Neuroscience Letters 472 (2010) 148–152

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Effects of ipsilateral cerebellum ablation on acquisition and retention of classically conditioned eyeblink responses in rats

Takahiro Horiuchi [a], Shigenori Kawahara [b,*]

[a] Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[b] Graduate School of Science and Engineering, University of Toyama, 3190 Gofuku, Toyama 930-8555, Japan

ARTICLE INFO

Article history:
Received 11 September 2009
Received in revised form 28 January 2010
Accepted 28 January 2010

Keywords:
Eyeblink conditioning
Cerebellum
Learning
Memory

ABSTRACT

The ipsilateral cerebellum to the trained eye has been reported to be essential for acquisition and retention of the conditioned response (CR) in rabbit eyeblink conditioning. Although pharmacological studies have suggested its important roles in other species too, to what degree does eyeblink conditioning in rats depend on the ipsilateral cerebellum is not clear. In this work, we ablated the ipsilateral cerebellum in rats before or after conditioning to examine its roles in acquisition and retention of the CR. In the first experiment, rats received ablation of the ipsilateral cerebellum and recovered for more than 3 weeks. They then underwent eyeblink conditioning for 7 days with a tone and a periorbital electrical shock. Consistent with other previous reports, hemicerebellectomized rats showed significant impairment compared to sham-lesioned rats. However, the hemicerebellectomized rats acquired CRs to some degree, and the acquired CR showed adaptive timing. In the second experiment, rats received the hemicerebellectomy after acquiring CR by 7 days of conditioning in a delay paradigm. After more than 3 weeks of recovery, they were again conditioned in a delay paradigm. Rats with ipsilateral cerebellar lesions showed severe impairment in retention of the pre-acquired CR; however, they reacquired CR to some degree during the subsequent reconditioning sessions. These results suggest that the ipsilateral cerebellum plays an important role in rat eyeblink conditioning as well but that other brain regions can partially compensate for its removal.

Classical eyeblink conditioning is one of the most extensively studied models for associative learning [4,5,18,20]. In this task, a corneal air puff or periorbital shock (unconditioned stimulus, US) is delivered to the eye to elicit a reflexive blink (unconditioned response). When a preceding neutral conditioned stimulus (CS) such as a tone is repeatedly paired with a US, animals blink in response to a CS. This is known as a conditioned response (CR), and it occurs in a carefully timed manner before a US. Cumulative evidence has clearly shown that the cerebellum and brain stem play a critical role in acquisition and retention of the CR in delay eyeblink conditioning in rabbits. On the basis of the results from lesioning [13] or reversible inactivation [2] of the deep cerebellar nuclei, the ipsilateral cerebellum to the trained eye was concluded to be essential for CR acquisition, retention, and expression. Similarly, CR acquisition and retention in rats were impaired after permanent lesions of the bilateral cerebellar nucleus [11,17] or reversible inactivation of the ipsilateral cerebellum [7]. In addition, infant rats that received unilateral lesions of the ipsilateral cerebellum on postnatal day 10 or 20 were unable to acquire CR during 3-day conditioning [6]. Although one of the previous studies reported personal observations that large unilateral cerebellar nuclei lesions did not diminish pre-acquired CR in some rats [17], other studies suggested that the ipsilateral cerebellum might be essential for CR acquisition and retention in rats in the same way it is in rabbits [6,7].

In the present study, we investigated the effects of ipsilateral cerebellum ablation on acquisition and retention of CR in delay eyeblink conditioning in adult rats. Sixty-one 10-week-old male Wistar rats (Japan SLC, Inc., Hamamatsu, Shizuoka, Japan) were used. The animals were housed in standard plastic cages with free access to food and water in a colony room with a 12-h light/dark cycle. All of the experimental procedures were performed during the light period of the cycle in accordance with the guidelines established by the institutional animal investigation committee at the University of Tokyo and the NIH Guide for the Care and Use of Laboratory Animals. Efforts were made to optimize the comfort as well as minimize the use of the animals.

We performed 2 types of lesion experiments: pre-conditioning lesions for testing learning ability, and post-conditioning lesions for testing memory retention. In the pre-conditioning lesion experiment, rats were randomly assigned to receive either ipsilateral cerebellar lesion (CBL group) or sham surgery (sham lesion group). Rats were anesthetized with sodium pentobarbital (65 mg/kg i.p.,

* Corresponding author. Tel.: +81 76 445 6871; fax: +81 76 445 6697.
E-mail address: kawahara@eng.u-toyama.ac.jp (S. Kawahara).

0304-3940/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2010.01.076

Kyoritsu Seiyaku, Tokyo, Japan). Isofluran (1–2%, Abbot Japan, Osaka, Japan) was also used when necessary. After removal of the overlying skull and dura, the ipsilateral cerebellum was ablated by aspiration to the level of the deep cerebellar nuclei. The resulting cavity was then filled with sterile gel foam (Pfizer Japan Inc., Tokyo, Japan) and the wound edges were sutured. The animals were then injected with ampicillin (100 mg/kg i.p., Meiji Seika, Tokyo, Japan) and warmed until they moved spontaneously. The sham lesion group received the craniotomy but not the cerebellar ablation. After 3 weeks of recovery, 4 Teflon-coated stainless-steel wires (#7910; A-M Systems, Carlsborg, WA, USA) were subcutaneously implanted in the left upper eyelid under anesthesia with sodium pentobarbital (65 mg/kg). These wires were soldered to the pins of a connector that was secured to the skull with dental acrylic resin and stainless-steel screws. Two to 4 days after surgery, spontaneous eyeblink frequency was measured for 2 days and then the rats were conditioned for 7 days. A daily session consisted of 100 trials grouped into 10 blocks, each of which included 9 CS–US paired trials followed by 1 CS-alone trial. Trials were separated by a variable inter-trial interval pseudo-randomized between 20 and 40 s with a mean of 30 s. The CS was a 350-ms tone (5 kHz, 85–90 dB) with a rise/fall time of 10 ms. US consisted of a 100-ms periorbital shock (1.5 mA, 100 Hz square pulses) that was delivered through a pair of electrodes implanted in the left upper eyelid. CR was monitored through electromyographic (EMG) activity recorded at a sampling rate of 10 kHz with another pair of implanted electrodes. Rats in the CBL and sham lesion groups received either paired conditioning or pseudo-conditioning. In paired conditioning (CBL: $n = 11$; sham: $n = 7$), the CS preceded and co-terminated with the US. In pseudo-conditioning (CBL: $n = 7$, sham: $n = 9$), a pseudo-randomized stimulus-free time (between 6 and 20 s) was interposed between the CS and the US.

In the post-conditioning lesion experiment, rats first received the surgery for implanting the wire electrodes as described above. After 2–4 days, spontaneous eyeblinks were recorded and then the rats were conditioned for 7 days in the paired conditioning paradigm. The rats whose mean percentage of CR-containing trials (CR%) during the last 2 days of acquisition sessions exceeded 70% were divided into 2 groups and received either sham surgery (sham, $n = 12$) or ipsilateral cerebellum ablation (CBL, $n = 15$) on the next day of the last acquisition session. At least 3 weeks after the surgery, the rats were conditioned again for 10 days to test their memory retention and reacquisition.

The EMG data were analyzed as described previously [19]. Briefly, mean + SD of the amplitudes of the EMG signal for 300 ms before the CS in 100 trials was defined as the threshold and was used in the following analysis. In each trial, average EMG amplitudes exceeding the threshold were calculated for 300 ms before CS onset (pre-value) and for 100 ms before US onset (CR value). If the pre-value was less than 10% of the threshold, the trial was regarded as a valid trial. Among the valid trials, a trial was assumed to contain CR if the CR value exceeded 10 times the pre-value. In CS-alone trials, the period for CR value calculation was extended by 100 ms to the end of the expected US. The percentage of CR-containing trials in the valid trials (CR%) was expressed as mean ± SEM. In the post-conditioning lesion experiment, we also calculated the retention ratio by dividing the CR% in the first reconditioning session by that in the last acquisition session. To show the temporal CR pattern, the EMG amplitude data of each rat were averaged over the valid trials for each day. These trial-averaged EMG amplitude data were normalized by the time-averaged values for 300 ms before the CS onset. CR onset latency was defined as the latency from the CS onset to the time when the EMG amplitude exceeded 10 times the pre-value for the first time. CR peak latency was defined as latency from the CS onset to the time of maximum EMG amplitude between

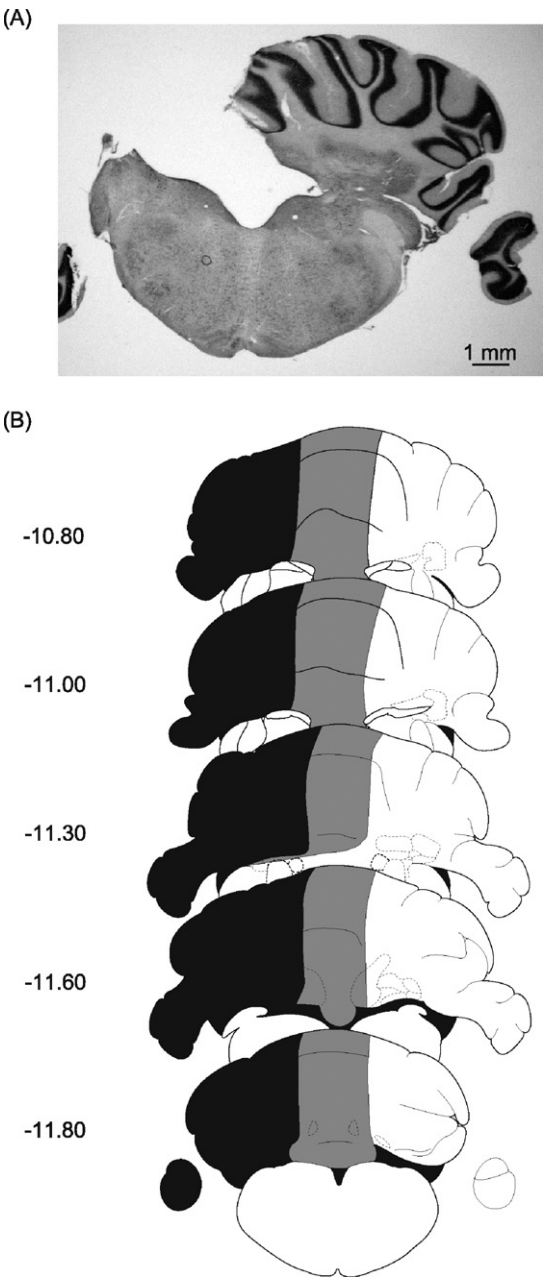

Fig. 1. Histological evaluation of ipsilateral cerebellum ablation. (A) Typical coronal sections stained with cresyl violet through the cerebellum and brain stem in the CBL group. (B) Line drawings of the coronal sections showing the extent of the cerebellar lesions in the CBL group. The black and gray areas indicate the smallest and largest lesions, respectively. Numbers to the left indicate stereotaxic coordinates relative to bregma [15].

50 ms after CS and US onset. These latencies were calculated in all trials that were judged to contain CR.

After all of the behavioral experiments were completed, the rats were intraperitoneally injected with an excess amount of sodium pentobarbital (130 mg/kg) and perfused intracardially with 0.9% saline followed by phosphate-buffered 10% formalin. The brain was removed from the skull and stored in 10% formalin for a few days. After infiltration with 30% sucrose, the brain was frozen, sectioned at 60 μm, and stained with cresyl violet. The largest and smallest lesioned areas were reconstructed from the sections according to the stereotaxic atlas of the rat brain [15].

Statistical significance was determined by a one-way or two-way analysis of variance (ANOVA) with repeated measures or by a