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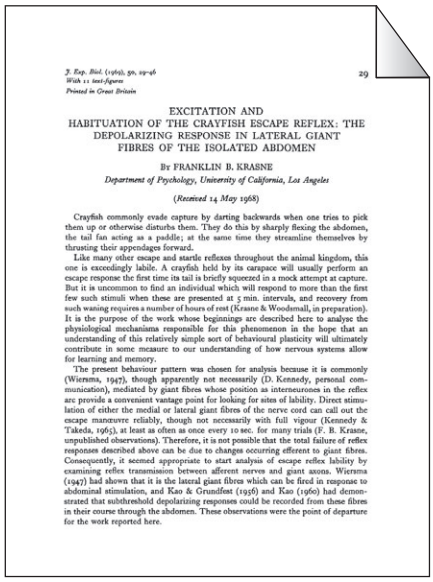
EXCITATION AND HABITUATION OF CRAYFISH ESCAPE



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EXCITATION AND HABITUATION OF THE CRAYFISH ESCAPE REFLEX: THE DEPOLARIZING RESPONSE IN LATERAL GIANT FIBRES OF THE ISOLATED ABDOMEN

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Crayfish commonly evade capture by darting backwards when one tries to pick them up or otherwise disturbs them. They do this by sharply flexing the abdomen, the tail fan acting as a paddle; at the same time they streamline themselves by thrusting their appendages forward.

Like many other escape and startle reflexes throughout the animal kingdom, this one is exceedingly labile. A crayfish held by its carapace will usually perform an escape response the first time its tail is briefly squeezed in a mock attempt at capture. But it is uncommon to find an individual which will respond to more than the first few such stimuli when these are presented at 5 min. intervals, and recovery from such waning requires a number of hours of rest (Krasne & Woodsmall, in preparation). It is the purpose of the work whose beginnings are described here to analyse the physiological mechanisms responsible for this phenomenon in the hope that an understanding of this relatively simple sort of behavioural plasticity will ultimately contribute in some measure to our understanding of how nervous systems allow for learning and memory.

The present behaviour pattern was chosen for analysis because it is commonly (Wiersma, 1947), though apparently not necessarily (D. Kennedy, personal communication), mediated by giant fibres whose position as interneurones in the reflex arc provide a convenient vantage point for looking for sites of lability. Direct stimulation of either the medial or lateral giant fibres of the nerve cord can call out the escape manœuvre reliably, though not necessarily with full vigour (Kennedy & Takeda, 1965), at least as often as once every 10 sec. for many trials (F. B. Krasne, unpublished observations). Therefore, it is not possible that the total failure of reflex responses described above can be due to changes occurring efferent to giant fibres. Consequently, it seemed appropriate to start analysis of escape reflex lability by examining reflex transmission between afferent nerves and giant axons. Wiersma (1947) had shown that it is the lateral giant fibres which can be fired in response to abdominal stimulation, and Kao & Grundfest (1956) and Kao (1960) had demonstrated that subthreshold depolarizing responses could be recorded from these fibres in their course through the abdomen. These observations were the point of departure for the work reported here.

Donald Edwards discusses Franklin B. Krasne's 1969 paper entitled: Excitation and habituation of the crayfish escape reflex: the depolarizing response in lateral giant fibres of the isolated abdomen.

A copy of the paper can be obtained from http://jeb.biologists.org/cgi/content/abstract/50/1/29

This paper describes one of the first attempts to analyze the synaptic basis for the release of an animal's fixed action pattern, which controls a behavior such as the crayfish escape response. It is also one of the early demonstrations that the neural mechanisms of a simple form of learning, known as habituation, are located in the central synapses of the neural circuit that produced the behavior.

The tail flip behavior of crayfish was one of the simple but dramatic escape responses of animals, like the startle response of fish and the jet propulsion escape of squid, that drew the attention of zoologists and physiologists in the first half of the 20th century. More complex than simple reflexes, these responses result from a 'decision' reached by the animal in response to a specific sort of stimulus. Once triggered, the responses orchestrate the behavior of the animal's entire body. Finally, these escape behaviors are often found to be subject to simple forms of learning, including habituation, dishabituation and sensitization. For the physiologists, an additional attraction was that, in several animals, a 'giant' interneuron was key to the release of the escape behavior. In squid, investigation of the giant neuron and giant synapses led to the discovery of the basic mechanisms of the action potential (Hodgkin and Huxley, 1952) and synaptic transmission (Katz and Miledi, 1967). In fish, studies on the Mauthner cell and its eight nerve inputs led to the discovery of ephaptic inhibition, in which a positive extracellular potential created around the axon hillock effectively hyperpolarizes the local cell membrane and so decreases its excitability (Furukawa and Furshpan, 1963). In the crayfish, the story began with Wiersma's identification of the lateral giant interneuron as key to triggering the tail flip escape in response to a sharp tap on the animal's abdomen (Wiersma, 1947; Wiersma, 1938), and was followed in a few years by the description of both rectifying electrical synapses and depolarizing inhibition at the synapse between the lateral giant interneuron and the giant motoneuron (Furshpan and Potter, 1959; Furshpan and Potter, 1957).

In each of these animals, a single spike in the giant neuron was found to be sufficient to evoke the entire escape behavior, or fixed action pattern. These discoveries provided strong support for the hierarchical decision architecture proposed by Nikolaas Tinbergen (Tinbergen, 1951), and helped promote the notion of the 'command neuron', first articulated for the circuit that controlled swimmeret beating in crayfish (Wiersma and Ikeda, 1964). The lateral giant interneuron and the medial giant interneuron appeared to fit this notion, as each excited overlapping but distinct sets of motoneurons with a single spike to produce, respectively, an upward and a rearward escape response (Mittenthal and Wine, 1973; Wine and Krasne, 1972).

Just as the lateral giant interneuron-mediated escape appeared to provide a system in which the neural control of fixed action patterns could be studied, it also appeared to provide a system where questions about the neural bases of behavioral habituation could be asked. At the time of Krasne's paper (Krasne, 1969), it was unclear whether learning was mediated by intrinsic changes to the neural circuits that controlled specific behavior patterns, as suggested by Donald Hebb (Hebb, 1949), or whether, as in the still new digital computers, learning resided in special circuits that could interact with circuits controlling behavior (von Neumann, 1958). For crayfish escape, the relevant question was whether habituation of the escape response occurred because the afferent pathway to the lateral giant interneuron, or the lateral giant interneuron itself, became less excitable with repeated stimulation, or because increasingly strong inhibition was imposed on the lateral giant interneuron circuit from elsewhere in the nervous system. Both possibilities were attractive. The first agreed with the then

popular view that synapses were the likely site of plasticity, whereas the second was suggested by the fact that strongly habituated responses could be dishabituated by a sensory stimulus that had no direct effect on escape behavior.

Franklin Krasne approached these two questions in the same simple, direct manner (Krasne, 1969). He used a microelectrode to penetrate the initial segment of the lateral giant interneuron axon where postsynaptic potentials could be recorded in response to shock of nerves containing sensory axons that innervate the periphery. A single, brief shock evoked a fast-rising excitatory postsynaptic potential in the lateral giant interneuron that consisted of several depolarizing waves whose amplitude and latency varied directly and inversely, respectively, with the strength of the shock. The first of these waves, labeled 'alpha' occurred with such a short latency that it seemed likely to result from a direct, or monosynaptic, input from the primary afferent axons stimulated by the shock. The later waves, particularly the second or 'beta' wave, also increased with the stimulus shock, but often to a greater degree than the alpha wave. This suggested that if the alpha excitatory postsynaptic potential was monosynaptic, the beta excitatory postsynaptic potential was produced through a pathway that was di- or tri-synaptic, involving as yet unidentified interneurons that were likely excited by some of the same primary afferents responsible for the alpha excitatory postsynaptic potential. Moreover, the individual contributions of some of these interneurons to the beta excitatory postsynaptic potential were identifiable when the excitatory postsynaptic potential experienced a step-like variation in amplitude between stimuli as the shock was slightly increased or decreased. Finally, the alpha excitatory postsynaptic potential was always subthreshold for firing the lateral giant interneuron, whereas the beta excitatory postsynaptic potential, riding on the declining phase of the alpha excitatory postsynaptic potential, could reach the lateral giant interneuron firing threshold with a sufficiently strong stimulus.

These results led Krasne to conclude that (i) many primary afferents converged on the lateral giant interneuron, each to create only a small excitatory postsynaptic potential, such that even when they were synchronously active they could not excite the lateral giant interneuron; (ii) many of the same afferents also excited a set of mechanosensory interneurons that also converged on the lateral giant interneuron. These created larger excitatory postsynaptic potentials which, when summated by synchronous excitation, could excite the lateral giant interneuron.

The second experiment was the same as the first, except that the stimulus was repeated at a constant interval. If an individual stimulus was superthreshold, the first few stimuli of a series would each excite the lateral giant interneuron, but later stimuli would not. Because each lateral giant interneuron spike would trigger an escape tail flip in a freely behaving animal, these responses were the neural correlates of a behavioral habituation of the tail flip escape response to repetitive stimulation.

Once the lateral giant interneuron no longer fired, the beta excitatory postsynaptic potentials that had triggered the lateral giant interneuron spike became apparent. These excitatory postsynaptic potentials continued to fall in amplitude with repeated stimulation until they reached a plateau level of response that was characteristic of the stimulus frequency. The response declined as the different components of the beta postsynaptic potential first increased their response latency and then failed altogether, suggesting that the presynaptic interneuronal spikes would follow the same dynamic.

The beta excitatory postsynaptic potential displayed two other characteristics of behavioral habituation: their amplitude would recover, along an exponential time course, with rest (i.e. no stimulation), and their amplitude would increase with an increase in stimulus intensity, but then decline to near the earlier habituated level. These experience-dependent changes of the beta excitatory postsynaptic potential were in contrast to the alpha excitatory postsynaptic potential, which experienced none of them, and retained an amplitude proportional to the stimulus intensity.

From these results, it was clear that changes in the afferent path to the lateral giant interneuron produced a decrease in the beta excitatory postsynaptic potential of the lateral giant interneuron in response to repetitive stimulation. Moreover, because the alpha excitatory postsynaptic potentials were unaffected, these changes appeared to be confined to the response of the interneurons in the afferent path that were presynaptic to the lateral giant interneuron, or to their synaptic contacts with the lateral giant interneuron. Experiments published elsewhere showed that these changes still occurred even when synaptic inhibition, which might have caused the changes in transmission, was blocked. However, these results did not appear to account for all habituation of the escape response, which was evident behaviorally at slow stimulus repetition rates where no effect on the beta excitatory postsynaptic potential was seen in the reduced preparations that Krasne was studying.

With two simple experiments, Krasne's paper reached two major conclusions. First, it completed the general outline for the afferent path to the lateral giant interneuron, and therefore for the entire escape circuit. This was one of the first, if not the first, polysynaptic circuits for a fixed action pattern that had been so described. Second, it demonstrated that much, but not all, of behavioral habituation of the escape response could be accounted for by synaptic depression within one limb of the afferent path that carries nervous signals to the lateral giant interneuron. As the paper suggested, descending inhibition has since been shown to be the other major contributor to habituation of the escape response when descending pathways from higher parts of the nervous system are intact (Shirinyan et al., 2006).

Krasne's description of both the afferent path to the lateral giant interneuron and the role and site of synaptic depression was sustained by Zucker's elegant study shortly thereafter, which identified the interneurons that produce the beta excitatory postsynaptic potential and showed that depression at synapses between the primary afferents and those interneurons accounts for habituation of the lateral giant interneuron's response (Zucker, 1972; Zucker et al., 1971). Moreover, Krasne's paper provided the foundation for many more papers that described a host of phenomena, including protection against reafference through presynaptic inhibition (Bryan and Krasne, 1977; Kennedy et al., 1974), mechanisms of serotonergic modulation (Antonsen and Edwards, 2007; Lee et al., 2008), and even long-term synaptic potentiation, a process linked in other animals to mechanisms of learning and memory (Tsai et al., 2005).

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