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Analysis and modeling of neural processes underlying sensory preconditioning

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ABSTRACT

Sensory preconditioning (SPC) is a procedure to demonstrate learning to associate between relatively neutral sensory stimuli in the absence of an external reinforcing stimulus, the underlying neural mechanisms of which have remained obscure. We address basic questions about neural processes underlying SPC, including whether neurons that mediate reward or punishment signals in reinforcement learning participate in association between neutral sensory stimuli. In crickets, we have suggested that octopaminergic (OA-ergic) or dopaminergic (DA-ergic) neurons participate in memory acquisition and retrieval in appetitive or aversive conditioning, respectively. Crickets that had been trained to associate an odor (CS2) with a visual pattern (CS1) (phase 1) and then to associate CS1 with water reward or quinine punishment (phase 2) exhibited a significantly increased or decreased preference for CS2 that had never been paired with the US, demonstrating successful SPC. Injection of an OA or DA receptor antagonist at different phases of the SPC training and testing showed that OA-ergic or DA-ergic neurons do not participate in learning of CS2–CS1 association in phase 1, but that OA-ergic neurons participate in learning in phase 2 and memory retrieval after appetitive SPC training. We also obtained evidence suggesting that association between CS2 and US, which should underlie conditioned response of crickets to CS2, is formed in phase 2, contrary to the standard theory of SPC assuming that it occurs in the final test. We propose models of SPC to account for these findings, by extending our model of classical conditioning.

1. Introduction

The capability of learning to associate between external sensory signals and to predict future sensory events plays critical roles in survival of animals in a changing environment. Associative learning of animals typically occurs in the presence of a biologically significant sensory stimulus that serves as a reinforcing stimulus. However, many animals including insects (Müller, Gerber, Hellstern, Hammer, & Menzel, 2000), molluscs (Kojima et al., 1998) and humans are also capable of learning to associate between relatively neutral stimuli in the absence of an external reinforcing stimulus, as has been demonstrated by the capability of sensory preconditioning (SPC) (Brogden, 1939). The SPC procedure consists of two phases (Rescorla, 1980). In phase 1, the subject is presented with two neutral sensory stimuli (conditioned stimuli, CS2 and CS1), and in phase 2, one of the stimuli (CS1) is paired with a rewarding or punishing stimulus (unconditioned stimulus, US). Then response of the animals to CS2 is tested (Fig. 1A). A significant learning score for CS2 indicates successful SPC.

The SPC procedure has been frequently used for analysis of associative process underlying learning (Dwyer & Killcross, 2006; Gewirtz & Davis, 2000), but the fundamental question of whether learning of association between neutral sensory stimuli in SPC occurs by the same learning rules and neural mechanisms as those of reinforcement learning remains unclear. Notably, whether neurotransmitters that mediate reinforcing signals in appetitive or aversive learning underlie formation of associations between neutral sensory stimuli has remained unsolved. One study in rodents showed that dopamine (DA) is released in the nucleus accumbens during training in phase 1 of an aversive SPC paradigm, as it is during aversive or appetitive conditioning, and the authors suggested that accumbal DA-ergic neurons serve as reinforcing neurons not only in aversive or appetitive learning (Schultz, 2007) but also in learning to associate between neutral stimuli (Young, Ahier, Upton, Joseph, & Gray, 1998). This suggestion, however, is not consistent with the finding in another study on rats that administration of a dopamine receptor antagonist before training in phase 1 of aversive SPC did not impair SPC (Nader & LeDoux, 1999).

In insects, evidence suggests that octopaminergic (OA-ergic) and DA-ergic neurons mediate appetitive and aversive reinforcing signals, respectively, in classical conditioning (honey bees: Farooqui, Robinson, Vaessin, & Smith, 2003; Hammer & Menzel, 1998; Vergoz, Roussel, Sandoz, & Giurfa, 2007; crickets: Unoki, Matsumoto, & Mizunami, 2005, 2006; fruit-flies: Aso et al., 2010; Schroll et al., 2006; Schwaerzel et al., 2003), although in *Drosophila*, critical

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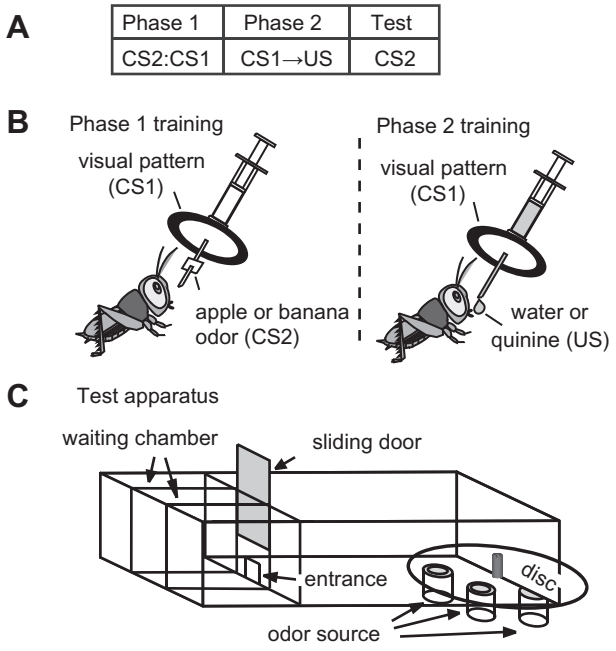

Fig. 1. Methods for SPC. (A) A table showing experimental procedures for SPC. (B) Training for appetitive or aversive SPC. In phase 1, an apple or banana odor (CS2) was simultaneously presented with a white-center visual pattern (CS1). In phase 2, the visual pattern (CS1) was associated with water (appetitive US) or quinine solution (aversive US). (C) Test apparatus. For the test of relative odor preference between apple and banana odors, a cricket was placed in the waiting chamber for acclimation and then allowed to enter the test chamber to freely visit apple and banana odor sources.

roles of DA-ergic neurons in mediating appetitive reinforcing signals have also be suggested (Kim, Lee, & Han, 2007; Liu et al., 2012; Burke et al., 2012). In crickets, moreover, we have suggested that OA-ergic or DA-ergic neurons also participate in memory retrieval after appetitive or aversive conditioning (Mizunami & Matsumoto, 2010; Mizunami et al., 2009).

In this study, we first established procedures for SPC in crickets, which allow long-term (1 day) memory retention after SPC training. In insects, SPC has been reported in honey bees (Hussaini, Komischke, Menzel, & Lachnit, 2007; Müller et al., 2000) and fruitflies (Brembs & Heisenberg, 2001; Guo & Guo, 2005), but not in any other species. Moreover, the effect of SPC has been found only shortly after training (<24 min) in these studies, which has hampered deeper analysis of SPC. Then, we studied the effects of pharmacological blockade of OA-ergic or DA-ergic transmission at various stages of the SPC procedure. Moreover, we addressed another fundamental question concerning SPC (Hall, 1996) of whether association between CS2 and US, which should underlie conditioned response of crickets to CS2, is formed in phase 2 or in the final test. Finally, we propose models of SPC to account for findings in this study.

2. Materials and methods

2.1. Insects

Adult male crickets, *Gryllus bimaculatus*, at 1 week after the imaginal molt, were used in this study. Three days before the start of the experiment, animals were placed individually in beakers and deprived of drinking water to enhance their motivation to search for water.

2.2. Procedures for SPC

SPC training consisted of two phases (Fig. 1A). In phase 1, an apple or banana odor (CS2) and a white-center and black-surround pattern (CS1) were presented at the same time to the animals. For presentation of stimuli, a visual pattern and a small piece of filter paper soaked with apple essence or banana essence were attached to the needle of a syringe (Fig. 1B), and the pattern and the paper were simultaneously presented near the head of the animal for 2 s. This trial was repeated 4 or 8 times with an inter-trial interval (ITI) of 1 min or 5 min. The procedure of phase 2 training (Fig. 1B) was the same to that of appetitive or aversive conditioning of a visual pattern described previously (Unoki et al., 2006). A visual pattern (CS1) was presented to the animal for 2 s and then a drop of water (appetitive US) or 10% quinine solution (aversive US) was given to the mouth. The trials were repeated 4 or 6 times with an ITI of 2.5 or 5 min. The interval between phase 1 training and phase 2 training was 5 or 60 min.

For control of the non-associative effect, one group of crickets was subjected to unpaired presentations of CS2 and CS1 in phase 1 and then subjected to paired presentations of CS1 and US in phase 2 (unpaired/paired or UP/P group), and another group was subjected to paired presentations of CS2 and CS1 and then unpaired presentations of CS1 and US (paired/unpaired or P/UP group). Unpaired presentations were performed in a pseudo-random sequence with an interval of 2.5 min, with the number of presentations of stimuli being the same as that in paired trials.

All groups of animals were subjected to odor preference tests before and after conditioning. We used the "operant testing" procedure, which is based on a high capability of crickets to transfer memory formed in a classical conditioning situation to an operant testing situation (Matsumoto & Mizunami, 2002; Unoki et al., 2005, 2006). In short, on the floor of the test chamber of the test apparatus, there were two holes that connected the chamber with two odor sources (Fig. 1C). Each odor source consisted of a plastic container containing a filter paper soaked with 3 μl solution of apple essence or banana essence, covered with fine gauze net. Three containers were mounted on a rotative holder and two of three odor sources could be located simultaneously just below the holes of the test chamber. Before the odor preference test, a cricket was transferred to the waiting chamber at the waiting position and left for about 4 min to become accustomed to the surroundings. Then the cricket was allowed to enter the test chamber and the test started. Two min later, the relative positions of the banana and apple sources were changed by rotating the container holder. The preference test lasted for 4 min. If the total time of visits of an animal to either source was less than 10 s, we considered that the animal was less motivated to visit odor sources, possibly due to a poor physical condition, and the data were rejected.

2.3. Procedures for aversive conditioning with quinine punishment

We newly developed a procedure for conditioning of a visual pattern with 10% quinine solution, the procedure being the same as that of aversive visual pattern conditioning with sodium chloride solution (Unoki et al., 2006). Either a white-center and black-surround pattern (white-center pattern) or black-center and white-surround pattern (black-center pattern) was used for conditioning. The procedure for the visual pattern preference test was the same as that described previously (Unoki et al., 2006). In short, two white-center patterns and one black-center pattern were presented on a grey sliding wall at the end of the test chamber, and the animal was allowed to freely choose between the two patterns during a test of 4 min in duration. If the total visiting time was less than 10 s, we considered that the animal was less motivated to visit patterns and the data were rejected.