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.

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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 airuff or periorbital shock. Initially, the US only evokes a reflexive eyeblink unconditioned response (UR). After hundreds of US–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 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, 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, GluR2/3–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 Snca 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 transynaptic mapping of eyeblink-related neurons in the cerebellar cortex in C57BL/6 mice revealed that the vermis and the simple (HVI) and ansiform (HVIl) 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 is 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 7–10 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
which there is a loss of neurons in the inferior olive histologically, show an impaired motor learning [46]. Sakamoto et al. reported that learned eyeblink responses (LER), during delay conditioning, were completely abolished when mesencephalal stem cells (MSCs) were injected into the RN in mice; however, the removal of MSCs led to the immediate acquisition of LER to the control level [47]. In line with this finding, Sun found [41] that the red nucleus is bilaterally labeled at the lateral rim in the research of transsynaptic tracing of eyeblink-related circuits in mice by injecting GFP-expressing Pseudorabies virus (PRV–152) into the mouse orbiculate oculi muscle; distinctively, he found that labeling was in the ventrolateral red nucleus which receives projections from the anterior interpontin. In addition, the facial nucleus and the deep cerebellar nuclei (DCN) also have been labeled [41].

There is convincing evidence that forebrain structures such as the hippocampus and medial prefrontal cortex (mPFC) that is critical in the trace paradigms are not necessary for DEC [48–50]. Interestingly, the role of hippocampus, which has long been associated with memory and motivated behaviors, is seemingly paradoxical during DEC. Although bilateral removal or lesion of the hippocampus does not affect CRs in DEC [50,51], the CA1 field of the hippocampus exhibits increased in pyramidal cell activity in response to the US and CS during DEC [52–54], and the hippocampus may play a role in the acquisition of long DEC with 1400-ms interstimulus intervals (ISI; the interval between the CS and US onsets) [55]. There is few convincing evidence showing that mPFC involved in DEC. Nevertheless Kawahara and Matoba reported [56] that mice ablated the ipsilateral cerebellum and the bilateral mPFC was significantly impaired during DEC, while mice ablated ipsilateral cerebellum or bilateral mPFC learned as well as intact mice, suggesting that mice require either the ipsilateral cerebellum or mPFC for successful DEC. Coincidentally, a recent study in our laboratory showed evidently that the mPFC was essential for the DEC with the soft tone CS but not for the DEC with the loud tone CS in guinea pig [57]. Thus, mPFC as a candidate is involved in the extra-cerebellar mechanism for DEC in mice is a conservative conclusion.

The amygdala, which has long been associated with memory, emotion, and motivated behaviors, is crucial in fear conditioning. However, only a few researchers pay attention to its involvement in DEC. By using the methods of lateral amygdala lesion or muscimol (MSC) injection into the lateral amygdala, Sakamoto et al. found [47] that the lateral amygdala plays important roles in the acquisition and expression of DEC in mice. Similarly, metabolic and lesion studies also show that the amygdala plays a role in DEC in rats [58–60]. Boele et al. proposed the Amygdala–Cerebellum–Dynamic–Conditioning Model (ACDC model), which indicates that the short-latency–response (SLR), a characteristic trace of the EBC in mice, may originate from the amygdala [17]. They believed that two facts can support their hypothesis: the first is that a few CS–US pairings is sufficient to establish SLRs; the second is that the latency (about 50–70 ms) to onset of the eyelid SLRs in mice during EBC is in line with the latency of signal convergent and output via the lateral amygdala during fear conditioning. A previous experiment by Buchanan et al. verified the anatomical connections between mPFC and amygdala [61]. They showed that projections from pre-limbic cortex (areas 32) and anterior cingulate cortex (areas 24) were primarily to the lateral, basolateral and basomedial nuclei, while infralimbic cortex (area 25) projected to the central nucleus, of amygdala. May be this is the basis of modulative function of amygdala on EBC. Taken together, it is reasonable to conclude that the amygdala involves in modulation of delay eyeblink conditioning in mice.

The above results lead to the conclusion that brainstem-cerebellar circuit plays an essential role in DEC while the amygdala modulates this process, and that the mPFC as a candidate is involved in the extra-cerebellar mechanism in modulating DEC in mice. Therefore, we modify Boele et al.’s [17] ACDC model of the essential neural circuitry for DEC into an Amygdala–Cerebellum–Prefrontal cortex–Dynamic–Conditioning Model (ACPDC model) in mice (Fig. 1).

4. The neural circuitry of TEC in mice

The TEC differs from DEC not only in its stimulus–free trace interval but also in its particular neural circuitries. Additional declarative knowledge may be required for TEC because the trace interval makes it difficult for the cerebellum to associate the CS with US due to the powerful inhibitory cerebellar networks [62]. Nearly 20 years ago, Solomon et al. found that rabbits subjected to aspiration lesions of the entire hippocampal formation could not acquire the trace conditioning, suggesting that the hippocampus was crucial for the initial acquisition of trace conditioning [63]. As far as we know, the hippocampus plays a time–limited role in memory retention after trace conditioning in mice [64]. It is similar to the result reported in rabbits in which the CR acquired recently requires an intact hippocampus for its retention, but the CR acquired remotely does not, the mechanism and time course of memory consolidation for TEC may be similar in mice and rabbits. Furthermore, researchers reported that area CA1 of the hippocampus had the greatest impact on TEC since it had a greater correlation with behavior than area CA3 in mice [65]. Another report showed that the NMDA receptor in the dorsal hippocampus was critically involved in the acquisition of CR in long–trace EBC in C57BL/6 mice [66]. It’s worth noting that the precise and timed activation of multiple hippocampal synaptic contacts during trace classical EBC has been confirmed in mice, showing that the acquisition of TEC was a multi-synaptic process in which the contribution of each synaptic contact varied in strength, and took place at different moments across learning [67]. Thus, hippocampus may play an essential role in TEC in mice as in other mammals.

The hippocampus is not the only neural circuitry of TEC in mice. Data from a mice model system of TEC indicated that forebrain regions act to bridge the trace interval between the CS and US in trace conditioning [1]. This was in line with the hypothesis of Weisz and Disterhoft [68]. Image analysis revealed a significant impairment in DBA/2 mice which had several deficits in the forebrain during the TEC. Analysis of CR temporal pattern demonstrated a significant difference in the onset latency, not the peak latency, of the TEC between C57 and DBA mice: C57 mice blinked earlier than DBA/2 mice did, though DBA/2 mice learned DEC as well [69]. Moreover, GluRRepsilon2 mutant mice showed no trend toward CR acquisition in the trace paradigm with a trace interval of 500 ms, indicating the forebrain was critically involved in successful learning of long–trace EBC [70]. The long stimulus–free interval involved in trace conditioning would require another structures, like pontine for conditioning to occur.

It is believed that the forebrain regions recruited to “bridge the trace interval” project to the pontine nuclei and from there to the cerebellar cortex and deep nuclei. The pontine sites may also be a site of modification by the forebrain, as suggested by Knowlton and Thompson [71]. In this way, the lateral pontine nuclei could function to bridge the trace interval as a relay of signaling CS presentation to the cerebellar circuit, and to insure that the CS input is projected to the correct area of the cerebellar cortex or deep nuclei for processing during learning.

Controversy still exists as to whether the cerebellar cortex participates in the TEC. Traditionally, the cerebellum is argued to be responsible for acquisition, storage, and retrieval of the CR in both trace and delay conditioning. This argument is supported by some lesion studies, in which the cerebellar cortex is proved to be involved in TEC. However, these studies are mainly limited by the
difficulty to remove the entire cerebellar cortex, and the possibility of disparate results due to partial lesion effects. Interestingly, the results from mice do not tally with the traditional viewpoint mentioned above. Results from Purkinje cell degeneration (PCD) mutant mice indicate that the essential neural circuitry for acquiring and extinguishing TEC involves connectivity that bypasses cerebellar cortex. Furthermore, several strains of mice with disrupted cerebellar cortical function that impaired DEC demonstrated normal TEC. In addition, Kishimoto et al. [21] found that mice with deficient in phospholipase Cβ4, a protein highly expressed in Purkinje neurons in the rostral cerebellar cortex, demonstrated severely impaired rostral cerebellar long-term depression (LTD) and delay conditioning, but intact TEC. This study together with their other work [23,72], indicated that LTD might be required as a neural substrate for delay but not trace conditioning.

In conclusion, the forebrain regions play an essential role in TEC based on the above results of TEC studies in mice. However, cerebellar cortex seems to be out of the neural circuitry of TEC in mice (Fig. 2).

5. The cellular and molecular mechanisms of DEC and TEC in mice

There has been limited data about the cellular and molecular mechanism of EBC due to its complexity, even though EBC is a seemingly simple form of associative learning. The application of transgenic mice has generated new perspectives about the neural substrates and molecular mechanisms. Based on studies using various mutant mice and pharmacological treatment, we sum up the molecular mechanisms of DEC and TEC in mice as follows.

5.1. Cerebellar parallel fiber (PF)-Purkinje cell (PC) LTD and LTP involvement in DEC and TEC in mice

Although a number of studies supported the view that the memory acquisition of EBC shares a common molecular mechanism with the cerebellar motor learning in the establishment of cerebellar PF–PC LTD, disputable results still existed in some studies of mice. Miyata et al. [73] reported that phospholipase C beta 4 (PLCβ4) mutant mice showed impaired DEC and impaired cerebellar cortical LTD, implying that LTD was correlative with DEC in mice. Emi et al. reported that intact PF–PC synapses and PF-LTD are necessary to acquire delay EBC in mice [74]. On the contrary, Welsh et al. indicated that PF–PC LTD under control of the climbing fibers was not required for general motor adaptation or the learning of response timing in two common models of motor learning for which the cerebellum has been implicated [75]. Similarly, Schonewille et al. propose that PF–PC LTD was not essential for DEC in mutant mice that target the expression of PF–PC LTD by blocking internalization of AMPA receptors [76]. In any case, although LTD at PF–PC synapses may not be essential for eyelid conditioning, they may indeed still contribute under physiological circumstances. On the other hand, it has been reported that cerebellar cortical PF–PC LTD deficient mice successfully acquired TEC [21–23,34], suggesting that the cerebellar cortical PF–PC LTD was not essential for this paradigm.

The potential involvement in DEC of postsynaptic LTP at the PF to PC synapse in cerebellum was excluded by recent studies from Schonewille et al. and Emi et al. [74,76]. Results of their studies indicate that intact PF–PC LTP is not necessary to acquire delay EBC in mice. Previously it was conjectured that PF–PC LTP induction might partially compensate for impaired LTD induction [77,78], indicating the potential role of LTP in eyelid conditioning. By the way,
5.2. Molecule involvement in DEC and TEC in mice

In recent years a series results concerning molecule involvement in DEC and TEC have been obtained from experiments with transgenic mice (Table 1). In a study using mice in which acid-activated ion channel (ASIC) was knocked out, ASIC was showed to contribute to DEC learning [81]. Similarly, both glia maturation factor (GMF)-knockout mice [46] and apolipoprotein E-deficient mice [82] showed defects in DEC. Furthermore, other studies indicated that PLCβ4 which is highly expressed in the PCs of the rostral cerebellum, was essential for delay conditioning, but not for trace conditioning [21,73]. A research using Cbln1-knockout mice showed that the Cbln1, a member of the C1q/TNF super family and predominantly produced in cerebellar granule cells, was involved in the delay paradigm, but not in trace conditioning [83]. On the other hand, studies using N-methyl-d-aspartate (NMDA)-type glutamate receptor mutant mice demonstrated a normal level and impairment of DEC learning in GluRpsilon1 (GluR1) mutant mice and GluR epsilon2 (GluR2) mutant mice, respectively. Moreover, severe impairment of TEC was observed in both GluR1 and GluR2 mutant mice with a trace interval of 500 ms [70,84]. Therefore, NMDA receptor GluR2 plays a more critical role than the GluR1 subunit in classical EBC. Recent study of metabotropic glutamate receptor subtype 1 (mGluR1) showed that the mGluR1a, a predominant splice variant in PCs, was required for the proper peri-synaptic targeting of mGluR1 and DEC [36]. According to data from the CaMKII T286A mutants, researchers suggested a potential hippocampal CaMKII-independent mechanism underlying the formation of temporal memory in TEC [66]. In addition, the activity-regulated transcription factor cAMP response element-binding protein (CREB), an essential component of the molecular switch that controls the conversion of short-term into long-term forms of plasticity, played a role in TEC in mice [79].

Pharmacological study (Table 2) found that both the GABA and NMDA receptors were involved in EBC in mice. Sakamoto et al. (2007) reported [85] that the C57BL/6 mice with muscimol (MSC: GABA-R agonist)-injection into the DCN showed significant impairment in the acquisition and retention of DEC. Further study suggested that GABA receptors in RN and amygdale [47], and in both ipsilateral and contralateral DCN, played critical roles in DEC [32]. However, involvement of GABA A receptors in TEC in mice has not been verified so far. In contrast, it has been demonstrated that NMDA receptors were involved in TEC. Takatsuki et al. demonstrated [86] that NMDA receptors were involved in CR acquisition of both DEC and TEC with short or long trace interval, in which long trace interval TEC depends more on NMDA-R than short interval TEC and DEC. Furthermore, their study using glutamate receptor subunit 62 mutant mice indicated that this receptor played critical roles in both acquisition and expression of 0-trace EBC [87]. In the same year, Sakamoto et al. found [67] that the NMDA receptor in the dorsal hippocampus was critically involved in the acquisition of the CR in long trace (500 ms) EBC in C57BL/6 mice. Then Rodríguez-Moreno et al. founded [88] that the CRs of TEC (with ISI of 250 ms) were severely impaired when the noncompetitive NMDA-receptor antagonist MK-801 was administered to mice. In addition, other substrates are also involved in the mice EBC.

The cholinergic systems play a pivotal role in learning and memory. There is considerable evidence for the involvement of the cholinergic system in EBC (both in DEC and TEC), primarily from pharmacological studies on rabbits or cats [89–92]. Interestingly, the result about the role of acetylcholine in EBC seems disparate between rats and rabbits. A research on acetylcholine release levels in rats revealed a dissociation in cholinergic activation of the mPFC and hippocampus during performance in trace but not delay appetitive conditioning [93]. In line with this finding, Rodríguez-Moreno et al. demonstrated [88] that nicotinic acetylcholine receptors enhanced glutamatergic transmission in hippocampal circuits involved in the acquisition of TEC with 250-ms interstimulus intervals in C57BL/6 mice, suggesting that acetylcholine promoted CRs of trace conditioning in mice. However,
Table 1
List of the molecule involvement in DEBC and TEBE from mice studies.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Description</th>
<th>Delay</th>
<th>Trace</th>
<th>Mice type</th>
</tr>
</thead>
<tbody>
<tr>
<td>αCaMK II</td>
<td>α-Calciim/calmodulin-dependent protein kinase II</td>
<td>Unknown</td>
<td>-</td>
<td>αCaMK II&lt;sup&gt;1294A&lt;/sup&gt; mutation mice</td>
</tr>
<tr>
<td>CaMK II/δ</td>
<td>Calcium/calmodulin-dependent protein kinase, δ</td>
<td>+</td>
<td>Unknown</td>
<td>C57BL/6 mice</td>
</tr>
<tr>
<td>Vamp1</td>
<td>Vesicle-associated membrane protein 1</td>
<td>+</td>
<td>Unknown</td>
<td>C57BL/6 mice</td>
</tr>
<tr>
<td>ASIC</td>
<td>The Acid-Activated Ion Channel as a key component of acid-activated currents</td>
<td>+</td>
<td>Unknown</td>
<td>ASIC null mutation mice</td>
</tr>
<tr>
<td>GMF</td>
<td>Gila maturation factor</td>
<td>+</td>
<td>Unknown</td>
<td>GMF-null mice</td>
</tr>
<tr>
<td>PKCδ</td>
<td>Protein kinase C, δ</td>
<td>+</td>
<td>Unknown</td>
<td>C57BL/6 mice</td>
</tr>
<tr>
<td>apoE</td>
<td>Apolipoprotein E</td>
<td>-</td>
<td>+</td>
<td>Mice lacking apoE</td>
</tr>
<tr>
<td>GluR1</td>
<td>GluR1 protein</td>
<td>-</td>
<td>+</td>
<td>GluR1 mutant mice</td>
</tr>
<tr>
<td>GluR2</td>
<td>GluR2 protein</td>
<td>+</td>
<td>+</td>
<td>GluR2 heterozygous mutant mice</td>
</tr>
<tr>
<td>GluR6</td>
<td>Cerebellar Purkinje cell-specific Glutamate receptor, δ2</td>
<td>+</td>
<td>-</td>
<td>GluR6 mutant mice</td>
</tr>
<tr>
<td>PLE34</td>
<td>Phospholipase Cβ4 (highly expressed in the Purkinje cells of the rostral cerebellum)</td>
<td>+</td>
<td>-</td>
<td>Phospholipase Cβ4 mutant mice</td>
</tr>
<tr>
<td>CbIn1</td>
<td>A member of the C1q/TNF superfamily and is predominantly produced in cerebellar granule cells</td>
<td>+</td>
<td>-</td>
<td>CbIn1 knockout mice</td>
</tr>
<tr>
<td>CREB</td>
<td>cAMP response element–binding protein</td>
<td>-</td>
<td>+</td>
<td>Bitransgenic pCaMKIIβ – tTA/tetO–VP16</td>
</tr>
</tbody>
</table>

The molecule involvement in delay and trace eyeblink conditioning from studies with various mice. “*” means positive correlation, i.e., this molecule involved in the paradigm. “−” means negative correlation, i.e., this molecule is not involved in the paradigm.

Table 2
List of the receptor involvement in DEBC and TEBE from pharmacological study in mice.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Drug-injected</th>
<th>Site-injected</th>
<th>Delay</th>
<th>Trace</th>
<th>ISI</th>
<th>Mice type</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA receptor</td>
<td>MSC, PTX</td>
<td>DCN</td>
<td>*</td>
<td>Unknown</td>
<td>/</td>
<td>C57BL/6 mice</td>
</tr>
<tr>
<td>GABA receptor</td>
<td>MSC</td>
<td>The lateral amygdala and RN</td>
<td>++</td>
<td>Unknown</td>
<td>/</td>
<td>C57BL/6 mice</td>
</tr>
<tr>
<td>NMDDA receptor</td>
<td>MK-801</td>
<td>Intraperitoneally</td>
<td>*</td>
<td>250 ms/100,500 ms</td>
<td>C57BL/6 mice</td>
<td></td>
</tr>
<tr>
<td>NMDDA receptor</td>
<td>APV</td>
<td>The dorsal hippocampi</td>
<td>++/++</td>
<td>500 ms</td>
<td>C57BL/6 mice</td>
<td></td>
</tr>
<tr>
<td>NMDDA receptor</td>
<td>MK-801</td>
<td>Intraperitoneally</td>
<td>Unknown</td>
<td>C57BL/6 mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ach receptor</td>
<td>R7R-2403</td>
<td>Intraperitoneally</td>
<td>Unknown</td>
<td>C57BL/6 mice</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The receptor involvement in delay and trace eyeblink conditioning in mice. “*” means an involvement of this molecule in the paradigm. “++” means an essential role of this molecule for the paradigm.

the contribution of acetylcholine to DEC in mice has not been investigated yet.

The above results indicate that molecular mechanisms underlying DEC and TEC in mice differ from each other, i.e., some distinct molecules involved in specific EBC paradigms, others are responsible for both. Further studies are required to elucidate the molecular neural substrates underlying EBC.

6. Conclusions

As mentioned above, this review focuses on the neural circuitry and cellular and molecular mechanisms of EBC, including both DEC and TEC in mice. As to DEC, we have demonstrated that brainstem-cerebellar circuit plays an essential role in DEC while the amygdala modulates this process, and that pPCa as a candidate is involved in the extra-cerebellar mechanism in mice, thus propose the Amygdala-Cerebellum-Prefrontal Cortex-Dynamic-Conditioning Model (ACPD model) for DEC in mice. As to TEC, the forebrain regions may play an essential role, whereas cerebellar cortex seems to be out of the neural circuitry in mice.

We have also reviewed the cellular and molecular mechanisms of EBC in mice. It should be noticed that the molecular substrates underlying delay and trace EBC are quite different, and further validation of unknown molecular mechanisms in EBC awaits. Furthermore, transgenic mouse model has disadvantages in some aspects, especially for its unpredictable and undocumented developmental effects on the transgenic manipulations. Moreover, many studies have shown that the transgenic manipulations are not as precise as they are initially claimed to be. Knocking out or overexpressing genes has many uncontrolled consequences within the cells. Nevertheless, Technological revolutions have recently brought new prospects in neuroscience research. More accurate and powerful techniques, like optogenetics and two-photon targeted patching technique, should be introduced to confirm the neural circuitry and the cellular and molecular mechanisms for both delay and trace EBC.

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