that underlie the observed correlations should contribute to our understanding of the metastatic process.

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The in vitro Classical Conditioning of the Gill Withdrawal Reflex of *Aplysia californica*

Abstract. *Associative learning has been demonstrated in a reduced siphon, mantle, gill, and abdominal ganglion preparation of* Aplysia. *The preparations learned to respond to a previously neutral stimulus as a consequence of training in a classical conditioning paradigm. Backward conditioning, presentation of the conditioned stimulus alone, or presentation of the unconditioned stimulus at some random interval after presentation of the conditioned stimulus failed to produce conditioning. This model system can be used to study the neural mechanisms underlying associative learning.*

If simple model systems such as the well-studied *Aplysia* preparation (*1*) exhibit associative learning (*2*), the neuronal mechanisms underlying learned behavior may be analyzed. Already much of our understanding of the neuronal mechanisms underlying two types of nonassociative learning—habituation and sensitization—have been obtained from the *Aplysia* model system (*3*). Although it has been difficult in the past to demonstrate associative learning in *Aplysia* (*4*), associative learning has recently been conclusively demonstrated

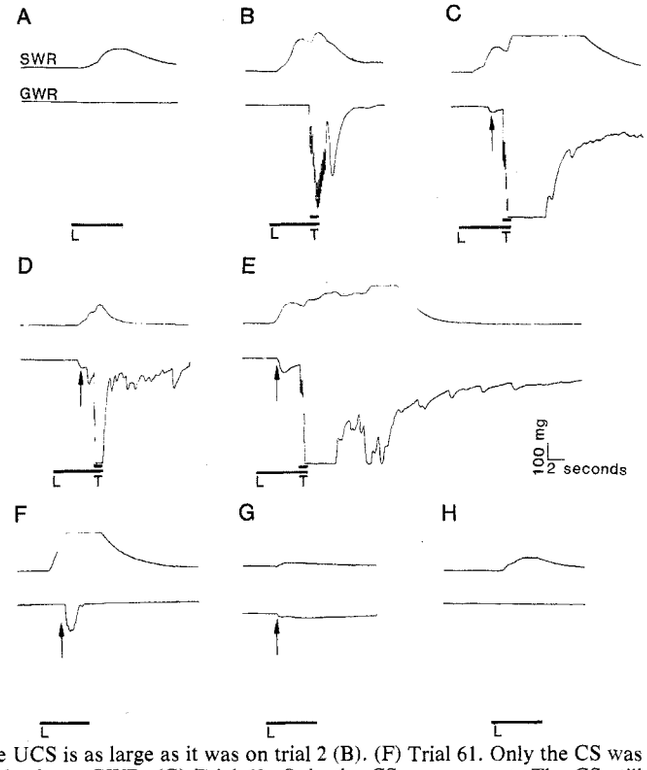

Fig. 1. Data from a preparation in the experimental forward conditioning group. (A) Trial 1. The CS [light (*L*)] was presented to the preparation on trial 1; it evoked a siphon withdrawal response (*SWR*), but did not evoke a gill withdrawal reflex (*GWR*). The CS is indicated by a bar under the tracings. (B) Trial 2. The first trial on which the CS was paired with the UCS [tactile stimulus (*T*)]. The CS evoked only an SWR. The UCS, however, evoked a large GWR as well as an SWR. The GWR evoked by the UCS did not readily habituate. (C) Trial 25. The CS evoked not only an SWR but also a small GWR (arrow). (D) Trial 40. (E) Trial 60. The GWR evoked by the UCS is as large as it was on trial 2 (B). (F) Trial 61. Only the CS was presented, and it evoked a large GWR. (G) Trial 63. Only the CS was present. The CS still evoked the GWR but its amplitude was smaller than it was on trial 61. (H) Trial 65. CS alone. It no longer evoked a GWR. Presentation of the UCS also led to sensitization of the SWR.

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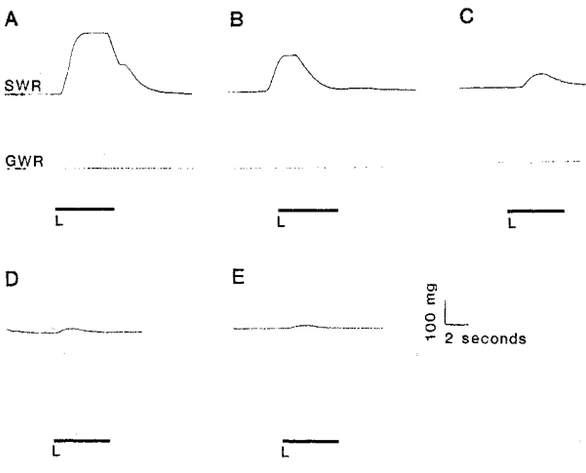

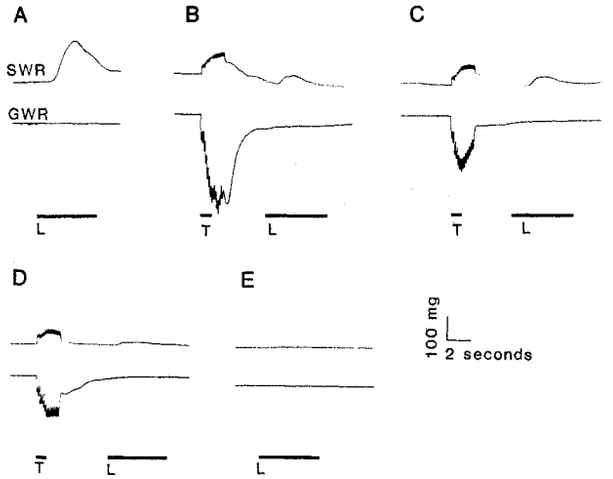

Fig. 2 (left). Representative preparation to which only the CS was presented. (A) Trial 1, (B) trial 20, (C) trial 35, (D) trial 65, and (E) trial 80. The SWR evoked by the CS habituated; even after 80 presentations of the CS, it did not evoke a GWR. Fig. 3 (right). Representative preparation from the backward conditioning group. (A) Trial 1 and (B) trial 2. The UCS preceded the CS and evoked a GWR and an SWR; the CS evoked only the SWR. (C) Trial 40, (D) trial 70, and (E) trial 80. In none of the backward conditioning preparations did the CS ever evoke a GWR even after 100 trials.

in intact molluscan preparations such as *Pleurobranchaea* (*5*), *Limax* (*6*), and now *Aplysia* (*7*). Since the neural circuits that mediate the conditioned behaviors in these animals have not been completely elaborated, what is required is an in vitro preparation that exhibits true associative learning. We have therefore focused our attention on probably the most-studied model system: the siphon, mantle, gill, and abdominal ganglion preparation of *Aplysia californica*. We now report that the gill withdrawal reflex (GWR) in this in vitro preparation can be classically conditioned. Thus, this system can now be used to study the neural mechanisms underlying associative learning, just as it has previously been used to study the mechanisms underlying nonassociative learning.

Twenty-five *A. californica* (150 to 250 g; Pacific Biomarine) were divided into four groups: experimental classical conditioning (N = 10); backward conditioning control (N = 5); conditioning stimulus (CS) alone (N = 5); random presentation control (N = 5) (*8*). The in vitro preparation consisted of the siphon, mantle, gill, and abdominal ganglion and has been described in great detail elsewhere (*9*). The siphon, branchial, ctenidial, and pericardial nerves were left intact; all other nerves and connectives were severed.

The CS was a 6-second light (photic) stimulus from 6-V microscope lamp, which illuminated only the siphon. The CS evoked only siphon withdrawal and not a GWR. The siphon response to the light is mediated by photoreceptors in the siphon (*10*). The unconditioned stimulus (UCS) consisted of a train (six per second for 1 second) of tactile stimuli (1.2 g) applied to the gill by the "tapper" (*11*). The UCS always evoked a GWR, which did not readily habituate.

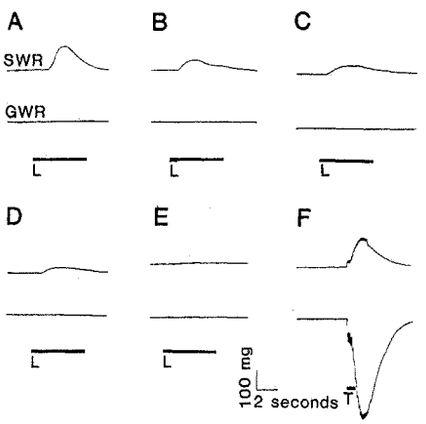

Fig. 4. Representative preparation from the random presentation group. (A) Trial 1, (B) trial 25, (C) trial 35, (D) trial 50, (E) trial 80. (F) The GWR evoked by the UCS between trials 24 and 25.

Trials occurred once every 5 minutes for at least 80 trials. In the classical conditioning group, the CS was presented and followed after 5 seconds by the UCS. The stimuli were terminated at the same time. In the CS-alone control group, the CS was presented alone. In the backward conditioning control group, the UCS was followed after 5 seconds by the CS. In the random control group, the CS was presented once every 5 minutes, and at a random time during that interval, the UCS was presented. A microprocessor system controlled the presentation of the stimuli to the preparation.

In the experimental classical conditioning group, the CS initially evoked only a siphon withdrawal response. However, after the CS and the UCS were paired, the CS evoked the GWR (which had initially been evoked only by the UCS) in seven of ten preparations by trial 25 (Fig. 1).

In order to demonstrate that this was a true example of associative learning and not of nonassociative learning, a series of control experiments were performed. In the CS-alone control group, even after 80 trials the CS did not evoke the GWR. Indeed, the only change that occurred was habituation of the siphon withdrawal response (Fig. 2). In the backward conditioning group, the CS did not evoke the GWR (Fig. 3). Finally, in the random presentation group, the CS did not come to evoke a GWR even after 80 trials (Fig. 4). Thus, according to the criteria developed for demonstrating associative learning in mammals set out by Rescola (*8*), associative learning has been demonstrated in the in vitro *Aplysia* siphon, mantle, gill, and abdominal ganglion preparation.

No systematic study has yet been attempted to determine the rate of extinction of the conditioned response, but it does seem to take at least five unpaired trials (Fig. 1, F to H). No effort has yet been made to show more rapid learning after extinction. We have also not at-

tempted to determine how long the learning would persist in this preparation.

In naïve preparations, the presentation of the CS does not normally evoke activity in gill motor neurons L_7 and LDG_1 (*10*). However, it may be that with classical conditioning the CS will evoke activity in these neurons. If so, a neural correlate of associative learning can be examined.

We have shown for what we believe to be the first time that a well-studied in vitro model system has the capacity for associative learning. All the advantages that the *Aplysia* siphon, mantle, gill, and abdominal ganglion preparation has had for the study of nonassociative adaptive behaviors, such as habituation and sensitization, can now be used to study the neural mechanisms underlying associative learning.

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2. Associative learning is defined in an operational manner and by control procedures [G. J. Mpitsos, S. D. Collins, A. D. McClellan, *Science* **199**, 497 (1978)]. Essentially, an initially ineffective stimulus comes to evoke a response initially evoked only by an innately effective stimulus, after the preparation has experienced the two stimuli in close temporal association.
3. Habituation and sensitization have been defined as types of nonassociative learning because temporal pairing of stimuli are not required to produce a response decrement (habituation) or a facilitated response (sensitization). Many believe, however, that habituation and sensitization are primitive forms of learning that may constitute the base of more complex learning (*1*). For an analysis of the neuronal mechanisms underlying aspects of habituation and sensitization see V. F. Castellucci, T. J. Carew, E. R. Kandel, *Science* **202**, 1306 (1978); M. Klein and E. R. Kandel, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3512 (1978).
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Immunization of Baboons With *Schistosoma mansoni* Cercariae Attenuated by Gamma Irradiation

Abstract. *Studies on the efficacy of a vaccine against schistosomiasis in young baboons (*Papio anubis*) disclosed that immunization with* Schistosoma mansoni *cercariae attenuated by gamma irradiation induced significant protection against subsequent infection with normal, viable* S. mansoni *cercariae. Such immunization resulted in reduced worm burdens (70 percent) and egg excretion rates (82 percent). These results support immunization as a potential method for schistosomiasis control.*

The most promising vaccines against schistosomiasis have been prepared with gamma-irradiated schistosome cercariae (*1–5*). Recently, however, Taylor *et al.* (*6*) reported that immunization with *Schistosoma mansoni* cercariae attenuated with low levels of irradiation (2.1, 2.4, or 6.0 kilorads) failed to induce significant resistance in baboons subsequently challenged with normal, viable *S. mansoni* cercariae. Schistosomiasis in the baboon is a chronic disease, as it is in man (*7*), and if a vaccine cannot be demonstrated to be effective in the baboon model, it is unlikely to be successful in man.

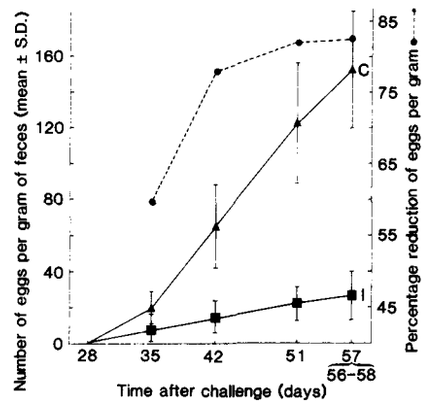

Fig. 1. Comparison of the mean fecal egg load in nine immunized (*I*) and ten nonimmunized (*C*) *Papio anubis* baboons. Each triangle and square dot and associated vertical line represent the mean and standard deviation. The dotted line represents the percentage reduction of eggs per gram of feces calculated according to the formula $[(C - I)/C] \times 100$. Fecal samples were obtained 7, 16, 28, 35, 42, 51, and 56 to 58 days after the baboons received the challenge dose.

The present study was conducted to determine whether attenuation of cercariae with high doses of ionizing radiation might yield an effective vaccine; such a vaccine had proved effective in NIH/Nmri CV mice (*5*). Nineteen young (6 to 8 kg) baboons (*Papio anubis*) from Kenya were used for the experiments. They were first subjected to three stool examinations (*8*) and to screening tests for circulating schistosomal antigen and antibody (*9*). Nine baboons (five males and four females) were immunized and ten baboons (five males and five females) were used as nonimmunized controls. For most studies of schistosome vaccines prepared with irradiation attenuated parasites, the mouse has been used; we therefore conducted a parallel monitoring experiment with 48 mice (6-week-old females of the NIH/Nmri CV strain).

Cercariae of *S. mansoni* (Puerto Rican strain) shed from *Biomphalaria glabrata* snails were attenuated (*10*) and used for

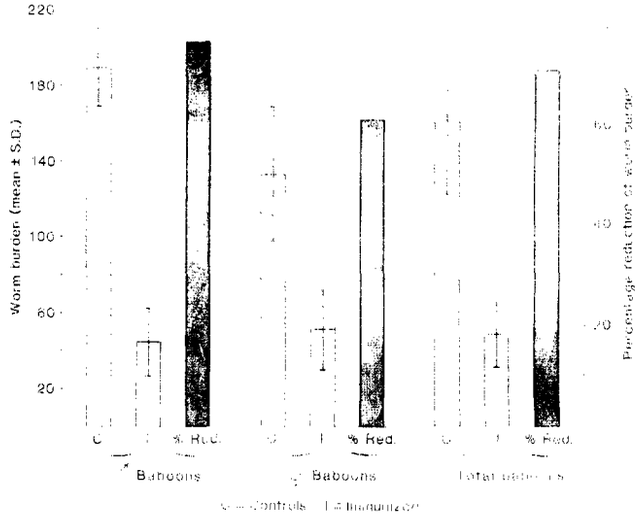

Fig. 2. Comparison of the worm burdens of nine immunized (*I*) baboons and ten nonimmunized (*C*) baboons challenged with 235 ± 22 normal *S. mansoni* cercariae. The open bars represent the mean (± standard deviation) worm burdens, and the shaded bars, the percentage reduction of the burdens calculated according to the same formula as that in the legend for Fig. 1.

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