Formation of Defense Behavior under Conditions of Functional Inactivation of the Median Cerebellar Cortex

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We studied the effect of functional inactivation of cerebellar vermis after injection of tetrodotoxin on the formation, consolidation/storage, and retrieval of long-term memory on the model of acoustic startle reaction extinction and freezing behavior in adult rats. Tetrodotoxin administered before training suppressed short-term sensitization of the acoustic startle reaction with subsequent impairment of long-term habituation and formation of conditioned freezing. Application of tetrodotoxin after training or before testing did not modulate the formation and reproduction of the studied forms of defense behavior. Two applications of the drug (before training and testing) caused no disorders in the long-term extinction of the acoustic startle reaction and freezing behavior, which attests to the possibility of dissociated training under conditions of cerebellar inactivation. These data indicate the involvement of the vermis cortex into the mechanisms of long-term memory of various forms of defense behavior.

Key Words: *cerebellum; startle reaction; conditioned fear; training; memory*

The role of the cerebellum in memory and training mechanisms is an important problem of modern neurophysiology [1,3]. Clinical studies and animal experiments showed that damage to the median cerebellum is associated with reduction of cognitive capacities irrespective of motor dysfunctions, this being paralleled by disorders in the formation of conditioned defense behavior and task performance associated with orientation and exploratory activity [1,8]. Removal or dysfunction of the median cerebellum disorders the long-term extinction of acoustic startle reaction (ASR) [7,15], a congenital generalized motor reaction to a sudden intensive acoustic stimulus aimed at inhibition of current activity and evaluation of the situation via activation of the sensorimotor systems [5]. The involvement of the cerebellum in the formation of freezing behavior functionally similar to ASR was demonstrated [12]. However, studies of both types of behavior under conditions of experimental dysfunction of the cerebellum are rare and their data are contradictory [10].

We studied the effects of functional inactivation of the vermis, induced by application of tetrodotoxin (TT), on the formation of ASR extinction and freezing behavior in rats.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (250-300 g) kept 3 per cage at 24°C and 12:12 day:night schedule. Experiments were carried out at 11.00-15.00.

Studies of short-term and long-term ASR habituation with simultaneous recording of freezing behavior were carried out using a Plexiglas box (15×10×17 cm) mounted on a platform equipped with a tension transducer connected to an autorecorder and computer for recording of the ASR

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amplitude. Acoustic stimuli were presented using a Russian amplifier 100Y-101. The parameters of the acoustic signal (wide-band noise, 500 msec duration, 110 dB vol.) were regulated by PC. The duration of freezing (complete immobility of the animal, including vibrissa movements) was recorded visually. The rats were adapted to the experimental box for 5 min 24 h before training. During the training session the animals were placed into the box and the freezing reaction was recorded for 5 min, after which 10 acoustic stimuli were presented at 20-sec intervals. Long-term extinction of ASR and conditioned freezing were tested by the same protocol [11] 24 h after training. Applications of TT were performed under light ether narcosis: TT (1 ng in 3.0 μl physiological saline) was applied (with a microsyringe) onto the cerebellar cortex through a pre-drilled hollow in the skull 3 mm caudally from the lambdoid suture along the arbitrary line continuing the median suture. The involvement of the cerebellum in the mechanisms of memory formation, retrieval, and consolidation for the studied defense behavior forms was studied on 4 groups of experimental animals. Group 1 consisted of rats treated with TT 1 h before training (n=32), group 2 rats (n=20) received the drug 15 min after training, group 3 rats (n=19) received TT 1 h before testing, and group 4 rats (n=22) received TT 1 h before training and 1 h before testing.

The animals of corresponding control groups received saline (3 μl) according to the same protocol. The number of rats in the control group corresponded to the number of rats in the corresponding experimental group.

The results were processed using analyses of dispersions (ANOVA), correlations, and regression using Statistica software.

RESULTS

The time of freezing in the experimental box before acoustic stimulation on the day of training is a complex indicator of orientation exploratory and passive defense behavior in a new setting, while the amplitude of the startle response to the first acoustic signal characterizes the orientation exploratory and active defense behavior during exposure to an intensive extra-stimulus under novel conditions [10]. Comparison of these two parameters shows the ratio of passive to active defense components under different experimental conditions.

Factor and correlation analysis revealed no significant regularities between freezing time and the amplitude of first ASR: Spearman's coefficient of correlation was r=-0.12 (p=0.49). Functional inactivation of the median cerebellum with TT before training did not modify the initial ASR and freezing time (Table 1), but a negative correlation between the freezing time and amplitude of reaction to the first stimulus was detected in rats after TT application (r=-0.59, p=0.0017). Hence, functional inactivation of the cerebellum before acoustic stimulation shifted the balance between active and passive defense behavior.

The dynamics of ASR amplitude during presentation of a series of acoustic stimuli in the training session is a result of several processes: sensitization, desensitization, habituation, and disinhibition [5]. Analysis of the data included calculation of regression coefficient (b_1) for amplitude (S) as a function of the number of stimulus in the training session (N) for the equation $S=a-b_1 \times N$ for the entire group and individually for each animal. Significant positive regression coefficient indicated predominance of habituation, while significant negative coefficient indicated predominance of sensitization; in mixed reaction b_1 coefficient was not significant and was taken for zero [9].

In our experiments the animals treated with TT before training exhibited more marked decrease in the amplitude during the training session (b_1=0.39±0.17; p<0.05) compared to the corresponding controls ($b_{1=}$0.17±0.13; p>0.1). In further experiments, the rats of the control and experimental groups were divided into subgroups with consideration for individual b_1 values: with predominant extinction, sensitization, or mixed type of ASR dynamics. It was found that controls were virtually evenly distributed into these three subgroups (Table 1), while in the TT-treated group sensitization was recorded in just three animals, which was less significant (p<0.05) than in the control. Hence, inactivation of the cerebellum after TT application before training led to inhibition of short-term ASR sensitization.

When analyzing long-term extinction of ASR we evaluated preservation or the pattern of changes in the reaction in the period period between training and testing (whether this level was retained or changed), as well as the dynamics of ASR amplitude during testing in comparison with the corresponding parameter during training. To this end, the difference between the amplitudes of the first response on the day of testing and the last response on the day of training was calculated for each subgroup of control and experimental animals (index of spontaneous recovery of the reaction, Ir) and the coefficients of regression of the amplitudes b_1 and b_2 for training and testing days, respectively, were compared. In all subgroups of control animals, Ir did not differ from zero (Table 1), in other words,

TABLE 1. Effects of TT Applied onto the Vermis of Adult Rats on the Dynamics of ASR and Freezing Behavior ($M \pm m$)

Group	Amlitude of first ASR	Index of ASR recovery	Regression coefficient b_1		Freezing time	
			initial	after 24 h	initial	after 24 h
Control ($b_1>0$), n=10	11.5±1.7	2.51±1.80	0.62±0.14	0.58±0.14	51.6±6.1	70.5±8.3
Control ($b_1<0$), n=12	13.5±2.1	-1.51±0.90	-0.57±0.12	-0.04±0.08	18.5±4.3	32.7±6.1*
Control ($b_1=0$), n=10	11.8±2.4	-0.96±0.51	-0.04±0.13	-0.07±0.14	10.3±7.9	32.2±8.1*
TT before training ($b_1>0$), n=14	14.1±2.6	2.52±1.90	0.86±0.28	0.41±0.18	25.8±8.4	37.8±7.1
TT before training ($b_1<0$), n=3	12.5±1.9	-1.51±0.80	-0.93±0.31	0.34±0.44	26.5±7.3	27.5±6.2
TT before training ($b_1=0$), n=15	13.3±2.3	4.2±1.6$^+$	-0.05±0.24	0.52±0.12	26.7±6.9	39.1±9.4
TT after training ($b_1>0$), n=7	13.2±2.2	3.12±2.10	0.63±0.11	0.63±0.18	33.4±7.2	56.8±8.3*
TT after training ($b_1<0$), n=6	11.9±1.8	-1.01±0.98	-0.48±0.09	-0.19±0.10	13.3±4.7	33.4±6.0*
TT after training ($b_1=0$), n=7	12.7±2.6	-1.03±0.67	0.08±0.07	-0.09±0.06	19.3±5.0	39.6±6.6*
TT before testing ($b_1>0$), n=6	13.4±1.4	1.5±1.1	0.57±0.10	0.45±0.09	30.5±7.9	52.6±8.3*
TT before testing ($b_1<0$), n=6	10.8±1.5	-1.5±0.88	-0.51±0.13	-0.13±0.07	19.1±4.9	33.9±6.8*
TT before testing ($b_1=0$), n=7	12.6±1.9	-1.23±0.96	0.03±0.2	0.11±0.07	21.1±4.2	36.5±6.1*
Double TT application ($b_1>0$), n=10	13.9±2.5	2.6±2.1	0.71±0.19	0.47±0.13	49.3±7.5	69.4±6.4*
Double TT application ($b_1<0$), n=3	12.4±1.9	-2.1±1.4	-0.69±0.15	-0.09±0.06	25.8±4.2	41.6±3.8*
Double TT application ($b_1=0$), n=9	11.8±1.8	-0.58±0.46	-0.04±0.07	0.11±0.07	21.3±5.8	39.7±4.1*

Note. $p<0.05$ compared to *the initial level, $^+$zero.

the attained level of reaction did not change. In rats exposed to TT application, a spontaneous increase in ASR was observed in the subgroup with $b_1=0$ (Ir>0, $p<0.01$), which indicated that the level of reaction was not preserved, while b_2 value increased in comparison with b_1 ($p<0.05$), which attested to retained capacity to further training. No differences from the control were observed in the two other subgroups of experimental rats. Hence, inactivation of the median cerebellum before training led to suppression of short-term sensitization of ASR with subsequent spontaneous increase in the reaction amplitude.

We observed prolongation of the freezing time before acoustic stimulation on the testing day in animals treated with saline in comparison with the parameter on the day of training (Table 1), which indicated the formation of conditioned fear of the situation. No appreciable prolongation of the freezing time was observed in the experimental group, and hence, functional inactivation of the median cerebellum before training disordered the formation of conditioned situation fear. According to published data, simultaneous prolongation of the freezing time and ASR amplitude are observed in the presence of increased fear and anxiety, but commonly there is no relationship between these processes [5]. A correlation between the index of the reaction recovery and prolongation of the freezing time was detected in the rats treated with TT (r= -0.36, $p<0.05$), but was absent in control rats (r= -0.19, $p>0.1$). Hence, training under conditions of the cerebellum inactivation is associated with shifted balance between extinction of active defense and formation of passive defense behavior. The data on the effect of TT application on the vermis after extinction session and 1 h before the testing session indicate that suppression of electrical activity of the median cerebellum did not impair consolidation and retrieval of long-term memory of ASR extinction and freezing behavior (Table 1).

Suppression of ASR sensitization after TT application before training indicates changed emotional status of animals. Impaired retrieval of memory formed against the background of changed emotional status and tested under common conditions can be due to the phenomenon of dissociated training [4]. In this case memory is reproduced if training and testing are carried out under the same conditions. We carried out experiments with double TT application: 1 h before training and 1 h before testing. Under these conditions TT had no effect on long-term extinction of ASR and dynamics of freezing reaction (Table 1). These facts suggest that suppressed electrical activity of the cerebellum before training is really linked with dissociated training. The involvement of the cerebellum in the mechanisms of dissociated training under the effect of, for example, ethanol, was described [13]. However, more profound studies of the role

of the cerebellum in the mechanisms of consolidation of long-term memory of various forms of defense behavior using inhibitors of protein synthesis and substances modulating the metabolic processes in nerve tissue are needed. Our results indicate that suppression of electrical activity of the median cerebellar cortex leads to impairment of the formation of long-term memory for various types of defense behavior because of changed balance between habituation/sensitization processes; in addition, it is essential for the passive/active defense behavior ratio.

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