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Review

The neural circuitry and molecular mechanisms underlying delay and trace eyeblink conditioning in mice

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HIGHLIGHTS

- The molecular mechanisms underlying DEC and TEC in mice differ from each other.
- Amygdala-Cerebellum-Prefrontal Cortex-Dynamic-Conditioning Model for DEC in mice is proposed.
- The forebrain regions may play an essential role in TEC of mice.
- Cerebellar cortex seems to be out of the neural circuitry of TEC in mice.

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ABSTRACT

Classical eyeblink conditioning (EBC), a simple form of associative learning, has long been served as a model for motor learning and modulation. The neural circuitry of EBC has been studied in detail in rabbits. However, its underlying molecular mechanisms remain unclear. The advent of mouse transgenics has generated new perspectives on the studies of the neural substrates and molecular mechanisms essential for EBC. Results about EBC in mice differ in some aspects from those obtained in other mammals. Here, we review the current studies about the neural circuitry and molecular mechanisms underlying delay and trace EBC in mice. We conclude that brainstem-cerebellar circuit plays an essential role in DEC while the amygdala modulates this process, and that the medial prefrontal cortex (mPFC) as a candidate is involved in the extra-cerebellar mechanism underlying delay eyeblink conditioning (DEC) in mice. We propose the Amygdala-Cerebellum-Prefrontal Cortex-Dynamic-Conditioning Model (ACPDC model) for DEC in mice. As to trace eyeblink conditioning (TEC), the forebrain regions may play an essential role in it, whereas cerebellar cortex seems to be out of the neural circuitry in mice. Moreover, the molecular mechanisms underlying DEC and TEC in mice differ from each other. This review provides some new information and perspectives for further research on EBC.

Contents

Abbreviations: CR, conditioned response; CS, conditioned stimulus; UR, unconditioned response; US, unconditioned stimulus DEC, delay eyeblink conditioning; TEC, trace eyeblink conditioning; mPFC, medial prefrontal cortex.

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1. Introduction

The increasing interest in eyeblink conditioning (EBC) has heightened the need for understanding its underlying neural circuitry and molecular mechanisms. Over the past decades, the research on EBC has mostly been conducted in rabbits, cats, and rats, and the neural circuitry of EBC has partially been established. However, results about the neural circuitry obtained by classical methods of lesion/inactivation or ablation are not completely reliable, due to incompleteness, excessiveness or imprecision of these methods, in the process of lesion/inactivation or ablation at a required neural site. Furthermore, as to research on molecular mechanisms of EBC, classical methods are insufficient to lead to the understanding about the underlying neural molecular mechanisms. Despite the disadvantage of high levels of non-associative responding and reflexive responding to the CS in mice, transgenic mice model has its advantages for research on molecular mechanisms of EBC. In recent years, great progress has been made by application of transgenic mice to investigate the molecular mechanism and the neural circuitry underlying EBC. By reviewing recent studies on EBC in mice, we compared the difference in the neural circuitry and molecular mechanisms of EBC between mice and other mammals, as well as the discrepancy in the neural substrates underlying both delay and trace paradigms.

2. Delay eyeblink conditioning (DEC) and trace eyeblink conditioning (TEC) paradigms

Classical conditioning of the eyeblink response is one of the most extensively investigated models of mammalian associative memory. During EBC, a behaviorally neutral conditioned stimulus (CS), usually tone (e.g., 1 kHz, 85 dB) or light, is paired with an unconditioned stimulus (US) such as a corneal airpuff or periorbital shock. Initially, the US only evokes a reflexive eyeblink unconditioned response (UR). After hundreds of CS–US pairings, the organism could learn to close the eyes in response to the CS before the onset of the US, which is referred to as the conditioned response (CR). According to the temporal relationship between the CS and US, there are two commonly used procedures in EBC: trace and delay paradigms. In DEC, the CS overlaps the US and the two stimuli terminate at the same time, which is in contrast to TEC, in which CS is followed by a stimulus-free interval before the US [1].

The essential neural circuitry for EBC, including DEC and TEC, has been delineated extensively and mostly established [2–4]. Strong evidence obtained in a variety of mammals suggests that DEC and TEC have similar input and output pathways, especially the same dependence on cerebellum and the related brainstem nuclei [4–6], the partially overlapping routes mediate these behaviors in the most efficient way. Signals of CS and the US are relayed via the mossy fibers originated from the lateral parts of the pontine nuclei and the climbing fibers originated mainly from the dorsal accessory inferior olive, respectively, and both signals are transmitted to the cerebellar interpositus nucleus as well as Purkinje cells of the cerebellar cortex [7–10]. Recent studies indicate that the medial geniculate nucleus and inferior colliculus are also involved in the auditory CS pathway [11–13]. The CR pathway is formed by the interposed nuclei projections via the red nucleus to the facial nucleus which innervates the eyelid muscles contraction [14]. The UR usually has two components: R1 and R2. While R1 is mediated by the circuit from the trigeminal nerve and nucleus to the facial nucleus, R2 is mediated by a superimposed loop through the reticular formation and/or cervical spinal cord [15–17].

It is widely accepted that the cerebellum and the related brainstem nuclei are necessary and sufficient to support DEC [4–6]. Yet the neural circuitry for TEC is much more complicated and has to be determined. Apart from the cerebellum and the related brainstem nuclei, other forebrain regions such as the hippocampus and medial prefrontal cortex may also be involved in TEC [18–20], to bridge the trace interval between CS and US by projection to the pontine nuclei and from there to the cerebellar cortex and deep nuclei [1]. On the other hand, the contribution of the cerebellar cortex to TEC remains controversial. Results from mouse and human suggest that the cerebellar cortex function is minimal during trace conditioning [21–25], while other studies indicate an involvement of the cerebellar cortex in this conditioning task [26,27].

3. The neural circuitry of DEC in mice

Cumulative evidence obtained from other mammals indicates an essential role of cerebellum and brainstem nuclei in DEC [4]. There is an agreement about the role of the cerebellar cortex in DEC in mice. Some findings indicate that CR acquisition has been disturbed in DEC in mGluR1 mutant mice, Purkinje cell degenerative (pcd) mice, GluRδ2-deficient mice, Purkinje cell-specific protein kinase C inhibitor over expressing mice, granule-cell specific reversible neurotransmission blocked (RNB) mice, Ngsk Prnp (0/0) mice and Purkinje neuron Scn8a KO mice, suggesting the importance of the cerebellar cortex in this paradigm [23,28–34]. Besides, the latest research indicated that both parallel fiber (PF)–Purkinje cell (PC) synapses and the metabotropic glutamate receptor subtype 1 (mGluR1, Grm1) in cerebellar Purkinje cells (PCs) are essential for DEC in mice [35,36]. Interestingly, results in C57BL/6 mice aged 4, 8, 12, 18, and 24 months suggest that individual variation in Purkinje neuron number is related to DEC in young organisms [37]. The researches of the eyeblink controlling regions in the cerebellar cortex in mice indicated that the responding areas are located at least in the simplex lobule and adjacent parts of lobule HVI [38]. The data obtained from other mammals by the metabolic and functional imaging have also revealed that the lobule HVI in cerebellar cortex is significantly activated during DEC [27,39,40]. A novel transsynaptic mapping of eyeblink-related neurons in the cerebellar cortex in C57Bl/6 mice revealed that the vermis and the simple (HVI) and ansiform (HVII) lobules are involved in conditioning eyeblink circuits [41]. In a word, cerebellar cortex seems to be responsible for the learning process of DEC in mice.

It's widely accepted that the interpositus nucleus, within the cerebellum, plays an important role in DEC [42,43]. In an experiment using metabolic marker of energy in rat, the radioactively labeled glucose analog shows a differential amount of activation in anterior and posterior portions of the interpositus nucleus, in delay and trace paradigms [27]. Recently, comparable studies have been conducted in mice. DEC studies shown that lesions of the anterior interposed nucleus (AIN) abolished well-timed cerebellar CRs in both wild-type and Fmr1 mutant mice [44]. Further gene research in C57BL/6 mice proposed that representative LATE gene (Vamp1, Camk2d, and Prkcd) expression was selectively increased in the AIN after 7-d paired training, suggesting that AIN has a crucial role in memory of EBC [45].

The participation of brainstem nuclei in DEC in mice is similar to that in other mammals. Glia maturation factor-knockout mice, in