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# Weakening Visual Function Enhances Auditory Fear Conditioning in Mice*

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**Abstract** As advanced cognitive activities, it is poorly understood if visual weakening affects auditory fear conditioning. Here we investigated the responses in auditory Pavlovian fear conditioning, using mutant *rd/rd*, *cl/cl* mice as visual weakening group. Freezing behaviors were recorded during fear conditioning, fear extinction, and extinction recall phases. The results indicated that mice with shape vision deprivation are more conducive to auditory fear conditioning. We discuss the possible neuro-mechanism of visual-auditory system interactions.

**Key words** weakening visual function, mice, auditory fear conditioning
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Auditory Pavlovian fear conditioning in rodents has long been an important model for associative learning and is used extensively to mimic various clinical features of anxiety disorders, such as post-traumatic stress disorder. After repeated pairing of a conditioned stimulus (CS, usually a tone) with an unconditioned stimulus (US, usually a footshock), the CS can elicit conditioned fear responses such as freezing, increased startle reflexes, autonomic changes, and behavioral response suppression. Fear responses can be extinguished by repeatedly presenting the CS without the US, which is termed fear extinction. Fear extinction in general is considered to represent new learning, which inhibits but not erases the memory for fear conditioning[1-4]. Fear conditioning and extinction are advanced cognitive activities, including learning and memory, as well as emotions. It is of great clinical significance to investigate the neural mechanisms of fear conditioning and fear extinction.

Conditioned learning has a higher neural process than simple conditioned reflex, which may involve more complex neural circuits. In mammalian sensory system, the main ways of perception are visual, auditory and olfactory information. Previous studies have shown that sensory systems do not work in isolation, they show interactions under some

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conditions, for example, visual cortex can become responsive to sound stimulation in blind animals[5-7], recordings from deaf animals show visual responses in auditory regions[8-9]. Similar results have been found in other systems as well, *e.g.* the olfactory[10-11], gustatory[12-14], and somatosensory cortices[15]. However, we don't know whether this kind of interaction exists in the more advanced neural process of conditional learning, and if so, what kind of influence it will have.

There are mainly two kinds of information transmitted by the visual system, one is the information with light on and light off, the other is the more complex information in the environment, such as shape information, color information, motion information, motion direction and speed. In previous studies, it is difficult to separate these factors due to the experimental technology, so simple methods are often used, such as enucleation, retinal destruction or optic nerve transection to block all visual information. But in recent years, due to the development of genetic engineering, we have better methods, such as mutant *rd/rd, cl/cl* mice. In this kind of mice, we can remove photoreceptors such as rod cells and cone cells from retina by genetic engineering, and keep basal ganglia cells intact. In recent years, it has been found that the basal ganglia cells of these mice can perceive light, but the spatial visual resolution of these mice is very low compared with wild mice[16]. In this case, we deprived the mice of shape vision to see if it had an effect on auditory conditioned learning.

In this study, we address the question in auditory Pavlovian fear conditioning, using mutant *rd/rd, cl/cl* mice. We compared their freezing behavior with wild type mice during fear conditioning, fear extinction, and extinction recall phases. The significance of these mutations is that these mice lack rod and cone photoreceptor, respectively, but both have functionally normal optic nerves and visual cortex. By using these mutations, it helps to avoid the damages of traditional invasive methods of analyzing brain functional anatomy, such as significant atrophy in the geniculocortical tracts.

## 1　Materials and methods

### 1.1　Subjects

Male wild type C57BL/6 (WT) mice, and mutant *rd*/*rd*, *cl*/*cl* mice were obtained from the University of Science and Technology of China. After arrival, mice were housed separately, in a temperature and humidity controlled environment with free access to food and water. Housing rooms were illuminated on a 12 h∶12 h light/dark cycle with lights on at 7∶00 A.M. All behavioral procedures were performed between 11∶00 A.M. and 5∶00 P.M. The experiments were approved by the International Review Board (IRB) of the Institute of Psychology, Chinese Academy of Sciences, and were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering. Mice arrived one week prior to the start of experiments to acclimate to the colony room.

### 1.2　Behavioral apparatus

Four identical observation chambers (30.5 cm×25.4 cm×30.5 cm, Coulbourn Instruments, Allentown, PA, USA) were used. Each chamber was constructed of aluminum (two side walls and ceiling) and plexiglass (rear wall and hinged front door) and situated within a sound-attenuating cabinet. Acoustic CSs were presented by a speaker mounted on one side panel of the chamber. The bottoms of the training chambers were composed of stainless steel grid floors (rods were 2 mm in diameter and spaced 1 cm apart) connected to a scrambled shock generator and solid-state grid scrambler (Coulbourn, H13-15, the rods were arranged orthogonally) for delivery of footshock USs (the unconditioned stimulus). Both shock and tone deliveries were controlled by a computerized system. A video camera was mounted on the chamber ceiling to videotape behavior. To reduce the influence of context on cued fear conditioning, tactile, visual, and olfactory cues were manipulated to create two distinct contexts (A and B). Context A was the original environment described above. Additionally, a small yellow light (6 W) mounted opposite the speaker was turned on to provide illumination inside the chamber while the fluorescent room light was turned off. The chambers were sprayed with 75% ethanol before and after each use between animals. Context B was modified from context A. Black acrylic boards with nine circular holes (1.5-cm diameter) were fitted to the sidewalls of the chamber, the chamber light was changed to a white light bulb (6 W), the floor was replaced with a steel sieve-shaped plate (2 perforations/cm), and chambers were sprayed with diluted perfume (1%). Experiments in context B were conducted in the same room as those in Context A but with the room fluorescent lights on. Chambers were cleaned between each test.

### 1.3 Behavioral procedures

The behavioral procedures consist of four phases: habituation, fear conditioning, extinction, and test. All animals were habituated to context B (testing context) for 10 min without any stimulus (Day 1). Twenty-four hours later, fear conditioning (training) was conducted in context A (Day 2). Briefly, after 3-min habituation in this environment, animals were conditioned by exposure to five tones (CS: 30 s, 70 dB, 2 kHz) paired with 5 footshocks (US: 0.7 mA, 1.0 s). Each footshock coexisted with a tone. The average inter-trial interval (ITI) was 120 s (range between 90−150 s). One minute after the last CS-US pairing, animals were returned to their homecages. On Day 3, after 3-min habituation in the environment, all animals were subjected to fear extinction, animals received 10 non-reinforced presentations of the CS in context B with a fixed ITI of 60 s. One minute after the last CS, animals were returned to their homecages. On Day 4, after 3-min habituation in the environment, an extinction test was conducted in which the animals received 10 non-reinforced CS presentations with a fixed ITI of 60 s in context B. One minute after the last CS, they were returned to their homecages[17-18].

### 1.4 Behavioral data analysis

The percentage of time spent freezing was used as measurement criteria for conditioned fear, freezing is defined as the absence of all movements except those related to respiration[16-17]. Freezing was measured continuously during all behavioral sessions, including the pre-CS “baseline” period as well as during tone presentation. Freezing was quantified automatically using Freeze-Frame software (ACT-100, Coulbourn Instruments) and further confirmed by a blind test to group assignments. The level of freezing is expressed as percentage (% ) of time freezing during the 30-s tone presentation. Data on freezing behavior during conditioning, extinction, and post-test were analyzed by two-way analysis of variance (ANOVA) for repeated measurements (RT-ANOVA) for determination of main effects and interactions, followed by Newman-Keuls post hoc tests for pair-wise comparisons among groups. Statistical significance was $P<0.05$ for all tests. All data are presented as mean±SEM, and $n$ is the number of animals used.

## 2 Results

Figure 1 shows the mean group freezing levels by trial during fear conditioning or by trial block during extinction training and recall of extinction phases (2 trials as 1 block). The average freezing time increased significantly over the five CS-US pairings (conditioning trials) in the two animal groups ($F(4,72)=63.75$, $P<0.000\,1$), and there was no significant group effect ($F(1,18)=2.308$, $P>0.05$, by two-way RT-

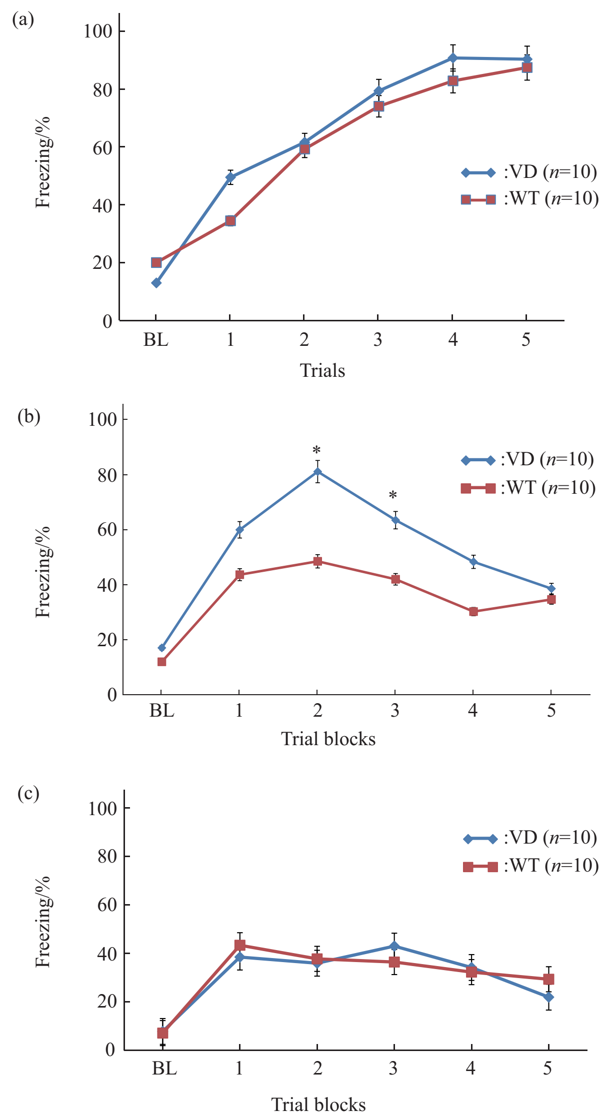


**Fig. 1 Effects of weakening visual function on auditory Pavlovian fear conditioning**

Mean group freezing levels by trial during fear conditioning (a) or by trial block during extinction training (b) and recall of extinction phases (c). Mutant *rd*/*rd*, *cl*/*cl* mice were used as shape vision deprivation group. Values were represented as $\bar{x} \pm s$. VD: shape vision deprivation group; WT: wild type group . *$P<0.05$.

ANOVA; Figure 1a). During extinction training (Figure 1b), the two groups initially exhibited high levels of tone-evoked freezing, which decreased progressively over successive tone alone presentations ($F(4, 72)=68.59$, $P<0.000\ 1$). Although both groups presented a decrease in the conditioned fear response across trial blocks ($F(4,72) = 10.396$, $P= 0.001$), post hoc comparisons revealed that shape vision deprived animals showed higher levels of freezing to the tone in trial block 2 ($P<0.05$), and trial block 3 ($P<0.05$) than the wild type animals. On the following day (recall of extinction, Figure 1c), the initial level of freezing returned to a high level but again decreased progressively during successive tone alone presentations ($F$, $P<0.001$). There was no significant group effect ($F$, $P>0.05$).

## 3 Discussion

In this study, we tested the effects of weakening visual function on auditory Pavlovian fear conditioning in mice. Our results suggest that decreased visual function enhances auditory fear conditioning in mice. This indicates that shape vision deprived animals were more likely to develop auditory conditioned fear than the wild type animals. In our study, we used transgenic mice, which have the same visual pathway as wild-type mice except for the damage of visual photoreceptors[16]. Therefore, the difference of visual information is the main factor affecting the mice to complete the task. Although compared with wild-type mice, light sensitivity may also have some impact, the deprivation of visual shape perception is the key factor, which means that in these mice, the effect of visual perception process is more important than that of sensory process.

Several studies have revealed that the plasticity of interaction between sensory systems are related to reorganization in the cerebral cortex involving the expansion of cortical areas in the deprived mode, and the expression of specific genes[19]. It has been shown that after suturing the eyelids of newborn cats, the auditory cortex extended to the visual cortex several years later[20]. Lippoldt's group found that deprivation of visual shape perception leads to enhanced hearing from the ability of the visual cortex to assume new responsibilities and participate in auditory tasks after visual loss[21]. After shape vision deprivation, compensatory behavior seems to be mediated by the reorganization of the visual and auditory cortices, which may be one of the causes in this experiment.

Previous studies[22-23] have shown that the integration of different sensory systems enables one system to compensate for the loss of sensation in another sensory system by developing new neural circuits in the non-deprived system. In this experiment, shape vision deprived mice were used. Thus, auditory pre-synaptic activity from the main sensory pathway can readily substitute for synaptic activity in the visual pathways. Auditory cues that shape vision deprived mice receive may be processed simultaneously in the auditory cortex and visual cortex, which is a reasonable explanation for the increased hearing ability under these sensory conditions.

Fear extinction in general is considered to represent new learning, which inhibits, but not erases, the memory for fear conditioning[24]. The visual function of the mice used in this experiment was weakened, but the interaction of the two sensory systems improved their auditory performance, without impairing the retrieval of extinction memory. Meanwhile, the results verify that conditioned fear and extinction are two different kinds of memory.

In our study, the fear conditioning task also involves an auditory perception process, that is, animals learn to distinguish pitch frequency. It raises an interesting question: at the same cognitive level, is the interaction of cross modal information the strongest? In our current experiment, we cannot answer this question, but this will be one of our next research contents, which will provide meaningful data for different levels of cross modal information processing research.

## 4 Conclusion

In conclusion, the present observations demonstrate that animals with shape vision deprivation have a greater disposition to establish auditory fear conditioning than wild type animals, suggesting that secondary sensory deficits can increase sensitivity in major sensory pathways. However, the specific neural mechanisms involved in this emotional associative paradigm need to be further explored.

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# 视觉功能减弱增强小鼠听觉恐惧条件反射*

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**摘要** 作为一种高级认知活动，视觉功能减弱是否影响听觉恐惧条件化学习目前还不清楚. 本文以突变体*rd*/*rd*、*cl*/*cl*小鼠为视觉功能减弱组，研究视觉功能减弱是否对听觉巴甫洛夫条件化恐惧反应有影响. 在恐惧条件化、恐惧消退和消除记忆再现阶段记录了僵直行为. 研究结果表明，视觉功能的减弱更有利于小鼠听觉恐惧条件化的建立. 文中讨论了出现此结果的可能神经机制.

**关键词** 视觉功能减弱，小鼠，听觉恐惧条件化

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