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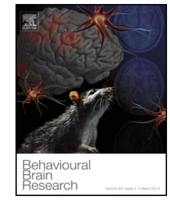

Research report

Transfer of classical eyeblink conditioning with electrical stimulation of mPFC or tone as conditioned stimulus in guinea pigs

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HIGHLIGHTS

- Transfer of eyeblink conditioning (EBC) occured between mPFC-CS and tone CS.
- Transfer of EBC for delay paradigm is much more effective than for trace paradigm.
- The effectiveness of mPFC-CS to establish EBC is higher than that of tone CS.
- The experience of learning to a new CS does not affect recall of the original CR to the early CS.

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ABSTRACT

Learning with a stimulus from one sensory modality can facilitate subsequent learning with a new stimulus from a different sensory modality. To date, the characteristics and mechanism of this phenomenon named transfer effect still remain ambiguous. Our previous work showed that electrical stimulation of medial prefrontal cortex (mPFC) as a conditioned stimulus (CS) could successfully establish classical eyeblink conditioning (EBC). The present study aimed to (1) observe whether transfer of EBC learning would occur when CSs shift between central (mPFC electrical stimulation as a CS, mPFC-CS) and peripheral (tone as a CS, tone CS); (2) compare the difference in transfer effect between the two paradigms, delay EBC (DEBC) and trace EBC (TEBC). A total of 8 groups of guinea pigs were tested in the study, including 4 experimental groups and 4 control groups. Firstly, the experimental groups accepted central (or peripheral) CS paired with corneal airpuff unconditioned stimulus (US); then, CS shifted to the peripheral (or central) and paired with US. The control groups accepted corresponding central (or peripheral) CS and pseudo-paired with US, and then shifted CS from central (or peripheral) to peripheral (or central) and paired with US. The results showed that the acquisition rates of EBC were higher in experimental groups than in control groups after CS switching from central to peripheral or vice versa, and the CR acquisition rate was remarkably higher in DEBC than in TEBC in both transfer ways. The results indicate that EBC transfer can occur between learning established with mPFC-CS and tone CS. Memory of CS–US association for delay paradigm was less disturbed by the sudden switch of CS than for trace paradigm. This study provides new insight into neural mechanisms underlying conditioned reflex as well as the role of mPFC.

Abbreviations: CR, conditioned response; CS, conditioned stimulus; UR, unconditioned response; US, unconditioned stimulus; DEBC, delay eyeblink conditioning; TEBC, trace eyeblink conditioning; mPFC, medial prefrontal cortex; SEM, standard error of the mean; IPN, cerebellar interpositus nucleus; PN, pontine nuclei.

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1. Introduction

Cross-modal transfer means that learning from one sensory modality facilitates subsequent learning with a different sensory modality [1]. The testing of cross-modal learning of conditioned reflex requires two successive processes: the initial training with a particular conditioned stimulus (CS, e.g., auditory or visual) named CS1 and, the subsequent training with a new CS of different sensory modality (CS2). Cross-modal transfer occurs when the acquired conditioned response to CS2 develops at an accelerated rate compared to the initial CS1 [2–4]. It is believed that this phenomenon results from the general transfer of the association between CS and unconditioned stimulus (US) rather than the stimulus generalization effect [5–7]. To date, characteristics and mechanism of cross-modal transfer still remain ambiguous. Recently, classical eyeblink conditioning (EBC) is often selected as an effective model in the study of cross-modal transfer [1,8–10].

Classical EBC is the simplest behavioral model widely used in the study of learning and memory [11–15]. It involves paired presentations of a behaviorally neutral CS (e.g., a tone or light) and an aversive US (e.g., a corneal airpuff or periorbital shock). According to the temporal relation between CS and US, EBC includes two basic paradigms: delay eyeblink conditioning (DEBC) and trace eyeblink conditioning (TEBC). In delay paradigm, the CS precedes, overlaps, and coterminates with the US. While in trace paradigm, a temporal gap exists between the offset of the CS and the onset of the US. Stimulation of peripheral sensors (e.g., auditory or visual stimulus) was often selected as the traditional CS for EBC establishment. Previous research about cross-modal transfer has been focused on cross-modal learning induced by different peripheral CSs. For example, transfer of cross-modal learning elicited by visual and auditory stimulus [6,16–18] or by tactile and visual stimulus [19–22], and so on. Recently, concerns have been raised about learning transfer across central and peripheral CSs in EBC, which can be considered as a special kind of cross modal transfer [23]. Leal-Campanario et al. [23] used electrical stimulation of the primary somatosensory cortex (S1 area, for vibrissae or hind limb) or peripheral as CS and paired with corneal airpuff as US to establish Pavlovian conditioning. After initial establishment of the conditioned eyeblink reflex, CSs switched immediately from central to peripheral (whisker pad) or vice versa. Their research indicated that the acquisition rate of evoked CRs to CS2 was significantly less disturbed if the applied sites for both CS1 and CS2 were located at the corresponding loci within somatosensory pathway (e.g., S1 areas for vibrissae vs. whisker pad), compared to CSs presenting to non-corresponding sites (e.g., S1 areas for limb vs. whisker pad). This two-way reciprocal switch does not affect the retrieval of the original CS–US associative memory. They believed that the occurrence of learning transfer was indicative of the presence of multiple distributed forms of associative learning, rather than restricting to small sets of cortical areas [23,24].

Although previous studies have already demonstrated the occurrence of learning transfer, a special kind of cross modal transfer, when CS switched across exerting stimulation to the primary sensory cortex and to the peripheral sensors, little is known about the transfer effect when CS shifts from stimulation of the associative cortex (e.g., mPFC) to the peripheral, or vice versa, and the difference between DEBC and TEBC in learning transfer. PFC is evolutionally the most advanced brain cortex participating in many critical cognitive processes, for instance, selective attention, motivation, working memory, plan making and behavior regulation [25–29], with vague related neural mechanism. We have already demonstrated that electrical stimulation of mPFC as a CS could successfully establish classical EBC [30]. The present study aimed to (1) clarify whether EBC transfer would occur when CSs shift across central (mPFC electric stimulation as a CS, mPFC-CS) and peripheral (tone as a CS, i.e., tone CS); (2) compare the differences in transfer effect between the two paradigms, DEBC and TEBC. Exploring the existence and the characteristics of the specific "cross-modal" transfer will contribute to providing insight into underlying neural mechanisms of conditioned reflex as well as the roles of mPFC.

2. Methods

2.1. Subjects

48 adult male albino Dunkin–Hartley guinea pigs (500–600 g), 4–5 months old were used in the experiment. The guinea pigs were individually housed in the standard plastic cages on a 12:12 light/dark cycle with free access to food and water. The room temperature was kept at 25 ± 1 °C. The procedures were approved by the Animal Care Committee of the Third Military Medical University.

2.2. Surgery

Approximately 1 week before training, guinea pigs were removed from their home cage and anesthetized with a mixture of ketamine (80 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). The anesthetized animal's head was secured in a stereotaxic apparatus. According to an atlas of the guinea pig brain [31], a longitudinal incision was then made to reveal the skull, onto which a Plexiglas headstage (1.0 cm × 1.0 cm × 0.5 cm), designed to secure the animal's head, was cemented with dental cement and four stainless steel anchoring screws. One small hole (diameter: 1.0 mm) was drilled on the right side of the skull centered on the right caudal mPFC at the following stereotaxic coordinates: anteroposterior (AP) +13.0 mm, mediolateral (ML) 1.0 mm relative to the frontal zero plane, and the midline sinus, respectively. Then, a bipolar stimulating electrode (No. 792500, A-M Systems, Sequim, WA, USA; coated diameter: 332.00 μm, bare diameter: 254.00 μm) was implanted into the right caudal mPFC through the hole and the electrode's tip was directed to the following stereotaxic coordinates: AP +13.0 mm, ML 1.0 mm, dorsoventral (DV) −2.5 mm to the skull surface (Fig. 1A and B). The stimulating electrode and guiding cannula were fixed to the skull with dental cement. Finally, a small nylon loop was sutured into but not through the edge of the upper left eyelid. In the present study, this loop is utilized to attach the upper left eyelid to a movement-measuring device. After the surgery, animals were allowed to recover for 1 week.

2.3. Behavioral procedures

All animals were firstly adapted to the experimental environment for three sessions at 30 min per session, immediately followed by early training (or pseudo-training) sessions (stage I), transfer training sessions (stage II), and recall session (stage III). During these sessions, animals were restrained in a Plexiglas container (25 cm × 15 cm × 15 cm) located in a sound- and light-attenuated chamber, and their heads were secured with blunt ear bars that pressed on the head stages. The left eye of the animal was held open in a confirmable position, with the nylon loop sutured into the left upper eyelid, which was linked to the high-resolution potentiometer (JZ101, XH, Beijing, China). The voltage level represented the eyelid position, with baseline manually calibrated to a constant value. Moreover, the animal's left lower eyelid was taped open. These measures ensured continual exposure of the animal's left cornea.

The 48 male guinea pigs were divided into 8 groups, including 4 groups for study of delay paradigm (Fig. 1C) and the other 4 for trace paradigm (Fig. 1D). In both studies of delay and trace paradigms, 2 groups (1 for experiment and 1 for control) were included for study of learning transfer from central to peripheral and another 2