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Formation of Long-term Olfactory Memory in the Cricket *Gryllus bimaculatus*

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Introduction

Insects live in constantly changing environments, where the availability of food sources varies with the seasons. Therefore, the ability to learn to associate a certain cue with an abundant food source must be of great significance for insects (Mizunami *et al.*, 1999). However, most previous studies on olfactory learning in insects have been restricted to the honey bee *Apis mellifera* and the fruitfly *Drosophila melanogaster*. It is obvious that studies in only two species are not sufficient to understand fully the principles of olfactory learning and its neural mechanisms.

Recently, we found that the cricket *Gryllus bimaculatus* (Matsumoto and Mizunami, 2000) and the cockroach *Periplaneta americana* (Sakura and Mizunami, 2001; Sakura *et al.*, 2002; Watanabe *et al.*, 2003) have a developed capacity for olfactory learning. Here we review recent progress on the olfactory learning capabilities of the crickets, which provide a solid basis for future studies of neural mechanisms of olfactory learning and memory.

Long-term retention of olfactory memory

Convincing reports of lifetime memory retention in insects have been limited to adults of social Hymenoptera. We have studied the time span of olfactory memory retention in the cricket (Matsumoto and Mizunami, 2002b). Third- or fourth-instar cricket nymphs were trained to associate one odor with water and another odor with saline solution. At 6 and 10 weeks after training, adult crickets exhibited significantly greater preferences for the odor associated with water over that associated with saline solution. The learned preference was altered when they were given reversal training at 6 weeks after initial training. We conclude that crickets are capable of retaining olfactory memory practically for their lifetime and of easily rewriting it in accordance with experience.

Temporal determinants of olfactory memory

We studied the time relationship between the conditioned stimulus (CS) and unconditioned stimulus (US) to determine long-term olfactory memory retention in the cricket (Matsumoto and Mizunami, 2002a). Crickets underwent appetitive, aversive or differential conditioning trials. In appetitive and aversive conditioning trials, odors of peppermint essence and vanilla essence were paired with a water reward and NaCl solution, respectively. In differential conditioning trials, peppermint odor was paired with water and vanilla was paired with saline solution. The odor preference of crickets was tested before and at 2 h, 1 day and 4 days after training by allowing them to choose between peppermint or vanilla sources. Differential and appetitive conditioning led to long-lasting memory with no significant decay from 2 h to 4 days after training, but memory formed by aversive conditioning had completely diminished 1 day after training. Studies using a differential conditioning procedure have shown that: (i) only four trials were sufficient to attain a saturated level of conditioning; (ii) conditioning was achieved when the conditioning stimulus was presented immediately or at 5 s before the onset of presentation of the US; (iii) the optimal intervals between trials were 2–5 min; and (iv) anesthetic treatment with CO_2 given immediately after training resulted in memory disruption but anesthesia-resistant memory was fully developed 20 min after training. It is concluded that differential conditioning procedure is especially effective for inducing this capability.

The dependency of memory retention on *de novo* brain protein synthesis was studied by injecting the protein synthesis inhibitor cycloheximide (CHX) into the head capsule (Matsumoto *et al.*, 2003). Injection of CHX inhibited 3H-leucine incorporation into brain proteins by >90% for 3 h. Crickets were trained to associate peppermint odor with water and vanilla odor with NaCl solution and were injected with CHX before or at different times after training. Memory retention up to 3–4 h after training was unaffected by CHX injection. However, the level of retention at 6–8 h after training was lowered when CHX was injected. CHX dissociates two phases of olfactory memory in crickets: an earlier protein-synthesis-independent phase (<4 h) and a later (>5 h) protein-synthesis-dependent phase.

Context-dependent olfactory learning

Conditioning in mammals often depends on the general 'context' in which association between CS and US occurs. In insects, the capacity for context-dependent learning has been demonstrated for visual pattern discrimination learning and for learning of the position of a visual target (see discussion in Matsumoto and Mizunami, 2004). However, no context-dependent form of olfactory learning, which is useful for the study of underlying neural processes, has been established in any insects.

We studied the capability of the cricket to select one of a pair of odors and to avoid the other in one context and to do the opposite in another context (Matsumoto and Mizunami, 2004). One group of crickets was trained to associate one of a pair of odors (CS1) with water reward (appetitive unconditioned stimulus, US+) and another odor (CS2) with saline solution (aversive US, US–) under illumination and to associate CS1 with US– and CS2 with US+ in the dark (Figure 1). Another group of crickets received training of the opposite stimulus arrangement. One day after the training for 3 days, the former group significantly preferred CS1 over CS2 under illumination, but preferred CS2 over CS1 in the dark and the latter group exhibited the opposite odor preference. Results of control experiments showed that background light conditions had no significant effects on memory formation or retrieval unless it was explicitly associated with US during training. Thus, the visual context affected learning performance only when crickets were required to use it to disambiguate the meaning of CSs and to predict USs.

An important question to be addressed in our ongoing studies is areas of the insect brain in which the olfactory CS pathway, the gustatory US pathway and the visual contextual stimulus pathway converge to form context-dependent olfactory memory.

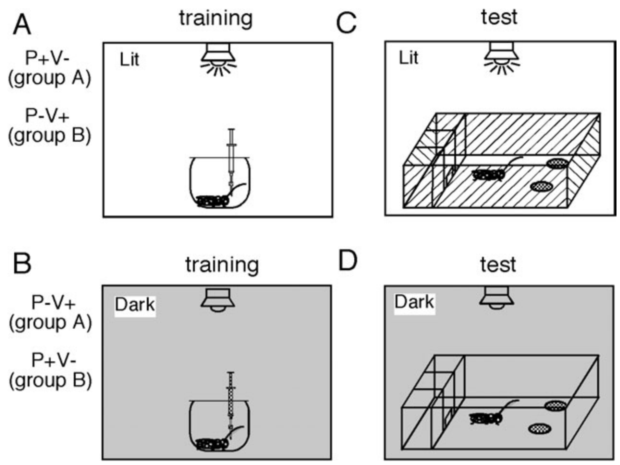

Figure 1 Diagrams of the experimental setup used to study context-dependent olfactory learning capability of crickets. Crickets were subjected to training or testing in a lit or dark condition. For training (A, B), each cricket was presented with peppermint or vanilla odor before the presentation of water (reward) or saline (non-reward) in a beaker. For the odor preference test (C, D), each cricket was allowed to freely choose between peppermint and vanilla sources in a box. For training or testing under illumination (A, C), a white fluorescent lamp on the ceiling was turned on. Crickets in group A received training to associate peppermint odor with water and vanilla odor with saline solution (P+ and V– conditioning trials) under illumination and that to associate vanilla odor with water and peppermint odor with saline solution (P– and V+ conditioning trials) in the dark. Crickets in group B received training with an opposite stimulus arrangement. Modified from Matsumoto and Mizunami (2004).

Conclusion and future perspectives

These results show that crickets have an excellent capacity for olfactory learning, characterized by quick acquisition, long retention and easy re-writing of memory. Crickets can be easily used for detailed pharmacological and electrophysiological studies and may emerge as excellent subjects for the study of the neural basis of olfactory learning.

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