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Research report

Cooling of the cerebellar interpositus nucleus abolishes somatosensory cortical learning-related activity in eyeblink conditioned rabbits

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Abstract

Nictitating membrane movement and multiple-unit activity in the somatosensory cortex were recorded from rabbits during paired ($N=6$) and unpaired ($N=5$) presentations of a tone conditioned stimulus (CS) and an airpuff unconditioned stimulus (US). A behavioural conditioned response (CR) to the CS and an accompanying neural response in the somatosensory cortex developed only in the paired group. Inactivation of the cerebellar interpositus nucleus abolished both the acquired CR and the accompanying neural response. However, the CS facilitated both behavioural and neural responses to the US during the inactivation. Thus, the absence of the CR could not be accounted for by the general inability of the CS to alter the behaviour constituting the CR or the activity of the somatosensory cortex. These findings suggest that the efferent copy of the signal related to the eyeblink CR is projected from the cerebellum to the cerebral cortical areas of the US modality.

Keywords: Eyeblink conditioning; Multiple-unit activity; Rabbit; Somatosensory cortex; Interpositus nucleus; Reversible inactivation

1. Introduction

Although the cerebrum is not necessary for the acquisition or maintenance of a classically conditioned eyeblink response (CR; e.g., Ref. [7]), plasticity in cortical sensory projection areas correlates with the emergence of the CR. Plasticity in the areas that are primarily involved in the processing of the unconditioned stimulus (US), as reflected by altered neural responses of those areas to a conditioned stimulus (CS) of a non-US modality [11,13,17], is of particular interest because two alternative accounts for such plasticity can be postulated. First, such plasticity may reflect either an association between the CS and the US that develops regardless of whether it drives a behavioural CR or not, as it does in sensory preconditioning (e.g., Ref. [12]). Second, such plasticity may relate specifically to the execution of the cerebellar memory trace for learned behaviour as has been found to be the case in various cerebral parts of the brain, including hippocampus [3] and ventrolateral thalamus [14] in addition to the interpositus nucleus (IPN) of the cerebellum [10], which is the putative centre of this trace [15].

Our previous study [17] favoured the latter alternative. In that study, a neural correlate of the eyeblink CR in the rabbit somatosensory cortex (SCx) was found to disappear on trials when animals spontaneously failed to produce the CR even though the CS continued to facilitate the unconditioned eyeblink response. This finding led us to further suggest that such spontaneous failures may reflect failures in activating the procedural memory trace, which has been postulated to be stored in the cerebellum [2,15] in this particular task.

In the present study, we aimed to replicate our previous finding but to control for the contribution of the cerebellar memory trace for the conditioned eyeblink response by inactivating the IPN in well-trained rabbits.

2. Method

2.1. Subjects

The subjects were 11 female adult New Zealand albino rabbits weighing 2.8–3.7 kg at the time of surgery. The animals were individually housed in metal cages on a 12-h light:12-h dark cycle with free access to food and water. All the

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experimental procedures were performed during the light portion of the cycle. Experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) regarding the care and use of animals for experimental procedures.

2.2. Surgery

The animals were anesthetized with an i.m. injection of ketamine–xylazine cocktail (Ketaminol, 50 mg/ml, 5.6 ml; Rompun, 20 mg/ml, 2.2 ml; physiological saline, 2.2 ml). The initial dosage was 1 ml/kg and the anesthesia was maintained by additional injections of 1 ml every 15–25 min. The anesthetized animals were placed in a stereotaxic instrument (Kopf Instruments) with the bregma landmark being placed 1.5 mm above the lambda landmark. A longitudinal incision was made to reveal the skull onto which the head stage designed to hold the minitorque potentiometer was cemented with dental acrylic using four stainless steel anchoring screws.

The recording electrode was implanted in the somatosensory projection area of the cerebral cortex (2.0 mm posterior and 7.0 mm lateral to the bregma; see Ref. [5]). Electroencephalography and multiple-unit activity (MUA) were monitored during the implantation procedure, and the electrode was lowered until typical cortical activity was observed. The implantation procedure has been described in more detail in an earlier paper [8].

The cooling probe was implanted using the co-ordinates 0.5 mm anterior, 6.5 mm lateral and 14.5 mm ventral to lambda following the same guidelines [8]. The construction of the cooling probe was modified after that presented in Ref. [18]. Briefly, the cooling probe consists of two stainless steel tubes one inside the other. The inner tube delivers the coolant approximately 1 mm from the tip of the probe, which is sealed by solder. The coolant exits through a plastic tube attached to the outer cannula at a Y-shaped junction. The coolant used was freon-like 1,1,1,2-tetrafluoroethane (KLEA R-134-A). The shaft of the cooling probe was not warmed, making it possible that cooling took place to some extent in the overlying cerebellar cortex.

At the end of the surgery, a nylon loop was sutured into, but not through, the nictitating membrane (NM) of the right eye. Analgesics (Temgesic, 0.3 mg/ml) were provided in the end of the surgery and additionally 8 h later if needed. The animals were given at least 1 week to recover after surgery before the actual experimental procedures.

2.3. Procedure and stimuli

During the experiments, the loop sutured to the NM was linked by a rigid stainless steel hook to the swivel arm of the minitorque potentiometer to measure the movement of the NM. The extension of the NM was transduced to voltage by the potentiometer (1 mm equalled 1 V). On the first day, the animals were adapted to the experimental situation by placing them in a Plexiglas restraining box [4] located in a soundproof conditioning chamber.

The CS was a tone (1000 Hz, 85 dB, 350 ms). The US was an airpuff (2.1 N/cm^2 source pressure, 100 ms) directed towards the cornea. Seven unpaired (UP) sessions consisted of pseudo-random presentations of the CS and the US so that they never co-occurred (explicitly unpaired treatment). The inter-trial interval (ITI) varied randomly between 15 and 25 s (mean ITI = 20 s). The UP group consisted of five animals. Each of seven classical conditioning (CC) sessions consisted of 80 trials (60 paired trials where the CS was followed by the US, 10 CS-alone and 10 US-alone test trials in a pseudo-random order). The stimuli in the paired trials were presented in a delayed fashion so that the onset of the CS was 250 ms earlier than the onset of the US, their offsets co-terminating. The ITI varied randomly between 30 and 50 s (mean ITI = 40 s). The cooling probe was activated in the sixth CC session. The CC group consisted of six animals.

The NM responses were averaged over subject, session and trial type. The CR and UR were measured as the maximum extension of the NM during a period of 100 ms immediately before or after the onset of the US, respectively. Any NM movement exceeding 0.5 mm during the CS period was counted as a CR. The criterion of learning was fulfilled when a robust CR was found in at least eight out of nine consecutive paired or CS-alone trials. Trials in which the NM movement exceeded 0.5 mm during the 100 ms prior to the administration of the CS were excluded from the analysis. During cooling, NM movements exceeding 0.5 mm were observed during the CS period in a few (1.1%) trials. Although these responses most likely were spontaneous NM movements, these trials were excluded from the analyses because it was possible that the temperature in the IPN had temporarily increased, resulting in an increase in blood circulation in this nucleus and, consequently, partial release in its activity.

2.4. Data-analysis

MUA was band-pass filtered (500–6000 Hz) and digitized on-line at the rate of 15,000 samples/s. Frequencies of spikes were calculated off-line using a custom-programmed DTVee for Windows program. After setting a spike frequency threshold (approximately 15 spikes/s), the spike frequency exceeding this threshold was counted per 10 ms bin. Subsequently, averages for three 100 ms periods per trial were computed by subtracting the mean of the 10 bins immediately before the trial from the mean of 10 bins from the period of interest. Given that learning-related neural responses elicited by a CS typically emerge close in time to the onset of the US, the average of spike frequency was computed for a 100-ms period preceding the onset of the US. Responses to the US in turn were measured as the average spike frequency for a 100-ms period following the onset of the US. In the analyses, analyses of variance (ANOVA) for repeated measures and *t*-tests for paired samples were used. In the ANOVAs, Greenhouse–Geisser-adjusted degrees of freedom for the averaged tests of significance were used whenever the sphericity assumption was violated.

2.5. Histological procedures

After the experiments, the animals were anesthetized with an i.m. injection of ketamine–xylazine cocktail and subsequently overdosed by an i.v. injection of pentobarbital. They were then perfused via the ascending aorta with saline followed by 10% formalin. Their brains were removed and fixed in 10% formalin solution for at least 1 week. The locations of the electrodes in the somatosensory cortex were verified after removal of the skull by visual inspection of the length of their penetration through the dura and of their visually detectable markings left to the surface of the cortex. Frozen coronal sections of 100 μm were taken from the site of the cooling probe. High-resolution digital images were taken for each section with a camera attached solidly above the cryotome. For verification of the probe tips, these images were displayed on a 20 in. colour computer screen and compared to the atlas of the rabbit brain [9].

3. Results

3.1. Histological results

Histological results are presented in Fig. 1. The tips of the recording electrodes were found to penetrate the primary projection area of the somatosensory cortex in all animals [5]. The tips of the cooling probes were found to be located lateral to the anterior IPN within the distance of 1.5 mm in all animals.

3.2. Effects of training

Fig. 2 depicts the mean NM response amplitudes and spike frequencies as a function of session. In the CC group, the learning criterion was exceeded by the fourth session at the latest in all animals. Both the behavioural and neural responses to the CS significantly increased in amplitude from the first to the fifth session [$F(4,20) = 6.27$, $P < 0.01$; $F(4,20) = 7.68$, $P < 0.01$, respectively] in the CC group. Such an increase could not be found in the UP group, showing that the mere exposure to the CS and the US was not sufficient for its emergence in the CC group. As illustrated in Fig. 3, the neural responses preceded the behavioural responses by about 30–40 ms within a trial.