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EXPERIMENTAL PAPERS

Active Avoidance Learning in Rats with Different Audiogenic Epilepsy Proneness

N. M. Surina[a,*], I. B. Fedotova[a], and I. I. Poletaeva[a]

[a] *Lomonosov Moscow State University, Biology Department, Moscow, Russia*
**e-mail: Opera_ghost@inbox.ru*

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Abstract—Learning difficulties are a fairly common comorbid complication in patients diagnosed with epilepsy. The relationship between the predisposition to epilepsy and the ability to learn is an important problem. The aim of the study was to analyze how the learning capacity differs in rats with different predispositions to reflex audiogenic epilepsy (AE). The success of active avoidance (AAC) conditioning in the shuttle box in rats of 3 strains was evaluated. These were rats predisposed to audiogenic epilepsy—the Krushinsky–Molodkina strain (KM), strain "4" (selected from a population of F2 hybrids of the KM strain and AE non-prone Wistars) and rats of "0" strain, selected for the absence of AE from the same population (i.e. these strains differ radically in AE-proneness, but have a similar genetic background). The training was conducted continuously for 5 days (20 presentations per day). Experiments have shown significantly more successful acquisition of this skill in rats of the "0" strain: in total, 75% of animals reached the learning criteria for 5 days of AAC training (70% AAC per day). The "4" strain rats indices were intermediate—41.7%, and only 1 from the 12 rats of the KM strain (8.3%) acquired the task according to this protocol. Thus, it was possible to identify more successful learning in rats, selected for the absence of AE seizures (strain "0") compared to rats of the KM and "4" strains (expressing tonic seizures of maximum intensity in response to sound exposure). The weakest acquisition was revealed in the KM strain, in which the selection process duration for AE proneness was significantly longer than in the rats of the "4" strain.

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Keywords: audiogenic epilepsy, Krushinsky–Molodkina strain, "0" strain, "4" strain, rats, active avoidance conditioning, inter-trial reactions

Abbreviations: AE—audiogenic epilepsy; KM—Krushinsky–Molodkina rats; AAC—active avoidance conditioning; CS—conditional stimulus; US—unconditional stimulus; L—latency; ITR—inter-trial reactions

INTRODUCTION

Audiogenic epilepsy (AE) in rodents, a phenomenon described for the first time more than 100 years ago, still requires the attention of neurophysiologists, since animal strains with a high predisposition to AE are a valuable model for the study of epileptogenesis [1]. The abnormal response of rodents to a loud sound can presumably be considered as an abnormally enhanced species-specific avoidance reaction [2]. It should be noted that despite a wide range of studies concerning on the mechanisms of AE and reactions of AE-prone animals to pharmacological agents, clear data on the ability of such animals to learn the conditioned reflex reaction of avoiding electric shock are lacking [3], although the role of epileptogenesis in learning other habits is evaluated quite successfully, for example, [4, 5]. In many studies, not the learning characteristics of AE-prone animals are evaluated, but the effect of the actual state after seizures on learning ability, for example [6, 7], while it should be considered that the exact moment of a seizure ending is not always possible to determine.

It should also be noted that if common learning disabilities occur in 2–10% of cases in human populations, in patients with epilepsy this proportion reaches 25% [8, 9], which determines the significance of such investigations.

Thus, the analysis of the learning capacity in rats of several genetic groups with different AE-proneness is of particular interest. Krushinsky–Molodkina (KM) rats is the world's oldest AE–selected rat strain with maximum expressivity penetrance of the trait among similar strains. The KM strain has "self-control strains" with a similar genetic background—these are the strains "0" and "4". They were selected for the absence of AE and for a high AE-proneness, being derived from the population of F2 hybrids from crossing rats of the KM strain with rats of the outbred Wistar population (specially selected for the absence of AE manifestations) [10]. The breeding of rats of these new strains had been started about 15 years ago, they have a similar genetic background with each other and partially with that of KM strain [11]. It should also be noted that the KM strain has been bred independently from the original Wistar population since the end of 1940, that is, for more than 70 years. In the selection rats for AE-non-proneness, the greatest "difficulty" was the breeding of strain "0", because the complete absence of an epileptiform reaction in response to the action of sound is usually detected in no more than 50% of animals in each generation. The aim of this study was to evaluate the AAC learning indices in rats of three genotypes with different AE-proneness—inbred KM strain, as well as in rats of strains "0" and "4".

MATERIALS AND METHODS

Experimental animals. In both series of experiments described in this paper, male rats of three strains were used—KM (n = 12), "4" (n = 12), and "0" (n = 12) at the age of 6 months. The animals were kept 6 animals per cage, with food (Laboratorkorm firm) and water ad lib, without prior handling procedure. AE-proneness was evaluated in arbitrary units according to the accepted standard [12] at the age of 3 months. The rats of the KM and "4" strains had maximum sensitivity to sound (score "4", tonic seizures of the whole body and limbs), while all rats of the "0" strain involved in the experiment had no AE seizures [10].

AAC training experiments were performed using 6-months old animals. In the 1st series, they were 12 rats of the KM strain, 10 rats of the "4" strain and 12 rats of the "0" strain, in the second series—12 animals from each strain.

The experimental schedule: AAC training was performed in an experimental box (60 × 30 × 30 cm) with two compartments and an electrified floor. The conditional stimulus (CS) was the light (400 lux, 1st series) or sound (80 dB, which did not cause AE seizures in rats of the KM and "4" strains, in the 2nd series). It should be noted that seizures in rats of the KM and "4" strains are provoked by a sound volume in 120 dB. The unconditional stimulus (US) was the switching on of the electric current to the grid floor in one of the halves of the experimental box (current strength about 0.6 mA).

The procedure of the AAC training: in 20–30 seconds after placing the animal in the box, the CS was turned on (isolated action during 10 s), after 10 s elapsed the US was turned on (the joint action with the CS+US lasted 10 s). The rat's transition into the safe box compartment turned off both stimuli. AAC was considered fulfilled if an animal entered the safe compartment before US switching on. Each animal was given 20 presentations per day and the calculation of the proportions (in %) of AAC and inter-trial reactions (ITR, i.e. the entrances into another compartment in the absence of stimuli) being performed. In the first series, the training was conducted for 7 days, in the second—for 5 days. The latencies (L) of the rat's transition into the safe compartment in the response of the CS and/or US were manually recorded. The durations of the interstimulus intervals were about 30 s. The experiments were conducted daily. Not only AAC reactions were recorded but also the "escape reactions" as well (when an animal moved to a safe compartment at the onset of

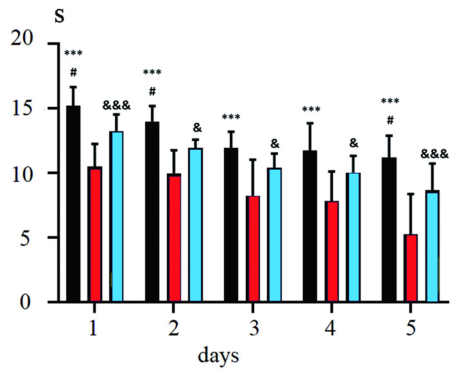

Fig. 1. Mean latencies (ordinate, s, mean ± st.err) of an animal transition into the safe compartment of the shuttle box in rats of 3 strains (*abscissa*: days of experiments). Black bars—rats of the KM strain, light gray—"0" strain, dark gray—"4" strain. ***—Significant differences from the indicators of the "0" strain, $p <$ 0.001, #—significant differences from the indicators of the "4" strain, $p < 0.05$, &, &&&—significant differences from the indicators of the "0" strain, $p < 0.05$ and 0.001, respectively (two-way ANOVA, post hoc LSD by Tukey).

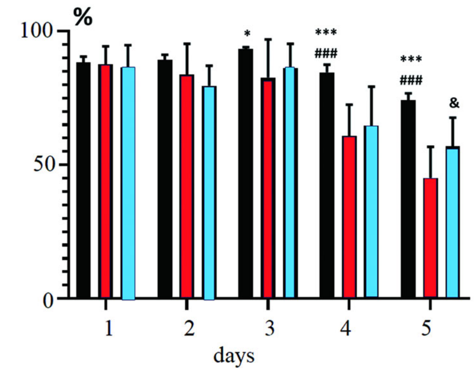

Fig. 2. The proportions of inter-trial reactions (ordinate, %) in respect to total CS-US presentations in rats of 3 strains. Designations as in Fig. 1. *, ***—significant differences from the "0" strain, $p < 0.05$ and 0.001, respectively, ###—significant difference from the "4" strain, $p < 0.001$, &—significant difference from the "0" strain, $p < 0.05$ (two-way ANOVA with post hoc LSD by Tukey).

US) thus the reaction L had been longer than 10 s).

Statistical analysis. The GraphPad Prism 8.01 program was applied using two-factor ANOVA (factors "training day" and "strain") with post hoc LSD analysis by Tukey.

RESULTS

The 1st series of experiments. Since the results of the AAC training procedure in rats of the three strains generally coincide (with more successful training in rats of the "0" strain), detailed data on series 1 are not provided. However, the important differences in the reactions of rats revealed in the two series should be noted. Rats of both strains in series 1 did not reach the learning criterion during 7 days of training (light as CS, 75% of AAC on the test day), but it was noted that the rats of strain "0" learned more successfully. At the same time, in this series of experiments, a fundamentally important phenomenon was revealed—in 5 KM rats and in 1 of "4" strain rat clonic and/or tonic seizures of the "audiogenic" type were noted during the interstimulus interval. Such seizures occurred mainly in the first days of training (from 1st to 3rd), but in one KM rat they occurred regularly until the 6th day of training (with daily experiments). It should be noted that this phenomenon, identified only in sound-sensitive rats, was observed for the first time.

The 2nd series of experiments. In this series, the sound was applied as a CS. On each of the five days of training, the L of the transition to the opposite box compartment was the longest in KM rats, and the shortest in rats of the "0" strain (Fig. 1). If the L was more than 10 seconds, this meant that the transition was made in response to the switching on the US and if the L was shorter than 10 seconds, then it was a reaction to the CS (sound).

A two-factor ANOVA of L indices of transition to a safe compartment revealed a significant influence of the "strain" factor ($F_{2, 165} = 88.26$, $p < 0.001$) and the "training day" factor ($F_{4, 165} = 34.91$, $p < 0.001$). The differences in the L indices between the KM and "4" strain rats were non-significant only in the 3rd and 4th days of training, whereas at other days the L was significantly shorter in rats of the "4" strain than in the KMs, which indicated their more effective acquisition of the reaction compared to the KMs. In rats of the "0" strain, L were significantly shorter in all days of training compared to those of KM and "4".

The main indices which indicated the learning capacity of the AAC were the reduction in the number of escape reactions (transitions in response to US onset) and an increase in the number of avoidance reactions (transitions in response to CS); as well as a shortening of the L of these reactions. To assess the learning process (the transition of the animal to a

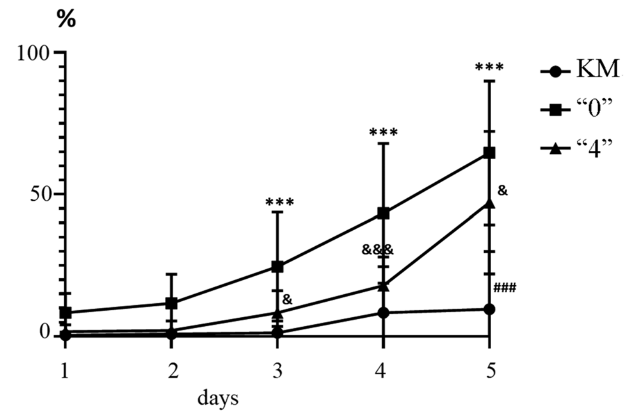

Fig. 3. Learning curves of active avoidance learning in rats of 3 strains (proportions of active avoidance reactions / 20 CS-US trial presentations, ordinate, %), along the days of experiments (abscissa). ***—The significance of the difference from this index for KM strain, $p < 0.001$, #—the significance of difference between KM and strain "4" indices. &, &&&—the significance of difference between the indices for strains "4" and "0", $p < 0.05$ and 0.001, respectively (two-way ANOVA, post hoc LSD Tukey test).

safe compartment in response to the CS), it is also necessary to analyze the dynamics of ITRs, i.e. transitions to another box compartment in the absence of CS and/or US (i.e. in time intervals when neither the CS nor the US were applied) (Fig. 2). The expression of ITR reflects the formation of a conditioned reaction to the "context", i.e., to the environment of the test. In this study, the proportions of ITR during the AAC achievement was determined as the ratio of the number of cases when ITR occurred to the total number of trials during this day (this number was 20). A two-factor ANOVA of data on the proportions of ITR revealed a significant influence of the "strain" factor, $F_{2,\,165} = 32.62$, $p < 0.001$, and the "day of training" factor, $F_{4,\,165} = 58.34$, $p < 0.001$. On the 1st and 2nd days of training, statistically significant interstrain differences in the proportions of ITR between the three groups were absent and thus the "context" reactions were similar. On days 3 and 4, the proportions of ITR in KM strain rats were significantly higher than in "0" strain (moreover, on day 4, this proportion significantly differed from the "4" strain indices). On the 5th day of training, the proportion of ITR in "0" strain rats was significantly lower than in KMs and "4", and the highest value of this index was in KM rats. ANOVA revealed differences in both the "strain" factor ($F_{2,\,165} = 32.62$, $p < 0.001$) and the "day of training" factor ($F_{4,\,165} = 58.34$, $p < 0.001$). On the first day of training, the scores of ITR (manifestations in relation to 20 CS—US pairings) in 3 strains did not differ, and the ITR indices in KM vs "0" strain were significantly different starting from the 3rd day of training (3rd day—$p < 0.05$, 4th and 5th days—$p < 0.0001$). It is interesting to note that according to this index, during the 3rd–5th days of training scores of the "4" strain were "intermediate" (Fig. 2). They displayed significantly less ITR than the KMs (days 4 and 5, $p < 0.001$) and slightly more than the rats of the "0" strain (day 5—$p < 0.05$). ITR, i.e. conditioned reactions to the context were more clearly expressed in rats of the KM strain, and their extinction (as a manifestation of associative abilities) proceeded slower in these rats than in rats "4" and "0" strains.

A two-factor ANOVA (with post hoc LSD by Tukey) for the ITR indices revealed differences in both—the "strain" factor ($F_{2,\,165} = 32.62$, $p < 0.001$) and the "day of training" factor ($F_{4,\,165} = 58.34$, $p < 0.001$). There were no statistically significant interstrain differences in these proportion between the three groups during 1st and 2nd days of training. On days from 3 to 5, the proportions of ITR in KM rats were significantly higher than in "0" strain rats. On the 5th day of training, the proportion of ITR in "0" strain rats was significantly lower than in "4" strain, while the highest value of this index was in KMs. The AAC was registered when an animal moved to another part of the box in response to CS—the sound. The learning curves (Fig. 3) are based on the proportions of these reactions on each of the training days. The largest proportion (in %) of successful AAC during all days of training was in rats of the "0" strain, while in KM rats there was no increase in this index up to the fifth day.

Two-factor ANOVA revealed, that this trait is under significant influence of the "strain" factor ($F_{2,\,165} = 48.29$, $p < 0.001$) and the "training day" factor ($F_{4,\,165} = 39.10$, $p < 0.001$). Figure 3 shows that on day 5, the proportion of AAC was significantly lowest for KM strain rats and the highest for "0" strain.

During 5 days of AAC training, the learning criterion (70% AAC per day) in total reached in "0" strain—9 from 12 rats (i.e. 75% of animals), in "4" strain—5 from 12 rats of the (41.7%), and in KM strain—1 from 12 rats (8.3%). In other words, the rats of the "0" strain learned more successfully than the rats of two other strains. According to the φ

Fischer test (estimating the significance of differences for alternative proportions), there were significantly more individuals in strain “0” which reached the learning criterion than in strain KM ($p < 0.001$) and they were more numerous in strain “4” (tendency). In KM strain rats, the learning indices were the lowest (according to the φ Fischer test, they significantly lower than in “4” strain, $p < 0.05$).

DISCUSSION

Thus, despite the differences in the dynamics of AAC acquisition, the data from the two series of experiments gave generally similar results—AAC learning of the strain “0” rats had been more effective than in KM and “4” strains, and this could be considered as a well-established fact. The convulsive seizures in KM tats in the inter-trial intervals in the 1st series of experiments, whereas in the 2nd series this phenomenon was absent, can be attributed to epigenetic effects associated with high inbred status of the KM strain [13]. One must also take into account that using light as a CS in the 1st series could be a provoking effect on the development of seizures mimicking the AE type (being the new type of stimulation which activated behavior). It worth be noted as well that despite the high intensity of AE seizures in rats of “4” strain (tested 3 months before the present experiments), and lack of differences from that of the KM strain, the strain “4” AAC learning capacity was slightly higher than that of KMs. This could be explained by common genetic background of “0” and “4” strains in comparison to the genetic background of KMs. In other words, these data once again emphasize the importance of taking into account the polygenic determination of AE in rats and the phenomenon of the influence of genetic AE-proneness on development of conditioned protective reaction in rats deserves further analysis. The dynamics of the L transition to the safe compartment during training days also indicates the faster reaction of “0” strain rats, even during the first day of training, when escape reactions prevailed in many animals the (i.e., reactions to the onset of US and not of the CS). These data allow to admit the more successful adaptation in “0” strain rats to test environment compared to KM and “4” strains—that is, their inhibition of reactions to the “context” and the development of the response to CS. In the literature, concerning the conditioning the reactions to electric shock, this aspect of learning was poorly analyzed, since the authors were focusing on the inhibition dynamics of contextual reactions using a predominantly unilateral avoidance reaction and evaluating the role of forebrain structures in the expression of these traits [14]. It should be mentioned that similar experiments were previously conducted to compare the AAC learning capacity in KM and “0” strain rats, but the primary data had been lost. However, a more successful learning capacity of “0” strain rats was also found in that study. The problem AE-proneness should be considered separately from the effect of seizures on learning ability, since the genetic AE-proneness is associated with the genomic and transcriptomic peculiarities in the corresponding genotypes [15–17]. The development of AAC in rats of the Brazilian Wistar Audiogenic Rat (WAR) strain [18] also demonstrated their significantly less successful learning than that of the control initial Wistar rats. This is comparable to the data obtained in the study presented. Less successful learning (but not AAC directly) was demonstrated in GEPR rats (AE-prone, selected from the Sprague-Dowley population [19]. It can be assumed that in AE-prone rats, the stress reaction development peculiarities to serial pain stimulations also affect associative processes. But, as shown in the presented work, in KM and “4” strain rats reactions to experimental context (i.e., inter-trial reactions) were expressed quite successfully. It is possible that stress reaction peculiarities play the significant role in the differences in AAC acquisition. The level of corticosterone in blood plasma in “0” strain rats in the background (i.e. without sound action) was significantly higher than in KM rats [20]. It is possible that the stress response pattern in the “0” strain, selected for the lack of AE, was the factor that determined the advantages of the “0” strain rats in the development of AAC. It should also be noted that the effect of sound, which did not cause seizures in “0” strain rats and Wistars, was not accompanied by the increase in corticosterone levels, while in rats of the KM strain, such an increase occurs/ noted in 30 minutes after seizures [20]. It can be assumed that the effect of sound *per se* does not cause a stress reaction. However, in general, the relationship between AAC acquisition and AE remains an important problem for pathophysiology. Previously, we provided a number of evidence that the AE phenomenon may be a consequence of the presence in rodents of an innate intensed reaction to avoid strong sounds [2], which should also be considered evaluating the detected features of AAC learning in this study.

CONCLUSION

Experiments with rats of three strains in conditioning of active avoidance of electric shock in the shuttle box revealed more successful acquisition in rats of the "0" strain (selected for the absence of seizures in response to strong sound) compared with rats of the KM and "4" strains, in which the effect of intense sound exposure causes tonic seizures of maximum intensity.

AUTHORS' CONTRIBUTION

The idea of the work and the planning of the experiment (Surina N.M., Fedotova I.B., Poletaeva I.I.), data collection (Kondratova S.N., Surina N.M., Nikolaev G.M.), data processing (Kondratova S.N., Surina N.M., Poletaeva I.I.), writing and editing of the manuscript (Poletaeva I.I., Surina N.M.).

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COMPLIANCE WITH ETHICAL STANDARDS

Experiments with animals were conducted in accordance with international recommendations on biomedical research with laboratory animals and were approved by the Commission on Bioethics of Lomonosov Moscow State University, Protocol no. 12 dated March 3, 2014).

CONFLICT OF INTEREST

The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

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