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In Search of an Animal Model of Autism Spectrum Disorders

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Abstract

In search of an animal model of autism spectrum disorders, the acoustic startle response elicited by 110 dB 10-ms pulses was studied at different periods of animal development in mouse lines selectively bred for high (HA) and low (LA) swim analgesia. Animals used in this study were obtained from our colony of Swiss-Webster mice selectively bred throughout 56-59 generations for the magnitude of analgesia. ASRs were assessed in 240 mice (both males and females), exposed to a sequence of twenty 10ms white noise pulses (10 ms, 110 dB SPL) presented in random order, at 70 dB white noise background. Mice from four successive generations were studied only once at the postnatal day P30, P60, P90, and P120 respectively for each generation. Statistical analysis revealed significant differences between HA, LA, and control (C) animals. From the second month of life, the ASR amplitudes differed between the mouse lines in the order of HA > Control > LA. Within all lines studied the male subjects startled more than the females. The differences may be accounted for the traits obtained during selective bred and the HA line can be recommended as a model for autism spectrum disorders.

Keywords: Acoustic startle; Animal model; Anxiety; Emotional development; Ontogeny

Introduction

The Acoustic Startle Response (ASR) is considered to be a promising neurophysiological test for translational research in psychiatry [1]. In particular, anxiety disorders are a group of mental disorders characterized by significant anxiety and fear [2]. Fear is an emotional state induced by perceived danger or threat and the magnitude of the startle reflex is an efficient index of anxiety level [3,4]. Increased ASR latency and higher reactivity to weak acoustic stimuli were found in children with autism spectrum disorders [5-7]. Such alterations of the ASR are known to develop typically in children before 8 years of age [1]. This is a common sensory-perceptual abnormality that interrupts their behavioral adaptations [5]. ASR became a convenient model for the study of a variety of physiological functions as the processing of sensory information, learning, memory, and the control of emotional behavior [8]. In particular, it was proposed a kind of subtle opioid-dopamine interaction might be essential for the sensorimotor gating of the acoustic stimuli [8]. The startle response is a jerk evoked by abrupt contraction of trunk, limb, neck and head muscles in response to a strong and unexpected stimulus of various modality. In laboratory rodents, the startle reflex is commonly elicited by a brief intense sound pulse. The response can be reliably quantified by measuring forces exerted on the ground while the animal is startling. Being a relatively simple oligosynaptic reflex, the ASR is subject to modulatory influences of various origins which decrease or augment its magnitude [9,10]. Such phenomena as potentiation of ASR by a conditioned fear stimulus, habituation, sensitization and inhibition by a no startling prepulse are believed to reflect the plasticity of the sensory systems involved in the animal's adequate responding to environmental cues (for review see [3,8]). Particularly, the ASR seems to be a fundamental motor response that is necessary for the animal's adjustment to its environment.

The startle in a natural environment, where it is elicited by sudden warning signals, initiates freezing or flight reaction. Therefore, the ability to perform an efficient startle seems beneficial for the animal's survival in dangerous circumstances, like a predator's attack or intraspecies competition [3]. Much evidence shows that in rodents the pattern of ASR can also

depend on the genetic makeup of the animal. In particular, we have shown that the magnitude of the ASR differs between mouse lines selectively bred in our laboratory for divergent magnitudes of analgesia produced by a 3-min swim in 20 °C water [11,12]. The selection that continued throughout over 50 generations and focused on the magnitude of the swim analgesia allowed to differentiate the mouse lines with the High Analgesia (HA) and with the low analgesia (LA). Both mouse lines substantially differ in acoustic startle responses. ASR amplitudes differed between the mouse lines in the order of HA > Control > LA [8]. The higher amplitude of ASR, taken together with the lower open-field activity of HA mice, can be interpreted in terms of higher background anxiety level, compared to the LA line [8,13]. We traced changes in ASR characteristics in young and adults' mice of HA and LA lines. The HA line is characterized by the augmented response to acoustic stimuli, similarly to patients with autism spectrum disorders who commonly exhibit atypical behavioral responses to sensory stimuli [7]. Over 96% of children with autism report hyper and hypo-sensitivities in multiple sensory modalities [7]. Our previous study documented also the effects of the animal's sex and age on the normalized ASR magnitude. The observed differences may be accounted for emotional development and sensitivity to auditory stimuli [8,14,15]. Characteristics of ASR (delay and amplitude) and its modulation are known to be stable in adult animals. However, little is known about the stability of these indexes in young animals in the period of development. Continuing this line of research, in the present study we focused on the startle response in ontogeny. Thus, by monitoring the ASR in longitudinal studies, we can compare the emotional development in selectively bred mouse lines and the controls. Towards this aim, we assessed and analyzed the ASR characteristics in 3 lines of mice in four successive months of their life.

Material and Methods

The protocols of the experiments and the selection procedure were approved by the Ethics Commission of the Institute for Genetics and Animal Breeding, Polish Academy of Sciences. The rules of intramural humane care of laboratory animals were strictly observed according to Polish law. Young animals used in this study were obtained from our colony of Swiss-Webster mice selectively bred throughout 56-59 generations for the high and low magnitude of analgesia induced by 3-min swimming in 20 °C water [11,12]. Litters of newborn mice were reared by their mothers. Then, the animals lived in socially stable groups of same-sex littermates 4-6 to a cage at 12:12 light/dark photoperiod and the ambient temperature of 21±1ºC, with unlimited access to murine chow and water. A total of 240 animals (sixty mice from each generation: 30 males and 30 females) of the HA and the LA line, including randomly bred controls (C), were tested for the ASR amplitude. The ASR responses were measured on 30 postnatal days (month 1 in generation 56), and subsequently: at P60 (month 2 in generation 57), P90 (month 3 in generation 58) and P120 (month 4 in generation 59). For details of the experimental design see Table 1.

Table 1: Experimental design and characteristics of the experimental groups.

Experimental Session	ASR1	ASR2	ASR3	ASR4
Mice generation	56	57	58	59
Age (months)	1	2	3	4
Mean Body Mass (±S.D.) Males [g]	29.8±2.2	33.9±2.8	37.6±3.2	38.9±3.3
Mean Body Mass (±S.D.) Females [g]	27.56±2.1	31.6. ±2.6	33.2±2.9	34.4±3.1

ASR testing was performed in a Coulbourn apparatus (Coulbourn Instruments, Allentown, PA) equipped with four force-sensitive platforms placed in a sound-proof ventilated chamber. Ten-millisecond white noise pulses (110 dB SPL) were used as the startle stimuli. Four animals placed in plastic cages were tested simultaneously. Before each test, the cages were thoroughly washed and wiped to eliminate odors. An adaptation period of five minutes was allowed before testing. During the experimental session, a sequence of 20 ten-millisecond acoustic pulses, separated by a pseudo-random interstimulus interval (ranging between 5 and 60 sec), was presented to the animals. The startle responses were assessed in a 100 ms window triggered at the onset of the acoustic stimulus and sampled at 400 Hz. The ASR amplitude was normalized to an animal's body mass. The data were analyzed with Statistical v. 6.0 (Stat Soft, U.S.A.) software. Three-way analysis of variance (ANOVA) taking line, sex and animal age as independent measures. The startle force magnitudes did not significantly vary between the twenty individual tests within the ASR sessions. Therefore, mean startle magnitude computed from the twenty ASR tests in each session was taken as a dependent variable in these analyses. Detailed comparisons, where appropriate, were made with LSD test. Significance was accepted at $p< 0.05$.

Results

Before each experimental session, animal body weight was measured, and this value was used then to compute the normalized value of the ASR response. Such values were calculated as the mean ASR amplitude (from twenty individual repetitions of ASR stimuli) divided by body mass of the animal. Mean values of body weight for male and female mice during each monthly session are presented in Table 1. The results of the descriptive statistics are summarized in the graphic form in Figure 1. Analysis of variance showed the effect of animals:

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(i) Age ($F_{3,216}$ = 7.29, p≤0.001),

(ii) Line ($F_{2,216}$ = 21.8, p≤0.001), and

(iii) Sex ($F_{1,216}$ = 12.3, p≤0.001) on the ASR amplitude.

Additionally, several interactions reached the level of significance. In particular: age and line interaction ($F_{6,216}$ = 2.76, p≤0.02), age and sex ($F_{3,216}$ = 3.32 p≤0.03), and line x sex interaction ($F_{2,216}$ = 8.14, p≤0.001). Generally, mice of HA line responded with greater amplitudes in comparison with control and LA group except for one-month-old HA males. The HA male group exhibited the greatest increase of the normalized startle amplitude in the successive trials reaching maximum (1.35 ± 0.8) at the age of 3 months. Startle response in LA mice was characterized by a decline of the normalized amplitude with the minimum at the age of three months, both for males (0.17 ± 0.06) and females (0.3 ± 0.08). The decline, however, was less pronounced in LA males, which generally responded more readily to acoustic pulses as compared with LA females. The gradual decline of the normalized ASR amplitude during the observation was seen in the control group. Both male and female mice of the C group exhibited a very similar pattern of changes. The results of the ASR assessment in the three lines of selectively bred mice are governed in Figure 1.

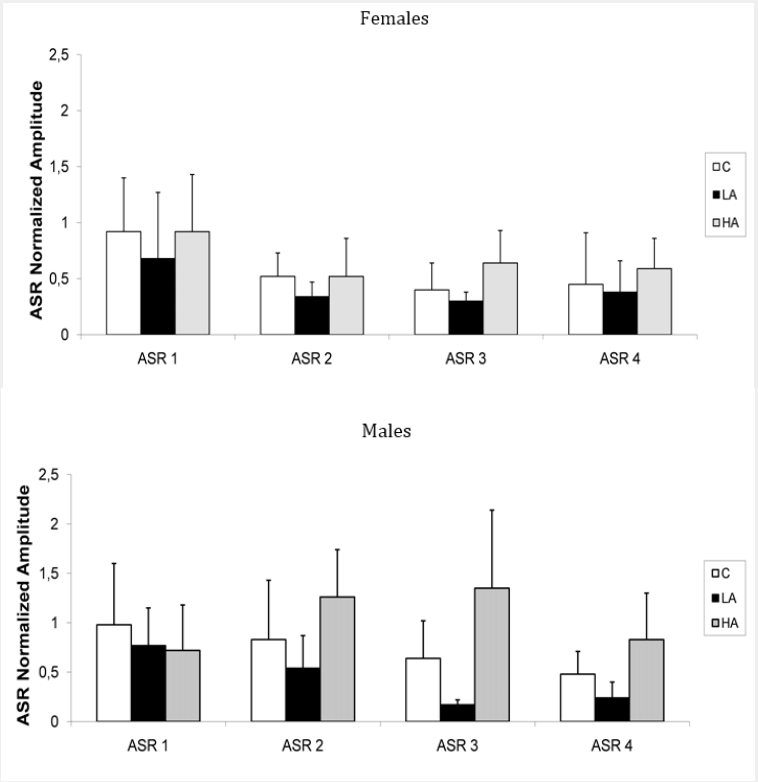

Figure 1: Normalized ASR amplitude (force magnitude normalized to body weight) in control (C), low- (LA), and high- (HA) swim analgesia in female (upper panel) and male (lower panel) mice at the age 1-4 months (ASR1-4). Error bars represent standard deviation.

Discussion

Recent studies of children with ASD suggest that comprehensive investigation of the ASR and its modulation, including the startle response to weak startle stimuli, peak startle latency, and pre-pulse inhibition (PPI), may contribute to an understanding of the impairment of the neural circuitry in children with autism spectrum disorders and its comorbid behavioral problems [1]. Animal studies investigating the development of sensorimotor gating in early development may contribute to the prevention of psychiatric disorders [1]. The ASR and its modulation can provide a well-established global neurophysiological index for translational research in psychiatric disorders [1]. Selective bred in mouse lines results in anomalous processing of acoustic stimuli in the adult auditory system. In the present study, the behavioral consequences of selective bred are assessed in 1-4-month-old mice using the ASR. Our findings demonstrate that selectively bred mouse pups exhibit altered behavioral responses to ASR stimuli in adulthood, indicating anomalies in intensity coding and loudness perception. Present results confirmed previously described differences in ASR characteristics between lines and also additionally documented effects of animal's age and sex. The normalized ASR amplitudes differed between the mouse lines in the order of HA > C > LA. This pattern was well pronounced in HA males' mice between their second and fourth postnatal month. It documents different emotional development between male

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and female animals within each selectively bred line. The most significant differences were observed between the first and the second month of life. Since we used the mice from 4 successive generations, the effect of generation should be also taken into account. In our previous study, we documented, however, that the between-line differences in ASR magnitude were present and consistent in all generations [7].

Therefore, observed in this study differences may be accounted for:

a. Motor [16,17],

b. Auditory [18,19], and

c. Emotional [8,15] development.

The ASR is an involuntary motor response following a strong and unexpected acoustic stimulus. It can be reliably assessed in rodents with fully developed neuro-muscular control. The motor development is dependent on both animal's age and motor activity. Usually, motor development is slowed down in less active animals. Compared to the LA line, HA mice were found less active in the open-field test [8]. Therefore the HA mice appear less active than LA mouse [8] and should exhibit a lower ASR amplitude. In our previous study, however, we have documented that the ASR magnitude in adult mice is significantly higher in the HA than in the LA line [8]. Therefore, motor development cannot fully explain our present results pointing rather a deficiency in the emotional development of the HA line. In contrast to LA mice, HA mice seem to exhibit greater emotionality in various behavioral tests [8]. Since the HA mice appear less active than LA mice in the open field test, we should link their higher startle with enhanced emotionality. The HA mice when exposed to forced swimming at certain temporal/temperature parameters, display a depressive-like behavior sensitive to antidepressant treatment [12]. The next factor that can be used to explain differences between lines is the development of their auditory system. Rodents are born with an immature auditory system [18-21]. For instance, in rats, first responses to acoustic stimuli appear on the 10th postnatal day. Later on, the auditory brainstem response thresholds improve rapidly, approaching adult values between days 24 and 36. Therefore maturation of the rodents' hearing function then proceeds up to the 4th–6th postnatal week [18]. For this reason, our first assessment of the ASR was carried on in month-old mice assuming that their auditory system is fully developed.

Also, the sex of animals may be accounted for the results [14,22]. Differences in the ASR characteristics of male and female rats during ontogeny were previously documented [22]. Generally, male subjects responded with a greater ASR amplitude. The differences were attributed to sex-dependent differences in the development of muscular mass and power between male and female rats. In conclusion, we can posit that the pattern of ASR responses in our lines of mice should be attributed to inherited traits. In conclusion, the HA line of mice can be recommended as an animal model for autism spectrum disorders.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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