscle contraction in response to an abrupt, loud sound. The magnitude of ASR is assessed by measuring the ground force of reaction evoked by a controlled acoustic stimulus. Although ASR is a relatively simple behavior, it is sensitive to a variety of experimental conditions. For this reason, startle became recently an important tool in investigations of the mechanisms of sensory information processing, learning, memory, emotions, nervous control of emotional behavior and movement control (for review see Koch 1999). The majority of data concerning characteristics, mechanisms and genetic setup influencing ASR were collected on rats, mice and humans. Few other mammals, like ferrets, cats or hamsters were tested for ASR (Gruner 1989, Moore 1982, Sasaki et al. 1998) and as yet, no such studies were performed on marsupials.

The short-tailed Brazilian opossum (*Monodelphis domestica*) is a recently introduced laboratory animal (Fadem et al 1982). Our earlier research of the behavior of opossum in the elevated cross-maze and open field, including reaction to new objects (Wesierska and Turlejski 2000, Wesierska et al. 2003), showed that its behavior differs substantially from the rat. Opossums overcome fear evoked by novelty and stop their defensive behavior faster than rats, thereafter approaching and investigating new objects more frequently and for a longer time. These differences between opossums and rats indicate lower level of fear in the opossums. As the startle reaction is augmented by fear and anxiety (Hebb et al. 2003) and is believed to be a good test of emotionality of animals, we decided to compare the startle response of the opossums and rats to learn more about the differences in emotionality between these two species. Except of the species differences in startle characteristics we were also interested in the sex differences in the test.

All experimental procedures were conducted with the approval of the Committee for Ethics of Animal Experimentation of the Nencki Institute of Experimental Biology and were in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Twenty gray short-tailed opossums (10 males and 10 females) from the breeding colony of the Nencki Institute of Experimental Biology were used in this experiment. They were 5–7 months old and their mean body mass was 77.9 ± 17 g (range 48–100 g). Acoustic startle reactions of the opossums were compared with those of twenty 2–3 months old Wistar albino rats (10 females and 10 males, mean body mass 88 ± 18 g, range 80–114 g). Opossums are born at a much earlier developmental stage than rats and develop at a slower rate. At the age of 5–6 months they reach sexual maturity and their body mass is about 70–90% of the adult (which is 70–90 g for females and 80–120 g for males). Therefore, our rats were at a developmental stage approximately equivalent to that of the opossums.

Testing was performed in a ventilated, double-walled sound-attenuating chamber (Coulbourn Instruments). For the time of testing the animals were placed singly in small plastic cages (180 × 85 × 90 mm) closed at the top with aluminum bars. Four animals were tested simultaneously. Each cage was placed on a separate force registering platform that recorded the vertical reaction force of the animal's startle responses. Testing proceeded in the darkness and the adaptation period of five minutes was allowed before the testing started. A sequence of acoustic pulses separated with pseudo-random intervals was presented to the animals. Each acoustic stimulus was a 10 ms long pulse of white noise with 2 ms rise/fall times and 6 ms plateau. The peak intensity of the pulses was 110 dB SPL. Each animal received a total of 20 stimuli during a single session.

Signals from the platform were amplified, rectified, filtered with the 40 Hz low pass filter, and then sampled at a frequency of 4 kHz. Four hundred millisecond sequences of data, starting at the onset of the acoustic stimulus, were stored in the computer for a further off-line analysis. The investigated parameters of ASR (peak amplitude of the first response and the latency to peak) were determined automatically with a computer program. Because of large body weight differences within and between species, the ASR amplitude had to be normalized. The amplitude of the startle as measured in newtons [N], was divided by the animal's body weight given in [N], resulting in the relative ASR amplitude. This method of normalization was previously used by the authors and described in details (Błaszczyk and Tajchert 1996). Each of the two parameters of startle response was analyzed using one-way ANOVA (Statistica v. 5.0, StatSoft Inc. USA).

The normalized responses of both female and male opossums were similar and of a moderate amplitude

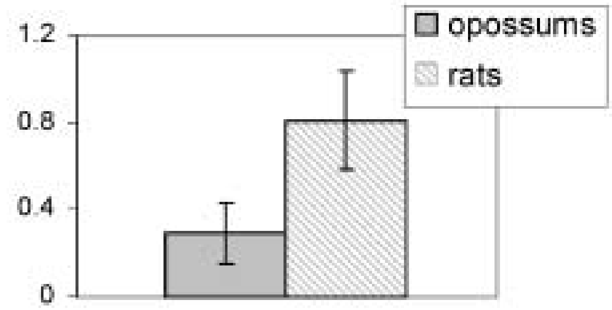

Fig. 1. Mean (± SD) values of the normalized amplitudes of ASR [N/N] in the investigated groups of opossums and rats

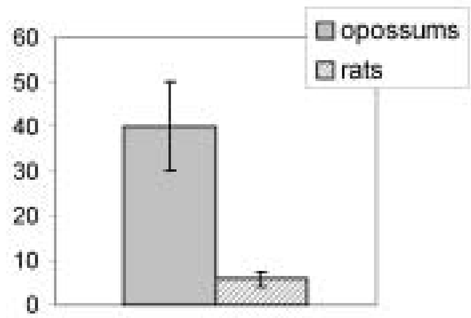

Fig. 2. Mean percentages (± SD) of the freezing responses (i.e., responses with the normalized ASR amplitudes lower than 0.3) in the opossums and rats

(females 0.30 ± 0.14, males 0.28 ± 0.15). As there were no sex differences (one-way ANOVA, P=0.48), the data for all opossums were pooled together and the statistical analysis was repeated on the whole set of data. The mean value of the ASR amplitude for all opossums was 0.29 ± 0.14 (Fig. 1). Similarly, rats did not show any sex differences in the amplitude of ASR and the data for both sexes were pooled together. Relative amplitude of responses in the whole group of rats was much higher than in opossums (mean 0.81 ± 0.23). The difference between the means for peak amplitudes of ASR of the opossums and rats was statistically highly significant ($F_{1,38}$=72.2, P<0.0001).

Another frequently used ASR index is the probability of eliciting responses with amplitudes lower than an arbitrary level, in our case 0.3. The higher value of this index indicates an increased tendency for the freezing response. Proportions of ASR with the relative amplitudes higher and lower than 0.3 were significantly different in the two investigated species (Fig. 2). In the opossums 40% of the startle reactions were below the threshold and were therefore classified as freezing responses. Proportions of freezing reactions were similar in both sexes of opossums (41% in females and 39.6% in males). In contrast, in rats 94.2% of the relative amplitudes of ASR were above the 0.3 threshold and therefore rats showed freezing reactions in only 5.8% of trials. Latencies of ASR were not statistically different between the two species and between sexes within species and equaled 52.8 ms and 53.5 ms in rats and opossums, respectively.

The results of our experiments showed a clear difference in the startle pattern between the opossums and Wistar rats. Our rats very consistently showed strong startle reactions, even though this line was not selectioned for any trait. Relative amplitudes of the opossum's startle responses were significantly lower and their probability of freezing significantly higher. Other authors found that almost half of their rats exhibited tendencies for either freezing or a specific movement pattern (Plappert et al. 1993). Reactions of our rats and opossums were very consistent and the probability of difference between the two species was two orders of magnitude higher than the probability of differences between rodent strains reported by other authors (Plappert and Pilz 2002, Plappert et al. 1993, Trullas and Skolnick 1993). Therefore, we conclude that the difference would be evident in comparison with any rat strain.

The magnitude of the startle response frequently correlates with behaviors known to reflect subject's anxiety or responsiveness to stress. For example, magnitude of ASR is increased by fear (Plappert and Pilz 2002) and it correlates highly with the length of freezing after the first exposure to the open field, which is another response driven by fear/anxiety (Leaton and Borszcz 1985). It is also negatively correlated with the proportion of time the rats spend in the open arms of the elevated plus maze (Trullas and Skolnick 1993).

During first exposure to the elevated plus-maze opossums entered the open arms more frequently and stayed there longer than rats (Wesierska and Turlejski 2000) and in the open field they showed more exploratory activity and less defensive reactions than rats and in response to novelty switched their behavior from defensive to exploratory more rapidly (Węsierska et al. 2003). All those findings are consistent with their ASR amplitudes being lower than in the rats. This suggests that in both species all those measures of behavior may depend on a balance of the same factors: the level of fear/anxiety and intensity of exploratory drive. However, this balance is different in each species.

Such comparisons are relatively safe if different strains of the same species that are kept in the same conditions are compared. In such case the differences reflect mainly the innate, genetic differences influencing emotionality of the strains (Fernandez-Teruel et al. 2002). However, when comparisons are made between species, such conclusions must be treated cautiously, because the startle mechanisms were frequently undergoing deep modifications, in relation to evolution and ecology of the species. Therefore, the same level of fear may evoke very different reactions in various species. Some of those differences could be also attributed to a different pattern of motor behavior. Our informal observations show that opossums initially react to novelty with either freezing or investigation that involves leaving the safe place. If the fear exceeds a certain level (especially in response to an unexpected sound), they show a very fast startle and fast, even frantic escape, followed by another freezing episode. Also, when in a safe shelter, they tend to freeze even when scared or touched. Therefore, opossums may have a different behavioral pattern of response to new and/or endangering situations than rats, and the very context of our experiments (narrow, enclosing space and darkness) could have increased the probability of their freezing reactions. These possibilities have to be investigated further before a final conclusion could be drawn.

In spite of all those reservations, it seems safe to conclude that all three behavioral tests (cross-maze, open field and ASR) point to a different balance of defensive and exploratory activity in the opossums and rats, with less fear-driven reactions, prevalence of freezing in the defensive reactions and a higher threshold for escape in the opossums. This different emotionality of the two species may result from the different ecology and/or their different evolutionary history.

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