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Research report

Limited impairments of associative learning in a mouse model of accelerated senescence

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HIGHLIGHTS

- Senescence-accelerated mouse P8 (SAMP8) can acquire limited trace eyeblink conditioning (TEC).
- SAMP8 can acquire limited discriminative TEC compared with SAM resistant/1 (SAMR1).
- Both SAMP8 and SAMR1 failed to acquire reversal learning of discriminative TEC.
- Impairments of aging on associative learning are incomplete in SAMP8.

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ABSTRACT

Research concerning impairment of associative learning during aging remains limited. The senescence-accelerated mice (SAM) prone/8 (P8) has been proposed as a useful model for the study of aging, and SAM resistant/1(SAMR1) is its control as a normal aging strain. Classical eyeblink conditioning has long been served as a model of associative learning. In order to explore the effects of aging on associative learning in SAM, the present study successively tested three paradigms of eyeblink conditioning in SAMP8 and SAMR1: classical single cue trace eyeblink conditioning (TEC), discriminative trace eyeblink conditioning and reversal learning of TEC. Behavioral performance indicated that SAMP8 could acquire limited single-cue trace eyeblink conditioning task and two-tone discrimination trace eyeblink conditioning with a relative lower acquisition rate compared to SAMR1. Both SAMP8 and SAMR1 failed to acquire reversal learning of discriminative TEC, and SAMP8′ startle reflex to tone CS was lower than SAMR1. These results indicated that the impairments of aging on associative learning were incomplete in SAMP8.

1. Introduction

Along with intensifying trend in the population aging, malfunction of the aging process is becoming important concern. Age-dependent deficits in learning and memory are pathognomonic of the aging process and animal models with such deficits are useful in gerontological research. The senescence-accelerated mouse (SAM), which was established through phenotypic selection from a common genetic pool of AKR/J strain of mice, was an animal model for studying senescence and age-associated disorders. Among its prone sub-strains, the SAM/prone8 (SAMP8) mouse strain has an inherited age-related impairment of learning and memory, while age-matched SAM/resistant1 (SAMR1) shows normal [1]. Many reports provide insight into mechanisms of the cognitive impairment in SAMP8, which could be related to alterations of the gene expression, protein abnormalities, oxidative stress, and other brain anomalies [2–6]. Abundant behavioral data have indicated that SAMP8 animals present evident deficits in spatial learning and memory (water maze task), the passive avoidance task, object recognition test, and the contextual fear conditioning [7–12]. However, research of associative learning capabilities in SAMP8 remains limited.

Classical eyeblink conditioning is one of the extensively investigated models of mammalian associative learning, which involves paired presentation of a behaviorally neutral conditioned stimulus (CS) and an unconditioned stimulus (US). According to the temporal relationship between the CS and the US, there are two commonly used procedures in eyeblink conditioning: trace and delay paradigms. Simple delay eyeblink conditioning (DEC) depends on

Abbreviations: CR, conditioned response; CS, conditioned stimulus; DEC, delay eyeblink conditioning; PFC, prefrontal cortex; SAM, the senescence-accelerated mice; TEC, trace eyeblink conditioning; SEM, standard error of the mean; UR, unconditioned response; US, unconditioned stimulus.

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brainstem-cerebellar circuit [13–16] whereas trace eyeblink conditioning (TEC) depends on intact forebrain structures such as the hippocampus and medial prefrontal cortex [17–23], nevertheless, it also requires interactions of hippocampus and/or prefrontal cortex if complicated discrimination reversal of eyeblink conditioning is involved [24,25].

The research of associative learning during aging has been so far conducted on eyeblink conditioning mostly in AD model mice [26–29] and few in SAM. However, the spontaneous model of early aging like SAM, relative to the transgenic AD model mice, has unique advantages in the research of senescence and age-associated disorders due to its inherited aging phenotype. Determining whether SAMP8 can successfully acquire the TEC would be helpful for further research of associative learning during aging. In a recent excellent research on aging [12], SAMP8 animals seem incapable of acquiring trace eyeblink conditioning across 5 conditioning sessions (100 trials each), which was attributed to significant deficit in long-term potentiation (LTP) at the CA1-medial prefrontal synapse. A weaker and brief tone was used as a CS (70 dB, 20 ms) while an electrical shock applied to the supraorbital nerve and presented 250 ms later after CS onset was used as US in their research. It should be noted that a relative high-intensity tone CS (loud CS, usually around 85 dB) was used usually in previous TEC research [27,30–33]. Except for intensity of CS, the CS length within a train is another important parameter affecting learning, with several previous aging studies using 250 ms or longer [27,32,34]. Ewers et al. reported that 12-month-old APP + PS1 mouse model of AD could successfully learn the trace eyeblink conditioning, in which the CS was a 250 ms, 85 dB white noise burst separated by a 250 ms stimulus-free interval between CS and US [27]. These considerations led us to propose that relative high-intensity tone and longer CS length may be necessary for acquiring of the TEC in SAMP8 animals.

In order to explore the effects of aging on associative learning in SAM, here, we set out to investigate the behavioral capability of SAMP8 and SAMR1 in acquiring TEC with an optimal tone CS (85 dB, 250 ms), then, we further checked the learning capability of discriminative trace eyeblink conditioning and the reversal learning of discriminative TEC in SAM. The present results provided direct evidence that SAMP8 could acquire limited single-cue TEC with optimal CS, SAMP8 could establish limited two-tone discriminative TEC compare with SAMR1, but both SAMP8 and SAMR1 failed to acquire reversal learning of discriminative TEC.

2. Methods

2.1. Subjects

A total of 22 male mice (11 R1, 11 P8) at 8 months of age were used in this study. Animals were obtained from the Experimental Animal Center in First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, and were individually housed in standard plastic cages on a 12-h light/dark cycle with constant temperature ($21 \pm 1\,^{\circ}C$). Animals were allowed *ad libitum* access to commercial mice chow and water. All of the experiments were performed between 8:00 A.M. and 6:00 P.M., during the light portion of the cycle. The experimental procedures were approved by the Animal Care Committee of the Third Military Medical University and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23). All possible effort was made to optimize the comfort and to minimize the use of the animals. The mice were divided into two experimental groups [i.e., SAMP8 ($n = 11$), SAMR1 ($n = 11$)], according to the type of the senescence-accelerated mouse strain (SAMP8 or SAMR1).

2.2. Surgery

The animals were allowed to remain undisturbed in their cages for 1 week prior to surgery. Mice were anesthetized with Pentobarbital Sodium (50 mg/kg, i.p.; Shanghai, China). The head of the anesthetized animal was secured to a stereotaxic apparatus (SR-6N, Narishige, Tokyo, Japan) with lambda positioned 1.0 mm ventral to bregma. A longitudinal incision was then made to reveal the skull, onto which a Plexiglas headstage (0.8 cm × 0.8 cm × 0.25 cm), designed to secure the animal's head, was cemented with dental cement and three stainless steel anchoring screws.

2.3. Apparatus

Eyelid movements were measured by a high-resolution spring-return potentiometer (JZ101, XH, Beijing, China) that was attached via a thread lead that was hooked through the nylon loop that was sutured into the left upper eyelid. A speaker that was placed 60 cm above the animal was used to deliver a tone CS, while a plastic pipe placed 5 mm from the animal's left eyeball was used to deliver a corneal airpuff US. Presentations of the CS and US were controlled by a homemade computer-monitored system. An eyelid movement mechanogram and markers of the applied stimuli were digitized at a sample rate of 10 kHz by a data acquisition system (RM6280C, Cheng Yi, Chengdu, China) and were acquired using the built-in software (v. 4.7). A Windows PC was used to store and analyze the behavioral data.

2.4. Design and procedures

Following postoperative recovery, all of the senescence-accelerated mice were trained with a classical trace eyeblink conditioning (phase I). The CS was a 250-ms, 3-kHz pure tone with a intensity of 85 dB (range in conditioning chamber, 83–87 dB; tested by sound level meter, type 2240, Brüel & Kjær, made in Denmark), followed 500 ms from CS onset by a 3.0-psi (measured at the tip of the plastic pipe), 100-ms corneal airpuff as a US (Fig. 1B). Furthermore, intervals between paired CS–US presentations were separated at random by 30 ± 5 s. In total, 1 habituation, 10 conditioning and 1 extinction sessions (100 trials each) were presented to each animal across 12 successive days. For habituation and extinction sessions, only the CS was presented, also for 100 trials at intervals of 30 ± 5 s.

After phase I, animals were trained with discriminative TEC (phase II, 7 sessions) and reversal learning (phase III, 7 sessions) successively (Fig. 1A). Each session in phase II and phase III consisted of 50 trials of a 250 ms, 85 dB, 3-kHz tone CS, and 50 trials of a 250 ms, 85 dB, 1-kHz tone CS, one of which (CS+) presented and followed 500 ms from CS onset by a US (3.0-psi, 100-ms corneal airpuff) and the other of which (CS−) was presented alone without the US (in phase II: 3-kHz tone+/1-kHz tone−; in phase III: 1-kHz tone+/3-kHz tone−). The intertrial interval (ITI) varied randomly in the range 30 ± 5 s. Trials were presented in random order, with the restriction that there could be no more than three trials of the same type (CS+ or CS−) in a row.

During all of the experimental sessions, animals were restrained in small cylindrical plastics containers (12 cm × 3 cm) located in sound- and light-attenuating chambers, and their heads were secured with blunt earbars pressing on the headstages. 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 spring-return potentiometer. The voltage level represented the eyelid position in which the baseline be manually calibrated to a constant value. Moreover, the animal's left lower eyelid was taped open. These two measures were made to insure continual exposure of the left cornea.