

## The Crayfish: background and related neurological research pertaining to Cheliped (Claw) research.

Crayfish belong to the Decapoda crustacean order with 10000 species. There are two superfamilies of crayfish: Astacoidea and Parastacoidea. The Astacoidea contains the families Astacidae and Cambaridae and Parastacoidea contains the family Parastacidae. Astacidae and Cambaridae occur in the Northern Hemisphere and the Parastacidae are confined to the Southern Hemisphere. They live in areas in Europe, Australia, New Zealand, North and South America and Asia. Lacking a water tight exoskeleton, yet having gills, the crayfish has done well in establishing many colonies in areas of aquatic, semi-aquatic and high humidity environments. Only 10 percent of crayfish are freshwater species. The freshwater crayfish is a omnivore who primarily feeds on aquatic vegetation, other invertebrates, and associated detritus; particles of decomposing plant and animals.<sup>1</sup> They are most vulnerable to predators during juvenile and post molting states of life. When first hatched, crayfish face predators include dragonfly nymphs, water bugs and water beetle larvae. Later fish, various mammals and birds regularly prey on the crayfish.<sup>1</sup> In many areas of the world they are considered an luxury food item. Maintaining the crayfish can be done by feeding them lettuce, carrots, watercress, potatoes, bananas, tomatoes and each other to name a few.<sup>1</sup> The crayfish has been a good subject for studies on all aspects of innervation including identifying neuromuscular junctions and their differences, axon types, muscle types, neurotransmitters, behavior, and long term modification of nerve and muscle types.

A reported characteristic of arthropods is the polyneuronal and multiterminal innervation of the muscles. Polyneuronal refers to the innervation of a muscle fiber by more than one motor axon. Multiterminal refers to the innervation of

the muscle fiber by more than one neuromuscular junction. The various types of innervation on the muscle fiber is seen in figure 1 below.

### **SOMETHING IS MISSING**

By testing end plate potentials and their uniformity across the muscle, P. Fatt and B. Katz confirmed polyneuronal and multiterminal innervation of crayfish skeletal muscle in the claw in 1953.<sup>2</sup> They found that with muscle innervation, they were able to record nearly identical end plate potentials (EPPS) all along the length of the muscle fiber. They also found when stimulating the dactylopodite with the fast excitatory (FE) or the slow excitatory (SE) axons, that both created an equal EPPS on the same muscle fibers. It was noted that there was two ways of conducting the stimulus throughout the muscle; electrotonic currents and distributed nerve endings. The electrotonic ability of muscle was found to have an average length of .9mm by Fatt and Katz in an earlier paper.<sup>3</sup> Because their readings were separated by more than .9 mm for each trial, Fatt and Katz concluded the EPPS were the result of the many distributed nerve endings.

Movement of crayfish consists of walking and a backwards darting movement. During walking the Cheliped claw is held forward to balance the abdomen weight. When walking downstream they often attach the cheliped claws to the river bed to prevent being swept away. The cheliped is also used to control water movement over the body by sweeping the water over or under the main torso. The Cheliped is also used to capture prey and direct food to the mouth. They have a rigid exoskeleton which must be molted to allow for growth several times in their life span. Movement is isolated to the flexible articular membrane between the segments. The skeletal muscle allows movements by antagonistic pairs of muscle which extend across the segments attaching by tonofibrils. The contraction of the flexor and relaxation of the extensor allows

movement. In the appendages the movement between the 6 segments, (protopodite, ischiopodite, meropodite, carpopodite, propodite, dactylopodite), is limited to only one plane with each articulation between the segments having an different axis. Combined, the appendage can move in more than one plane. When reviewing research it is common for the cheliped limb to be limited to a description of the meropodite, propodite, and dactylopodite.

The muscles are innervated by motor and inhibitor neurons in varying amounts. During earlier studies<sup>5</sup> it was understood there was one excitatory neuron (OE) and one inhibitory neuron (OI) in the opener muscle of the dactylopodite. In later studies<sup>6</sup> a second inhibitory neuron called a common inhibitory (CI) was found to innervate the bender, extensor, accessory flexor and the closer muscle of crabs. Theodore Weins found in 1984 CI innervation of all seven muscle structures in the cheliped of the crayfish.<sup>7</sup> Weins found that the CI was able to reduce postural tension during locomotion and facilitated rapid movements. The method used in determining the innervation revolved around the stimulation of the CI nerve and simultaneously measuring the Inhibitory Junction Potentials (IJP's) of the bender and accessory flexor muscles which are innervated by the CI only, and the measurement of any potential changes in the tonic muscles of the flexor muscle. Wiens found matching IJP's in all three. The location of the neuromuscular junction varied in each muscle group. In the opener muscles of the dactylopodite, there was innervation in only a small bundle on fibers in the proximal end of the muscle. Measurements for similar IJP's didn't register CI for the opener muscle initially. By using a stress gauge on the dactylopodite and measuring tension with and without simultaneous CI excitation Wein was able to detect the reduced tension caused by an apparent innervation by the CI nerve. Further measurements with hook electrodes in all areas of the opener muscle then showed the location of the CI innervation. These results were gathered using the European crayfish *Astacus leptodactylus* and the American *Procambarus*

clarkii. The current understanding of claw muscle inhibition is shown below in figure 2.

### **SOMETHING IS MISSING**

Motor output in the cheliped can be antagonistic where OE and OI are activated together. The net behavior effect depends on their frequency ratio of the two neurons. (Ripley and Wiersma, 1953). This is explained by Wiens<sup>7</sup> as the result of motoneurons in the appendage serving more than one role, i.e. the opener of the dactylopodite also extending the propodite, using peripheral inhibition to limit interference with antagonistic muscles, the cross-connected synaptic claw motoneurons.<sup>7</sup> An example of cross-connected synaptic motoneurons is seen when the opener inhibitor (OI), slow closure excitor (SCE), and the fast closure excitor (FCE), mutually excite each other when one neuron is stimulated.<sup>7</sup>

The muscle is characterized as slow (tonic) and fast (phasic). The phasic and slow twitch muscles are prominent in the limb and abdominal muscles.<sup>13</sup> Phasic muscle are capable of fast contraction speeds and short periods of fast stimulation where glycolysis is the primary fuel supply. Oxidative metabolism is the primary fuel for the tonic muscle where slow and sustained contractions for longer duration are required.<sup>13</sup> If a researcher wanted to characterize the two muscle types by visual means, the phasic muscles are generally characterized by short sarcomeres in the muscle fiber and the tonic have longer sarcomeres. The sarcomere length in crustaceans varies between 2 to 20  $\mu\text{m}$  with phasic being less than 4 $\mu\text{m}$  and the tonic being greater than 6  $\mu\text{m}$ .<sup>12</sup> It is noted that there are many varying lengths in-between these general guidelines. The corresponding motoneurons for fast and slow excitation innervate the phasic and tonic muscles in the limb.<sup>13</sup> In considering the development of muscle and neuromuscular synapses it is found the

differentiation into phasic and slow twitch muscle types is preceded by the development of the neuromuscular synapse and may require this order.<sup>13</sup> The synapses grew in size during development and the excitator synapses developed faster than the inhibitory synapses in the lobster claw. this may be true for the crayfish as well.<sup>4</sup>

The activity of mitochondria in the neuron is a marker of what type of neuron you have. The slow axon's mitochondria vary in three ways: larger, different shape with a varicose look, is more prevalent throughout the axon. This was discovered using fluorescent dyes such as Rhodamine-123 and 4-Di-2-Asp which fluoresce more brightly with higher numbers of mitochondria. By blocking the oxidative metabolism through 2,4-dinitrophenol (DNP) the slow axons produced enhanced EPSP's briefly and then within five minutes EPSP's approached zero potential change. <sup>13</sup> The effect is attributed to the reduced ability of the axon terminal to regenerate and refill synaptic vesicles. Since the type of neuron determines the type of muscle how does the neuron change? Autotomy and seasonal changes requiring behavioral modifications may be some reasons a fiber changes between phasic and tonic types. G.A. Lnenicka found that fast axon terminals become more varicose in summer producing smaller and fatigue resistant EPSP's and return to filiform in shape in winter producing larger fatigable EPSP's.<sup>13,15</sup> Lnenicka and Atwood<sup>13,14</sup> conducted research on the normally inactive phasic closure muscles in captive *Procambarus clarkii* crayfish. They implanted electrodes which produced additional stimulation to the fast axon in the limb. The result was a long term change in the fast axon through increased mitochondrial numbers. Measurements done on the phasic muscle this axon innervated in the limb showed smaller EPSP's and higher fatigue resistance.<sup>13</sup>

This adaptive change is shown below in figure 3.

## **SOMETHING IS MISSING**

Neurotransmitters, neurohormones and their interactions play a role in the skeletal muscle of the crayfish. Inhibitory effect is seen in the Presynaptic neuromuscular synapse as “presynaptic inhibition”. This inhibition develops by the increased membrane conductance of the presynaptic terminal caused by increased inhibitory transmitter release.<sup>9</sup> In the excitatory motor axon terminal this results in a hyperpolarization potential causing in a lower amplitude of the EPSP which occurs after the IPSP. The neurotransmitters involved in the crayfish limb include Acetylcholine,  $\gamma$ -Aminobutyric Acid (GABA), and Glutamate.<sup>9</sup> The GABA is exclusively seen in the inhibitor motor neurons. There are four types of GABA receptors in the crayfish.<sup>9</sup>

1. Slowly desensitizing, B-guanidino-propionic acid (B-GP) insensitive found in the postsynaptic receptors of the opener muscles.
2. Slowly desensitizing, B-GP sensitive presynaptic receptors of the opener muscles.
3. Rapidly desensitizing, B-GP sensitive postsynaptic receptors of the closer muscles.
4. Rapidly desensitizing, B-GP slight sensitivity presynaptic receptors of the closer muscles.

The Glutamate neurotransmitter increases postsynaptic membrane potential by increasing permeability of  $\text{Na}^+$ ,  $\text{Ca}^{++}$  and  $\text{K}^+$ . This pushes the membrane potential towards zero or plus 50 depending on if the proximal or central muscle fibers are stimulated.<sup>9</sup> It is noted this difference is the result of central muscle fibers having no  $\text{K}^+$  channel and therefore slowing down conductance.<sup>10</sup> Large levels of glutamate at the neuromuscular synapse will result in desensitization as well and reduce the effect of the EPSP. The

inactivation of glutamate is the result of the uptake by glial cell that surround the synapses. The acetylcholine neurotransmitter doesn't release at the neuromuscular junctions involving skeletal muscle.<sup>10</sup> Some evidence of it has been found in the stomach muscles. The skeletal muscles are effected by the inhibition caused by GABA and the excitation caused by glutamate and the effects of three neurohormones: serotonin, octopamine, and dopamine. These neurohormones play a role on the pre and post synaptic side. Dopamine works against Octopamine and Serotonin by reducing tension in the muscles. The amines affect the second messenger cAMP in both the neurons terminal and the muscle which can result in increased  $Ca^{++}$  levels.<sup>10</sup> These intracellular  $Ca^{++}$  levels regulate the amount of neurotransmitter released. The amount of neurotransmitter released by the crustacean is small (less than 10 quanta per impulse) compared to the release of frog muscle ( hundreds of quanta per impulse).<sup>9</sup> This was found to differ because the neuromuscular synapse of the vertebrate was much larger than the crustacean due to the crustacean muscle having poly and multiinnervation of muscle fibers with smaller neuromuscular junctions.<sup>9</sup>

## References

1. Holdich, D.M. & Lowery, R.S. 1988. *Freshwater Crayfish Biology, Management, Exploitation*. Croom & Helm, London. QL444 .M33 F73
2. Fatt, P. & Katz, B. 1953. Distributed 'End Plate Potentials' of Crustacean Muscle Fibers. *Journal of Experimental Biology* **30**, pp.433-439
3. Fatt, P. & Katz, B. 1953. The electrical properties of crustacean muscle fibers. *Journal of Physiology*. **120**, pp.171-204.
4. CK Govind. 1982. Development of Nerve, Muscle and Synapse. In H.L. Atwood and D.C. Sandeman, *The Biology of Crustacea Vol 3*, pp. 185-203. Academic Press, New York.
5. van Harrveld, A. 1936. Doubly-, triply-, and quintuply-innervated crustacean muscles. *Journal of Comparative Neurology* **70**, pp. 428-432.
6. Wiersma, C.A.G. 1941. The inhibitory nerve supply of the leg muscles of different decapod crustaceans. *Journal of Comparative Neurology* **74**, pp. 63-79
7. Wein, T.J. 1985. Triple Innervation of the Crayfish Opener Muscle: The Astacuran Common Inhibitor. *Journal of Neurobiology* **16**, pp. 183-191
8. Weins, T.J. 1982. Small Systems of Neurons: Control of Rhythmic and Reflex Activities. In Atwood, H.L. & Sandeman, D.C. *The Biology of Crustacea Vol 4*, pp. 193-240. Academic Press, New York.
9. Atwood, H.L. 1982. Synapses and Neurotransmitters. In Atwood, J.L. & Sandeman, D.C. *The Biology of Crustacea Vol 3*, pp. 105-150. Academic Press, New York.
10. Taraskevich, P.S. 1975. Dual effect of l-glutamate on excitatory postjunctional membranes of crayfish muscle. *Journal of General Physiology*. **65**, pp. 677-691.
11. Bergren, W.R. and Wiersma, C.A.G. 1938. Chemical Changes In The Adductor Muscle of the Cheliped of the Crayfish in Relation to the Double Motor Innervation. *Journal of General Physiology* **22**, pp. 193-206.
12. Govind, C.K. 1987. Muscle and Muscle Fiber Type Transformation In Clawed Crustaceans. *American Zoologist* **27**, pp. 1079-1098.
13. Atwood, H.L. & Nguyen, P.V. 1995. Neural Adaptation in Crayfish. *American Zoologist* **35**, pp. 28-35.
14. Lnenicka, G.A. & Atwood, H.L. 1985a. Age dependent long-term adaptation of crayfish phasic motor axon synapses to altered activity. *J. Neurosci.* **5**, pp. 459-467.
15. Lnenicka, G.A. 1993. Seasonal differences in motor terminals. *Comp. Biochem. Physiol.* **104A**, pp. 423-429.