Role of Cerebellar Vermis in Memory Consolidation in Different Types of Defense Behavior

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Cycloheximide application on the cerebellar vermis 5 min after learning impairs consolidation of long-term extinction of the defense component of the acoustic startle response. Application 2 hours after training inhibits consolidation of extinction of orientation and exploratory components of the acoustic startle response and long-term fear memory in the experimental chamber before the start of acoustic stimulation. These findings indicate that the cerebellar vermis is selectively involved in long-term memory formation in certain types of defense behavior at different time after training.

Key Words: *cerebellum; memory; startle response; fear conditioning*

Involvement of the cerebellum in cognitive processes and emotions, apart from its role in motor functions, was demonstrated in clinical and laboratory studies [1,4,5]. However, the data on the role of cerebellum in long-term memory (LTM) consolidation and retention processes are contradictory [5,7,10]. Cerebellar vermis activity is crucial at the stage of formation of LTM in acoustic startle response (ASR) extinction, a congenital generalized active defense response of the organism to unexpected intensive stimulus [2,6].

The aim of the study was to investigate the role of the cerebellar vermis in LTM consolidation in defensive behavior. We used protein synthesis inhibitor cycloheximide (CHM) as a tool which impairs memory consolidation.

MATERIALS AND METHODS

The study was conducted on male Wistar rats weighing 250-300 g. The animals were kept 3 per cage at 24°C and 12:12 light cycle. Long-term habituation of ASR and freezing behavior were recorded in a plexiglas chamber 15×10×17 cm mounted on a platform equipped with a PC-connected strain gauge indicator, which was used for ASR amplitude detection. Acoustic stimuli (broadband noise, duration 500 msec, magnitude 11 dB) were presented using a loudspeaker and power amplifier controlled by PC. Duration of freezing (absolute immobility including whiskers movement) was recorded visually.

The rats were habituated to the experimental chamber for 5 min 24 h before training. In the course of extinction session, the animals were placed into the chamber and their freezing behavior was recorded for 5 min, after which 10 acoustic stimuli were presented with 20-sec intervals. Duration of freezing was also recorded during acoustic stimulation. After 24 h long-term extinction of ASR and freezing behavior were tested using the same scheme. CHM (50 μg in 3.0 μl saline) was applied on the cerebellar vermis with a microsyringe through a cannula preliminary implanted according to the following stereotaxic coordinates: L=0, AP=-11.8, H=-3. The animals from the control groups received 3 μl physiological saline.

We studied the effect of CHM applied on the cerebellar vermis at different time intervals after training on LTM consolidation of ASR extinction and conditioned freezing behavior playing a role similar to ASR (inhibition of current activity and activation of sensorimotor systems) [10].

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Analysis of long-term extinction consisted in estimation of spontaneous response recovery index: the difference between amplitudes of the last response during training and the first response during testing. Significant negative value of the index is indicative either of impairment of long-term habituation or of long-term sensitization. Intergroup variations of the dynamics of ASR was estimated by the value of regression coefficients of response amplitude (*Sn*) on stimulus number (*N*): *Sn*=A-bx*N* upon training (b1) and testing (b2), as well as by using analysis of variation with repeated measures (repeated measures ANOVA). The results were processed using ANOVA analysis of variation, correlation and regression analyses.

RESULTS

CHM application on the cerebellar vermis 5 min after training did not affect spontaneous ASR recovery (Fig. 1; Table 1). However, it significantly decreased regression coefficient b2 compared to the control value. Analysis of variation revealed significant difference between the control and experimental groups in the dynamics of ASR amplitude in the second half of the testing procedure, between stimuli 6 and 10 ($F(1, 4, 140)=4.067$, $p=0.0038$). No difference between the groups was observed in the beginning of testing procedure ($F(1, 4, 140)=0.71528$, $p=0.58$; Fig. 1). Neither before, nor in the course of acoustic stimulation, had CHM influenced the conditioned freezing memory consolidation (Table 1).

Significant spontaneous recovery of the response was observed 2 h after CHM administration, but no significant differences between the groups in the dynamics of the response in the second half of testing procedure ($F(4, 140)=0.83$, $p=0.69$) and coefficient b2 were revealed (Table 1). Experimental rats demonstrated impaired conditioned fear memory consolidation:

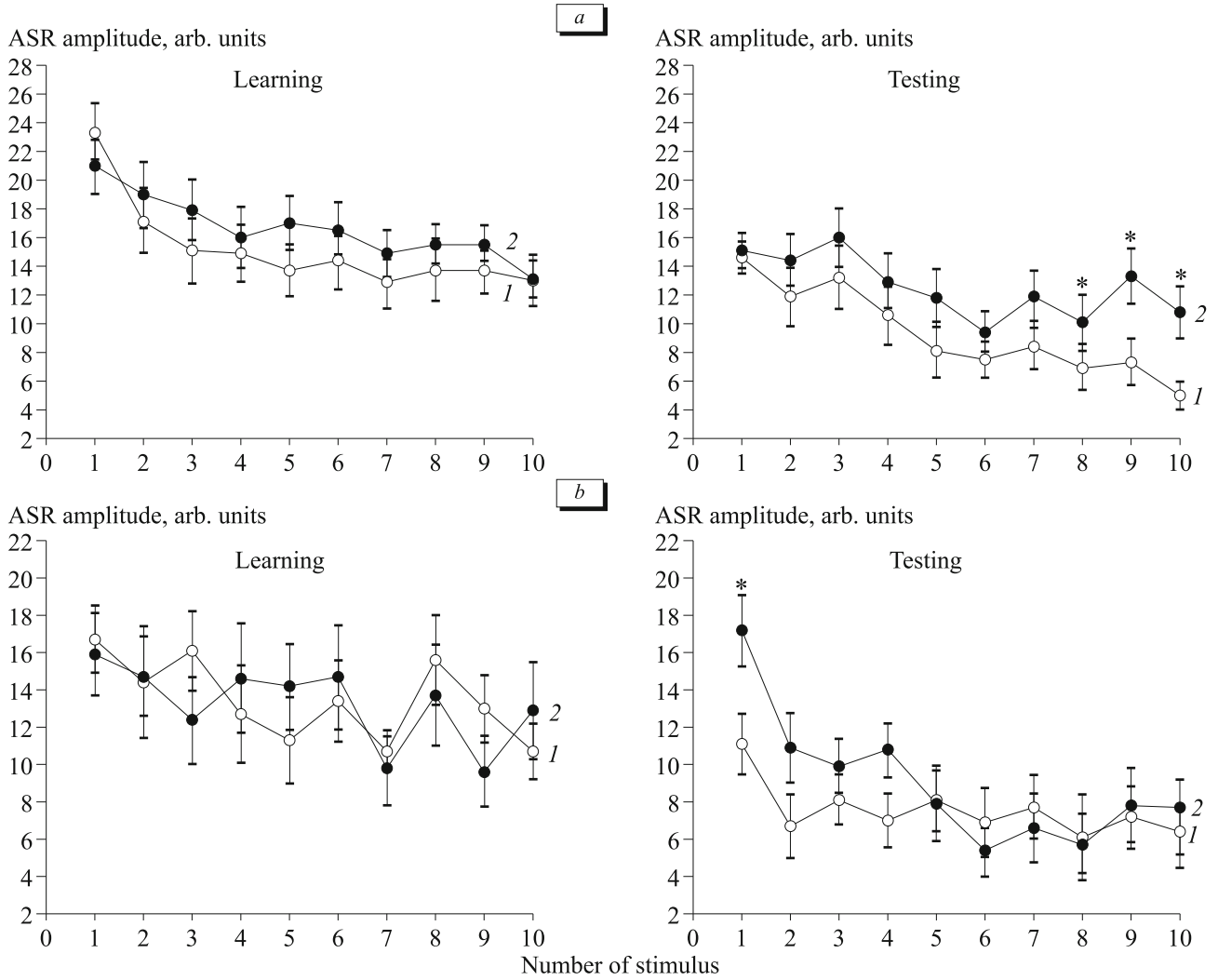

Fig. 1. Effect of CHM application on the cerebellar vermis on long-term extinction of ASR. *a*) 5 min after training; *b*) 2 h after training. *1*) control; *2*) CHM. *$p<0.05$ compared to the control.

duration of absolute immobility before the beginning of acoustic stimulation on the day of testing in experimental group was significantly shorter, than in the control, but did not differ from the same index on the day of training (Table 1). We revealed significant changes in freezing time against the background of acoustic stimulation on the day of testing as compared to the day of training in both control and experimental rats (Table 1).

According to published data, orientation and exploratory components prevail in ASR response to the first two testing stimuli, while defense component predominated in ASR response to testing stimuli 3-5 and declines by the end of the testing procedure [3,9]. Thus, CHM application to the cerebellar vermis 5 min after training inhibits LTM consolidation of extinction of ASR defense component, and CHM application 2 h after training inhibits consolidation of habituation of exploratory ASR component and LTM of freezing behavior.

In rats receiving CHM 2 h after training, a negative correlation between the index of response recovery and changes in the freezing time on the day of testing compared to the day of training was observed (Spearman correlation coefficient r=-0.53, p<0.05); this correlation was not observed in controls. It was previously shown that administration of tetrodotoxin into the cerebellar cortex before training, apart from impairment of long-term extinction of ASR exploratory component and freezing behavior, led to the appearance of a positive correlation between spontaneous recovery index and freezing time prolongation [2]. It can be hypothesized that the cerebellum is involved in the maintenance of optimum relationship between extinction of active defense behavior and formation of passive defense behavior at different stages of memory trace formation and participates in the realization of the modulating influence of sensory input from environment on extinction of exploratory ASR component.

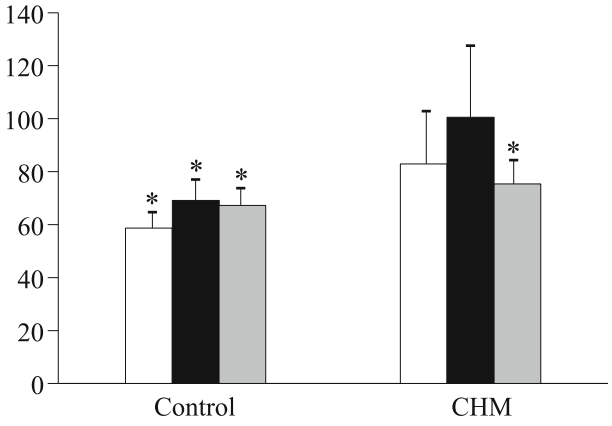

Fig. 2. Effect of CHM application on the cerebellar vermis 5 min after training on processes of long-term habituation and long-term sensitization of ASR. Ordinate: ASR amplitude during testing, % of corresponding values during learning. Light bars: mean ASR amplitude for session; dark bars: mean value of amplitudes followed by an increase in response; grey bars: mean values of ASR amplitudes followed by a decrease in response. *p<0.05 compared to initial level.

A mechanism explaining the role of cerebellum in consolidation of long-term extinction of ASR defense component can be hypothesized. Analysis of ASR dynamics (Fig. 1) during training and testing reveals nonuniform changes in response amplitude, *i.e.* its periodic fluctuations. The difference between amplitudes S (ΔSn) of the response to stimuli n and n+1 positively correlates with response amplitudes Sn (r=0.44, p<0.05); while the mean ASR amplitudes (S-) followed by negative ΔS providing sensitization of the response is lower than the mean ASR amplitudes (S+) followed by positive ΔS providing habituation (10.9±0.97 and 19.4±0.89 respectively). In control rats, the mean amplitude S, S+, S- decreased on the

TABLE 1. Effect of CHM Application on Cerebellar Vermis at Different Terms after Training on ASR Extinction and Freezing Behavior (M±m)

Time between training and administration	Group	n	ASR recovery index	Regression coefficient b		Duration of freezing before acoustic stimulation, sec		Duration of freezing against the background of acoustic stimulation, sec	
				initial (b1)	24 h later (b2)	initial	24 h later	initial	24 h later
5 min	Control	18	-1.55±1.3	0.55±0.23	1.03±0.36	58.6±8.2	94.4±7.0	107.3±8.9	110.1±10.0
	CHM	18	-1.96±1.1	0.67±0.33	0.07±0.9*	62.4±6.4	100.4±10.1	108.5±9.6	116.2±11.3
2 h	Control	19	-0.5±2.4	0.62±0.34	1.02±0.44	54.7±8.7	109.9±13.5	112.7±9.0	118.4±12.8
	CHM	19	-5.3±2.1*+	0.61±0.39	1.35±0.59	64.9±8.6	62.6±15.4*	106.2±9.9	112.0±13.1

Note. p<0.05 compared to: *control, +zero level.

day of testing compared to the day of training. In rats receiving CHM application 5 min after training, *S*- did not decrease during testing compared to training, while the dynamics of *S*+ changes did not differ from the control (Fig. 2). Thus, CHM application on the cerebellar vermis 5 min after training impairs plastic changes responsible for adaptive control of ASR sensitization in motor systems.

There are data on the involvement of the cerebellum into optimization of oscillatory eye movements in gaze holding [8,11] with a 20-40-sec period, which are maintained by activity of inner model, kept in memory in the form of circulatory excitatory inputs in the medulla oblongata and cerebellum. Cerebellar activity is also linked to optimization of oscillatory eye movements upon adaptation of vestibular-oculomotor reflex, which is controlled by information from the motor apparatus [5]. The cerebellum is involved in learning and memory mechanisms under condition of tight interaction with other structures. Memory trace properties can change in the course of memory consolidation and it can be transferred to other brain regions [5,7,10]. Obtained results indicate that the cerebellar vermis is selectively involved in consolidation of different types of defense behavior at different time points after training. It could be hypothesized that the revealed selectivity is caused by specific activation of separate afferent inputs and changes of activity profile inside structural and functional units of the cerebellar cortex (modules) [5,12].

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