Activation of opposing muscle groups in the crayfish claw

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Introduction

Arthropod motor innervation differs from vertebrate muscles in three respects: many muscles are supplied by an inhibitor axon, stimulation of which causes relaxation if the muscle is excited, each muscle is innervated by only a small number of motor axons, and polyneuronal innervation occurs where an individual muscle fiber may be innervated by more than one axon.[1] The arthropod muscle doesn't propagate action potentials throughout the muscle and rely on the frequency of stimulation and summation to control the amount of contraction produced. In dissecting the crayfish limb there is a primary nerve trunk and smaller nerve bundles innervating the opposing muscle groups. These consist of the motor excitor, motor inhibitory, and sensory nerves. They branch from the T1 thoracic ganglion in the central nervous system.[2] The four segments of interest in the claw and the abduction and adduction muscles of the dactylopodite are shown in figure 1a and 1b below:

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In these segments there are only seven muscles. They all are innervated by a common inhibitor.[3] There is a specific inhibitor for the extensor or stretcher muscle in the carpopodite and the opener of the dactylopodite. These innervations are shown below in figure 2.[3]

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The excitatory neurons are known to be of two types: fast and slow. They innervate the tonic (slow) and phasic (fast) muscle types. The excitatory innervation is shown in figure 3.[4] A characteristic of the arthropod nerves is their ability to branch off onto other muscle groups. This is seen in the carpopodite flexor and the claw abductor of the claw.

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By hook electrode placement on the excitatory nerve axons it is desired to stimulate the opposing muscle structures specific for the hinged finger of the claw called the dactylopodite.

Materials and Methods

Procambarus clarkii, red swamp crayfish, were purchased from the Waubun Laboratories in Schriever, Louisiana. They were kept in a container with 1/2 to 1" of declorinated water with individual homes for each crayfish made of 3" planter pots broken in two. Container was washed with normal tap water approximately every two days. No food was provided other than crayfish remains after both claws were removed. Physiological crayfish saline was prepared using van Harreveld's[5] crayfish solution containing (g/L): NaCl 12, KCl 0.4, $CaCl_2$ 1.5, $MgCl_2$ 0.5, $NaHCO_3$ 0.2. Cuticle scissors were purchased to cut through the chitinous cuticle. To detach the appendage an incision was made at the joint between the protopodite and the isciopodite. The claw will likely flex and abduction of the dactylopodite will occur after removal due to the continued activation of their excitor axons. With the dactylopodite turned downward, a section of the meropodite cuticle is removed from the ventral side. This is completed with care to keep incisions superficial to prevent damage to the underlying covering of the muscle and the nerve bundles. Start the incision at the joint between the isciopodite and the meropodite. Cut towards the distal end of the segment keeping the incision along the medial side the segment. Cut to within 1/8" of the mero-propodite joint and then begin cutting towards the lateral side of the segment. A cut should then be made back along the lateral side towards the mero-isciopodite joint again stopping an 1/8" before the joint. Before completing the rectangular cut with a cut across the proximal end of the meropodite, the dissection should be mounted to the forceps. Attach the claw by holding the isciopodite in one forcep and the carpopodite in the other. **Light** tension can be applied to the forceps while completing the removal of the cuticle but all tension should be **released** at least 5 minutes before any stimulation is applied. Crayfish saline was applied at this point to keep the dissection moist. With the claw secured, use a dissection microscope, surgical tweezers and a glass probe, gently separate the cuticle from the muscle attachments. Establish a crayfish saline drip with a rate of 1 drop every 10 seconds. With the cuticle gone there are two large opposing muscle groups seen. To ensure the prep will respond to stimulus the muscle should be stimulated directly by placing the hook electrodes on the muscle. Using the stimulus settings in table 1, stimulus of 5-10 seconds should produce claw movement. The main nerve trunk is located in the distal part of the meropodite running laterally. This trunk contains the excitatory neurons for the flexion of the claw and the slow and fast excitatory neurons to adduct the dactylopodite. Approximately in the middle of the meropodite segment is the branching of the very small bundle of nerves innervating the extensor muscle in the meropodite and then continuing laterally and superficially above the main nerve trunk towards the distal dactylopodite. After these bundles are identified your dissection is ready for stimulus. The accessory flexor muscle in the meropodite is located laterally to the main nerve trunk and should not be mistaken as a nerve bundle. On further dissections

after initial stimulation, the muscle structures in the meropodite with exception of the accessory extensor muscle which runs parallel and lateral to the main nerve bundle, are cut and removed. This is best done by using the glass probe to scrape the origin of the muscles away from their attachment to the cuticle. Then cutting the central tendon to which the muscles attach close to the distal end of the meropodite.

Experiments

Four experiments were performed: General stimulation of the nerve bundles to obtain a response in the abductor and adductor muscle of the dactylopodite; region specific stimulation of the nerve bundles to further isolate the location of the specific abductor and adductor nerves; separation of the main nerve trunk into smaller isolated bundles and stimulation of each bundle to further define the location of the excitor axons for the dactylopodite; repetitive stimulation with a latent period over time to determine if responses diminish with time.

General stimulation:

Using a fresh preparation, hook electrodes were placed on the main nerve bundle and stimulation was given at several different voltages, stimulation widths and frequencies.

Region specific stimulation of the main nerve trunk:

Using a fresh dissection the main nerve trunk was exposed. The bundles within the main nerve trunk were identified under the dissection microscope and stimulation was applied to each region of bundles observed to determine innervation of the limb muscles.

Separation of the main nerve bundle into smaller bundles with stimulation:

The flexor and extensor muscles in the meropodite were removed before manipulation of the nerve trunk. Using a glass probe with a fine flame smoothed tip, the main bundle was teased into smaller bundles to attempt to isolate the specific axons involved in excitation of the abduction and adduction of the dactylopodite. After the individual bundles were isolated, stimulation was applied to the bundle.

Repetitive stimulation of the nerve bundles over time:

Using a new dissection, the main nerve bundle and the small nerve bundle containing the abduction axons for the dactylopodite were identified. Stimulation was started 20 minutes after removal of the claw from crayfish. Stimulation was the same as the settings found successful in the first experiment. Every five minutes, stimulation was applied for 20 seconds and repeated until the claw failed to respond. The hook electrodes were not moved at any time during this experiment.

Results

In the general stimulation experiment initial attempts at stimulation resulted in blackening of the electrodes and burning the axon coverings. Damage was apparent by the sticking of the hook electrodes to the neurons when the electrodes are withdrawn. Hydrolysis also occurred and was apparent by the formation of bubbles on the surface of the immersed electrodes. By further attempts on fresh preparations at lower voltage and shorter more frequent stimulation bursts, adduction of the dactylopodite and flexor of the claw was achieved; see table 1 for the stimulus settings used for each trial. Knowing the nerve for abduction of the dactylopodite branches and innervates the extensor muscle in the meropodite[4], lightly moving the extensor muscle with a glass probe produced visible movement of the nerve bundle. Placement of the hook electrodes on this very small bundle and stimulation using the successful settings found for adduction, resulted in both the abduction of the dactylopodite and extension of the claw.

Table 1: General stimulation of nerves (main trunk & dactylopodite abductor bundle)

Burst Width (Seconds)	Duration of burst (sec.)	Time between bursts (sec.)	length of trial (sec.)	Pulse amplitude (V)	Loc. of Stimulus	Desc. of Response
1	1	1	60	5	main trunk	Rapid blacking of electrodes, production of bubbles, nerve stuck to electrodes, no movement
$1*10^{-1}$	$1*10^{-1}$	$1*10^{-1}$	20	2	main trunk	electrodes blackened, nerve stuck, no movement
$1*10^{-2}$	$1*10^{-2}$	$1*10^{-2}$	20	1	main trunk	initial adduction of dactyl.
$1*10^{-2}$	$1*10^{-2}$	$1*10^{-2}$	20	1	main trunk	same claw as above trial yet no response
$1*10^{-2}$	$1*10^{-2}$	$1*10^{-2}$	20	.5	main trunk	adduction of dactyl.
$1*10^{-2}$	$1*10^{-2}$	$1*10^{-2}$	20	.5	main trunk	adduction of dactyl., flexion of claw
$1*10^{-2}$	$1*10^{-2}$	$1*10^{-2}$	20	.5	main trunk	no response
$1*10^{-2}$	$1*10^{-2}$	$1*10^{-2}$	20	.5	abd. bundle	abduction of dactyl. and extension of claw
$1*10^{-2}$	$1*10^{-2}$	$1*10^{-2}$	20	.5	abd. bundle	abduction of dactyl. and extension of claw
$1*10^{-2}$	**$1*10^{-2}$**	**$1*10^{-2}$**	**20**	**.5**	abd. bundle	abduction of dactyl. and extension of claw

Highlighted settings were used for all further experiments unless otherwise noted.

In the nerve trunk region specific stimulation experiment, the main nerve bundle was visually categorized into three separate regions by the identification of three large bundles. Upon further inspection the first two regions most lateral in the meropodite each had large bundles with divisions into two smaller bundles each. The most medial region was as large as the two others but

didn't have easily identifiable divisions. Since it was possible to stimulate this region of the nerve trunk in different locations it was also given location reference letters. These bundles and their divisions into smaller bundles are shown in figure 4 below:

SOMETHING IS MISSING

Each small bundle section was stimulated by the hook electrodes to locate the fast and slow excitor axons for the adduction of the dactylopodite. The two electrode wires were allowed to touch and then were separated using the fine end of a glass probe to help limit the size of the area stimulated. During stimulation the settings found successful in experiment one were used with only changes in the voltage applied. The results are shown in table 2:

Table 2: Region specific stimulation of the main nerve trunk

Site of Stimulus	Stimulus Voltage	Response to stimulus
1 A&B	.3	Flexion of claw
1 A&B	.5	Adduction of the dactylopodite
2 A&B	.3	No response
2 A&B	.5	Slight flexor of claw
1 A&B	.3	N.R.
1 A&B	.5	fast adduction of dactyl. w/ flexion of claw
1 A&B	.3	N.R.
1 A&B	.5	N.R.
1 A&B	.3	N.R.
1 A&B	.5	fast adduction of dactyl. w/ flexion of claw
2 A	.5	abduction of dactyl.
2 B	.5	abduction of dactyl.
2 A	.5	abduction of dactyl.
2 B	.5	abduction of dactyl.
3 A&B	.3	N.R.
3 A&B	.5	abduction of dactyl.
3 A&B	.3	abduction of dactyl.
3 B	.4	abduction of dactyl.
3 B	.4	abduction of dactyl.
3 B	.4	flexion of claw
2 B	.5	adduction of dactyl.
1 B	.5	fast adduction of dactyl.
3 A&B	.5	extension of claw
2 A&B	.5	flexion of claw
2 A&B	.5	adduction of dactyl.
3 B	.5	extension of claw
3 B	.5	extension of claw

The experiment to separate the main nerve trunk resulted in teasing the trunk into 9 separate nerve bundles. Stimulation was applied to each individual bundle using a small glass probe to physically separate and insulate the bundle being stimulated. After stimulation the bundle was cut and the next bundle separated and stimulated. Throughout the experiment, stimulus produced no visible effect on the dactylopodite.

The repetitive temporal stimulation of the claw showed fast closure with a full adduction during the first 20 minutes. The remaining 40 minutes produced slower responses that successively produced less closure movement in the claw.

Conclusion

Because the arthropod muscle doesn't produce true propagated action potentials, the size of the action potential mattered less than the amount of individual stimulation's. Using the summation of these individual and rapid stimulation's the muscle was responsive. By placement of the hook electrodes across the nerve bundle the entire bundle was stimulated and adduction of the dactylopodite and flexion of the claw was achieved. The speed and amount of adduction or flexion was rarely identical in each dissection. The peripheral resistance characteristic of the inhibitor axons also being stimulated simultaneously, appears to be the cause of the variance seen in each stimulus attempt.

The results found when stimulating regions of the main nerve trunk showed apparent location of the specific excitor axons and even indicated where in the nerve trunk the fast axon was which innervates the adductor, (1A&B). This dissection appeared successful in the determinations of adduction and flexion until a second attempt with a new claw was performed. These locations failed to reproduce the same responses. The stimulation of abduction within the main nerve bundle, (2 A&B) were likely the result of the superficial abduction bundle getting in the way of stimulation of the main trunk. It is also possible that the nerve bundle doesn't always orient itself with the same side exposed after dissection. Twisting of the nerve trunk by the flexor and extensor muscles in the meropodite may play a role in changing its orientation in every dissection. It may also possible that the growth of the nerve trunk doesn't require specific orientation and each crayfish may have an entirely different orientation of it's nerve trunk.

The teasing and individual stimulation of the 9 nerve bundles produced no response and it is assumed that this dissection had nerve trunk damage caused by the dissection either in cutting the cuticle near the joint between the meropodite and the carpopodite, or removal of the flexor and extensor muscles in the meropodite. The recommendation is to consider leaving the muscles in the meropodite intact if future dissections failed. It would be helpful in manipulating the nerve trunk to push these muscles aside and secure them out of the way. This procedure holds the best promise in identifying which bundle(s) house the fast and slow excitor axon for the adductor.

The temporal stimulation showed evidence enough time exists to prepare and conduct experiments on the nerves. Further experiments would add to the one trial conducted and help confirm how long the dissection will perform with one stimulus setting. It is suggested that further experiments may include higher voltages after stimulation fails to produced the same results as the five minute test.

The crayfish dissection held many variables when attempted. However, these variables do not prevent usage of this animal as the research papers referenced show. It appears to require further repetitive dissection attempts with care to control how many variables are changed during each dissection. The success in producing a response within the claw by nerve stimulation will be encouraging when future dissections are done.

References

1. Aidley, D.J. 1971. *The Physiology of Excitable Cells*. Cambridge University Press, Great Britian.
2. Holdich, D.M. & Lowery, R.S. 1988. *Freshwater Crayfish Biology, Management, Exploitation*. Croom & Helm, London. QL444 .M33 F73
3. Wein, T.J. 1985. Triple Innervation of the Crayfish Opener Muscle: The Astacuran Common Inhibitor. *Journal of Neurobiology* **16**, pp. 183-191
4. van Harreveld, A. & Wiersma, C.A.G. 1937. The Triple Innervation of Crayfish Muscle and its Function in Contraction and Inhibition. *Journal of Experimental Biology* **14**, pp. 448-462