Chapter 20 – Crustaceans as Model Systems for Teaching Neuroscience: Past, Present, and Future

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<1> Abstract

In addition to their role as important preparations for research, crustaceans have served as model systems for teaching basic principles of signal transmission in nervous systems, particularly in student laboratory classes. We survey published and other easily available crustacean preparations used in neuroscience teaching, focusing on neuromuscular properties, synaptic transmission, neuronal excitability, sensory physiology, heart, respiratory, and intestinal muscle control, and neurogenesis. We also briefly describe select computer simulations, behavioral exercises, and commercially available exercises that contribute to neuroscience teaching, and insect neurobiology exercises that are often complementary. Finally we suggest future directions for development of crustacean teaching exercises.

<1> Introduction

Crustaceans are excellent models with which to teach physiology of nervous systems and introduce students to electrophysiological techniques. These preparations come from a long history of research, mainly concentrated on decapods (crabs, lobsters, crayfish), that continues to the present (Florey 1990, Weise 2005a, 2005b; this volume). There are advantages of using invertebrates instead of vertebrates in teaching laboratories (Deyrup-Olsen and Linder 1991). (1) They often illustrate a principle more clearly than vertebrate preparations because of the relatively small number of neurons in the nervous system, many of which are identifiable across preparations. (2) They can survive and remain physiologically active at room temperature for long periods without supplemental oxygen. (3) They are generally less expensive than vertebrates. (4) They are not subject to the same governmental regulations regarding purchasing, storing, and dissecting as are vertebrates. (5) Finally, crayfish in particular are farmed

commercially for fishing and food, so their removal from the environment does not damage local ecosystems.

Most crustacean teaching exercises came from neurobiologists who used the preparations in their research laboratories and brought them into teaching laboratories (Parfitt 2002, Atwood 2008). For example, some versions of exercises on crustacean neuromuscular junctions (NMJs) stem from Harold Atwood's physiology class at the University of Toronto beginning in 1965 (Atwood and Parnas 1968, Baierlein et al. 2011). One of us (RRH) studied development and regeneration of the crayfish NMJ for his dissertation work and later developed this preparation for the *Crawdad* teaching project (Wyttenbach et al. 1999). This chapter surveys published and other easily available examples of crustacean preparations used in teaching neuroscience. We focus on teaching exercises rather than on the research that led to their development; the original research can be found in other chapters of this and related volumes (Wiese 2002a, 2002b). This overview is intended to inspire further development and modernization of old and possibly forgotten teaching preparations, and suggest material for future educational development.

<1> Early Developments

Two early laboratory manuals were influential in development of crustacean teaching exercises: the *Laboratory Exercises in Invertebrate Physiology* manuals (Welsh and Smith 1949, 1960, Welsh et al. 1968) and the *Experiments in Physiology and Biochemistry* compilation (edited by G.A. Kerkut, starting in 1968). We start with an overview of these early efforts; specific exercises are described in more detail in the next section.

The first edition of *Laboratory Exercises in Invertebrate Physiology* (Welsh and Smith 1949) has a remarkably comprehensive offering of crustacean neurobiology exercises developed

for a semester-long physiology course at the Harvard Biological Laboratories. This edition included exercises on the pharmacological and neural control of decapod heart rates, facilitation of tension and contraction rate in crayfish leg muscle and claw opener muscle, interaction of excitatory and inhibitory innervation on contraction strength in decapod muscle, and extracellular recordings of crayfish caudal photoreceptor and responses to stimulation of tactile sensilla. The second edition (Welsh and Smith 1960) updated these exercises and introduced important new ones, including stimulus-response properties of the crayfish abdominal muscle receptor organs (MROs), measurement of the conduction velocity of action potentials (APs) from axons in decapod leg nerves and giant axons in the ventral nerve cord, and the effects of toxins and transmitters on spontaneous activity in the ventral nerve cord. This edition also introduced intracellular recording of muscle resting and synaptic potentials. In the first two editions, descriptions are short, with limited detail; much is purposely left unexplained so that students take responsibility for self-motivated exploration (Welsh and Smith 1949). The third edition (Welsh et al. 1968) updated earlier exercises and described protocols more fully. This edition suggested the crayfish abdominal superficial flexor muscle as a good preparation in which to match spontaneous APs of motor nerve with synaptic potentials of muscles, but did not develop the preparation further. A new exercise characterizing location and response properties of sensory interneurons in crayfish circumesophageal connectives and ventral nerve cord was added. This edition also added an appendix on electrophysiological equipment and techniques that is still helpful today.

The first and fifth volumes of the *Experiments in Physiology and Biochemistry* series contain exercises similar to those found in Welsh et al. (1968), but described in more detail. These include the innervation, synaptic physiology, and pharmacology of the neuromuscular

junction of the crayfish abdominal extensor muscle (Atwood and Parnas 1968); inhibitory-excitatory motor neuron interactions controlling claw muscle contraction (Hoyle 1968); changes in sign and amplitude of postsynaptic potentials under different nerve stimulation protocols (Hoyle 1968); neural control and pharmacology of the crayfish heart (Florey 1968a, 1968b); and a detailed examination of the crayfish caudal photoreceptor (Hermann 1972). There are also new exercises on the pharmacology of crayfish hindgut activity (Florey 1968c) and the excitability of lobster giant axons (Hoekman and Dettbarn 1972).

<1> The Crustacean Toolbox for Teaching Neurobiology

Thus, we find that most of the crustacean neurophysiology exercises commonly used today were present in the teaching literature nearly 40 years ago. In this section, we briefly describe published and easily available laboratory exercises that use crustaceans to examine nervous system structure and function. While our focus is on exercises that address physiology, many of these also incorporate simple staining to examine anatomical organization of nerve innervation patterns. See Table 20.1 for an annotated summary of selected crustacean physiology exercises that we describe below. [**Table 20.1 near here**]

<2> Neuromuscular Physiology and Synaptic Transmission

Most published crustacean lab exercises address neural control of muscle activity in decapods in a variety of neuromuscular preparations. The major learning goals address the transformation of the neural code into muscle contraction, patterns of muscle innervation, excitatory and inhibitory control of contraction, pharmacology of the nerve-muscle synapse, and basic synaptic properties including synaptic integration and short-term synaptic dynamics.

<3> Leg and Cheliped

The four neuromuscular physiology exercises in Welsh and Smith (1949) show how students can use simple techniques to examine important aspects of neuromuscular transformation and synaptic dynamics. Students use methylene blue staining to see that small numbers of axons innervate leg muscles, and that muscle fibers are innervated by multiple motor neurons. They stimulate motor neurons with simple bipolar metal electrodes poked through the shell of an isolated crayfish walking leg or cheliped. Dactyl closer muscle contractions are monitored by a transducer writing on a smoked drum! (Today, of course, the transducer would feed into an oscilloscope or computer with A/D board.) Students define nerve stimulation regimens that facilitate muscle tension, control the rate of contraction, and demonstrate the "ratchet" effect, whereby a single extra stimulus pulse added to ongoing repetitive stimuli causes a partial contraction that increases muscle tension. Another exercise examines excitation and inhibition of crayfish claw contraction with the same crude stimulation technique. Again measuring muscle contraction, students adjust stimulus parameters to recruit fast and slow muscle fibers and determine different thresholds for recruitment of excitatory and inhibitory innervation. Next, the motor nerve is split into finer bundles and movements of the leg tip are observed with stimulation of different groups of nerve fibers. Properties of twitch, tetanus, contraction rates, relaxation, and fatigue, and the synaptic sites of these properties, are examined by comparing contractions evoked by direct muscle stimulation with those evoked by nerve stimulation. The effects of temperature and insecticides on neurally evoked contractions are suggested as ways to examine environmental modulation of the NMJ. The second edition (Welsh and Smith 1960) suggests adding the inhibitory neurotransmitter GABA to examine its direct effects on claw

muscle contraction with and without the GABA receptor blocker picrotoxin. Hoyle (1968) describes a similar exercise with the crayfish claw opener muscle in more useful detail, with background information and guided interpretation, and also describes recording of muscle EMG activity (see also Welsh et al. 1968). In addition, Hoyle (1968) describes an intracellular recording exercise with the dactyl closer muscle to examine post-synaptic potential (PSP) facilitation. These basic exercises measuring muscle contraction are important because they introduce students to principles of motor control different from vertebrates. They also set the stage for later exercises that use the polyneuronal, multiterminal, and excitatory/inhibitory innervation of the crustacean muscle as a model system for vertebrate brain synapses (Wyttenbach et al. 1999). The intracellular recording exercises of Hoyle (1968) and Atwood and Parnas (1968; see below) start a new era in student laboratory physiology. The crustacean NMJ is now a model system to study fundamental properties of synaptic transmission and integration.

The classic leg and dactyl muscle preparation (Fig. 20.1) was recently re-described by Cooper and colleagues in video articles with extensive background and literature review. Dissection of the opener muscle preparation is described in detail, with a demonstration of short-term synaptic plasticity recorded intracellularly from the muscle. Extracellular “macro patch” recordings measure evoked and miniature PSPs and analyze the quantal content of transmitter release after visualization of single synaptic boutons with the fluorescent vital dye 4-Di-2-Asp (Cooper and Cooper 2009). The crayfish leg extensor muscle preparation is used to demonstrate high- and low-output nerve terminals that synapse on the same muscle fibers (Fig. 20.1A, B), but have very different synaptic output and short term dynamics. Differential stimulation of motor axons shows different intracellularly recorded EPSP amplitudes with different short term dynamics in the same muscle fiber (Fig. 20.1C), and extracellular macro patch recording

demonstrates differing quantal content of high and low output synaptic junctions. Again, the general innervation pattern of muscle is visualized with methylene blue staining before physiology, and the distribution of single synaptic boutons is observed with the fluorescent vital dye 4-Di-2-Asp (Fig. 20.1A; Wu and Cooper 2010). The American/Canadian section of the International Brain Research Organization (IBRO) also has a good description of the dactyl opener muscle preparation on its web site (IBRO 2011). [**Fig. 20.1 near here**]

<3> Abdominal Extensor

Another early student exercise for recording intracellularly from the crustacean NMJ appears in Atwood and Parnas (1968). They use methylene blue to study innervation patterns of the muscle and, after formalin fixation, compare sarcomere lengths in deep and superficial extensors. Intracellular recordings from muscle fibers and stimulation of motor nerves demonstrate facilitation and post-tetantic potentiation. The effects of GABA and strychnine on PSP amplitude and the ability of picrotoxin to block the effect of GABA demonstrate inhibitory input to the muscle (Atwood and Parnas 1968). Baierlein et al. (2011) provide an updated video version of the original lab exercise of Atwood and Parnas (1968). Cleland (2005) uses this preparation to pose the experimental question of transmitter identity. Students first apply toxins acting on different receptor types and note their effects on EPSP amplitude, muscle resting potential, and input resistance. Their results are correlated with the effects of these drugs pressure injected or iontophoresed onto the muscle surface during intracellular muscle recording.

<3> Abdominal Superficial Flexor

Several exercises in the *Crawdad* CD (Wyttenbach et al. 1999, Johnson et al. 2002) are based on the crayfish abdominal superficial flexor muscle (Fig. 20.2A) and its innervation. The student manual includes detailed video of dissections and recording methods, while the instructor's manual adds detailed setup instructions and sample results. The superficial branch of abdominal nerve 3 is purely motor and innervates the superficial flexor muscles in each segment (Fig. 20.2B). A series of four exercises are available.

(1) Extracellularly record spontaneous activity of nerve 3, sort APs by amplitude or energy density (arising from motor axons of differing diameters), determine the number of neurons innervating the muscle, and characterize activity patterns of each motor neuron. Tactile stimulation of the tail fan and pleopods demonstrate reflexes that differentially alter spontaneous activity in the motor neurons (Fig. 20.2C). Baierlein et al. (2011) describe a similar exercise that characterizes nerve 3 and synaptic activity in response to sensory stimuli.

(2) Backfill nerve 3 with cobalt to observe functional morphology and ganglionic location of the motor neurons.

(3) Match extracellularly recorded APs in the nerve with intracellularly recorded PSPs in the muscle, finding examples of selective polyneuronal innervation of muscle fibers and examining synaptic integration (summation and excitatory/inhibitory interactions), especially during sensory stimulation (Fig. 20.3A).

(4) Stimulate the nerve with pulse pairs and trains to elicit facilitation, depression, and post-tetanic potentiation (Fig. 20.3B), and investigate modulation of synaptic transmission, particularly by amines. The two exercises on synaptic transmission are also described in Paul et al. (1997), who adapted our Cornell laboratory class handouts for their use. [**Fig. 20.2 near here**] [**Fig. 20.3 near here**]

<2> Ionic Basis of the Resting Potential and Neuronal Excitability

The large fibers of crustacean claw, leg, and tail muscles are excellent preparations in which to determine the ionic basis of the resting potential. Using intracellular recording to examine the role of K^+ concentration in setting the resting potential of fibers in crayfish abdominal extensor muscle (Atwood and Parnas 1968, Baierlein et al. 2011) and superficial flexor muscle (Wyttenbach et al. 1999) has been described. Wyttenbach et al. (1999) also ask students to test the contributions of Na^+, Cl^-, and Ca^{2+}. In a more difficult preparation, students test the importance of K^+ and Na^+ for the resting potential of giant axons in the lobster nerve cord (Hoekman and Dettbarn 1972).

Welsh and Smith (1960) briefly present two exercises that examine axonal responses to nerve stimulation. Isolated crab or crayfish leg nerves are stimulated and the resulting AP activity recorded extracellularly. Reduced or calcium-free saline and DDT are tested for their ability to induce repetitive firing in sensory and motor neurons. In the third edition (Welsh et al. 1968), students examine the effect of stimulus strength and duration on nerve activity, and repetitive AP firing after long-duration stimuli. Absolute and relative refractory periods of compound APs and the maximum AP firing rate are determined. Students also explain the shape of recorded nerve activity after changing the distance between bipolar recording electrodes or crushing the nerve between electrodes. A similar but more detailed exercise described by Oakley and Schafer (1978) uses *Limulus* leg nerves, but they suggest that lobster or crayfish leg nerves can substitute. The lobster giant axons are used to examine the effects of membrane potential, external Ca^{2+} and Mg^{2+}, acetylcholine and some of its analogs, and procaine on stimulated AP

initiation, amplitude, and waveform (Hoekman and Dettbarn 1972). This detailed study of excitability is best suited to advanced students.

Spontaneous activity in semi-intact and isolated ventral nerve cords of crayfish is manipulated with nicotine, picrotoxin, and ethanol in Welsh and Smith (1960,1968). They suggest blowing tobacco smoke directly on the preparation to apply nicotine, not an acceptable delivery method in today's laboratory! (We would more likely purchase acetylcholine or other nicotinic receptor agonists from a chemical supplier.) Students compare the relative occurrence of small, medium, and large fibers in cross-sections of the ventral nerve commissures with the representation of small, medium, and large APs in their recordings of spontaneous activity.

Welsh et al. (1968) suggest determining AP conduction velocities by measuring travel time between two recording points a known distance apart in decapod leg and ventral nerve cords. Students compare preparations with different axonal diameters (leg nerves vs. ventral nerve cord), and under different recording conditions of external resistance. Robinson et al. (2011) update this exercise using the crayfish ventral nerve cord to show axon recruitment with increasing stimulus intensity and measure conduction velocity of the compound AP. Students also quantify absolute and relative refractory periods with twin pulse stimuli. The electrical synapse blocker heptanol is used to modify the waveform and velocity of the compound AP, showing the contribution of gap junctions to AP transmission between giant axons in the ventral nerve cord. Temperature changes can also affect the waveform and velocity of compound APs. Johnson et al. (2009) describe simultaneous measurement of conduction velocities of up to six motor neurons firing tonically in the branch of abdominal nerve 3 that innervates the superficial flexor muscle, while varying temperature (Fig. 20.4). The nerve is cut near its entrance into the muscle and stretched across the muscle to allow the longest distance between extracellular

recording points. An advantage of this preparation over the leg nerve and ventral cord is that velocities are determined for multiple identified axons of different diameters in the same recording, with no stimulation required. The disadvantage is the relatively short length over which to calculate velocity. These exercises are framed in the context of evolutionary pathways for regulating conduction velocity, such as axon diameter changes and myelination. [**Fig. 20.4 near here**]

<2> Sensory Physiology

The abdominal muscle receptor organ (MRO) of crayfish is a classic preparation with which to study sensory responses (Fig. 20.5). All published exercises using this proprioceptor investigate stimulus-response properties, different stimulus thresholds, and adaptation time courses of the slowly and rapidly adapting receptors. The preparation is described in limited to moderate detail in Welsh and Smith (1960) and Welsh et al. (1968). More development is found in the *Crawdad* CD (Wyttenbach et al. 1999, Johnson et al. 2002) and the video article by Leksrisawat et al. (2010). These descriptions include methylene blue staining for students to view the anatomy of the stretch receptors, and suggest further exploration of neuromodulatory actions on MRO responses. [**Fig. 20.5 near here**]

The propodite-dactylus organ of the decapod leg is another proprioceptor suggested for lab exercises. This organ is one of the series of joint receptors that monitor limb segment positions. It spans the joint between the last two leg segments and is composed of tens of bipolar sensory neurons sensitive to joint movement. Descriptions of this preparation by Welsh and Smith (1960) and Welsh et al. (1968) are very brief. Methylene blue staining is used to visualize

the organ, and extracellular recording during dactyl movement shows the response pattern, directional sensitivity, and adaptation properties.

The crayfish caudal photoreceptor is described as a student exercise by Welsh and Smith (1960) and Welsh et al. (1968). Unlike most preparations, which are best studied shortly after dissection, the authors suggest maintaining ventral nerve cords 12-24 hr before student experiments in order to reduce spontaneous activity of other cord neurons. Extracellular recording from the ventral nerve cord with focused illumination shows the location of light-sensitive interneurons, the latent period and after-discharge of the photoreceptor after illumination, the stimulus-response relationship, and the flicker-fusion response to repeated stimuli. In a much more detailed exercise, Hermann (1972) asks for a rigorous mathematical analysis of the caudal photoreceptor response. The students also examine the receptor's response after applying GABA and acetylcholine. The aim of this exercise is to determine the features of the receptor signal important to initiate a behavioral response such as walking.

Summed photoreceptor responses in eyes (electroretinograms, or ERGs) are easily recorded in many arthropods, including crustaceans. Olivo (2003) briefly describes recording crayfish ERGs with pin electrodes, with full details on his course web site (Olivo 2012). This exercise focuses on the role of second messengers in visual transduction, and students quantify stimulus-response relationships. A simple wick electrode placed on the crayfish eye, adapted from a lab exercise using *Limulus* eyes (Wald et al. 1962), would also record light responses (Olivo, pers. comm.).

<2> Central Neural Networks

The neural network generating rhythmic movements of pleopods in the crayfish tail is an excellent preparation that has not received the attention it deserves for teaching. It allows examination of a central pattern generating network, leading to comparisons and discussion of the mechanisms organizing repetitive rhythmic movement, such as locomotion, in all animals. The best description for a student exercise is on a course web site (Olivo 2012) with a brief published description in Olivo (2003).

Welsh et al. (1968) very briefly describe an exercise to characterize sensory interneurons in the crayfish ventral nerve cord and circumesophageal connectives. Students are directed to determine the response properties, location of receptive modalities and fields, and direction of signal travel in interneurons responding to tactile or visual stimuli.

Although not presented specifically as a student exercise, Herberholz (2009) describes a simple measure of central network activity that could easily be done by students. Wire recording and ground electrodes are positioned in a small water-filled chamber to record neural and muscular activity during escape responses elicited by disturbing a crayfish. This is combined with behavioral observations and/or high-speed video recording for later correlation with nerve and muscle activity. Students can record activity from neural networks that mediate strong escape responses to head and tail stimuli, and a weaker avoidance response to milder touch of the thorax. Results are discussed in the context of the organization of neural networks underlying each behavior and the social conditions that may alter the efficacy of the networks.

<2> Control of Heart and Respiratory Function

Crustaceans have been popular teaching models for cardiac and, to a lesser extent, respiratory physiology. The first edition of the Welsh and Smith series (1949) has several such exercises.

The cladoceran *Daphnia* is used to determine the sensitivity of heart and respiration rates to temperature changes through direct observation and calculation of the Q10 (Welsh and Smith 1949, 1960, Welsh et al. 1968). A version of this exercise has also been developed for high school students to examine the pharmacology of heart function (Biotechnology Institute 2002). However, most exercises examining heart function and its neural control use decapods. Welsh and Smith (1949) describe an exercise to monitor spontaneous heart rate in response to application of acetylcholine and epinephrine in the isolated heart. Students compare the effects of these transmitters on neurogenic crustacean hearts with their effects on the myogenic vertebrate hearts. In the 1960 edition, Welsh and Smith added serotonin and pericardial organ extract from crabs to be tested as potential hormonal controls of heart rate. Florey (1968a) describes the crustacean heart in more detail and a semi-intact preparation to study control of the crayfish heart. Since crustacean hearts are neurogenic, heartbeat frequency reflects the burst frequency of the motor neurons, contraction amplitude reflects the number of APs per burst, and contraction duration reflects the burst duration (Florey 1968a). Experiments include stimulating cardiac accelerator and inhibitory nerves separately or together to determine their effects on heartbeat parameters. A separate exercise combines nerve stimulation with pharmacological study of excitatory (glutamate, acetylcholine, atropine, eserine, and epinephrine), inhibitory (GABA and picrotoxin), and possible hormonal (serotonin) control of heart rate (Florey 1968b). The goal is to test hypotheses of transmitter identity and whether the transmitters/modulators act within the neural network for heart regulation, at the heart NMJ, or both.

A comparison of the pharmacology of the neurogenic heart of lobsters and the myogenic heart of clams, measuring heart rate and contraction strength during application of acetylcholine, GABA, and serotonin, is described in detail by Zamer and Shick (2005). Bierbower and

Cooper's exercise (2009) monitors heart and respiration rates in freely moving crayfish with extracellular electrocardiogram (ECG) recordings. This straightforward exercise is designed as a physiological measurement of the animal's response to environmental and social stressors that might not be obvious with strictly behavioral observations. Although specific protocols are not given, DiCecco et al. (2007) describe a lab course for engineering students that uses methylene blue to examine heart network anatomy, and intracellular recording to examine properties of lobster heart neurons. This is one of several invertebrate preparations that DiCecco et al. (2007) used to teach biomedical engineers dissection, standard laboratory practices, electrophysiology, application of engineering concepts to neural networks, and data acquisition and analysis. In our Cornell neurophysiology lab course, we have also seen growing interest by engineering students in hands-on neurobiology work over the past few years.

<2> Rhythmicity of Intestinal Muscle

The crayfish hindgut has been developed as a preparation to study the pharmacology of the enteric nervous system. Spontaneous contractions of intact (Cooper et al. 2011) or isolated (Florey 1968c, Cooper et al. 2011) crayfish hindguts are monitored under perfusion with various neurotransmitter candidates including acetylcholine, atropine, eserine, glutamate, GABA, picrotoxin, serotonin, and dopamine. Students observe the effects on the rate and strength of contraction, and construct dose-response curves for each effect. The Florey (1968c) exercise has detailed methods and guided exploration; the Cooper et al. (2011) exercise has excellent background information and video of the procedure. Both exercises frame the exercise in the context of transmitters acting both pre- and post-synaptically to shape motor output.

<2> Neurogenesis

Paul et al. (2002) use 5-bromo-2'-deoxiuridine to label newborn cells in the crayfish brain as a student exercise to examine the importance of environmental stimulation for brain development. (Fig. 20.6; see chapter 7 for more on this technique.) Students observe greater neurogenesis in animals given "enriched" environments (presence of many conspecifics, water aeration, frequent feedings, many objects for manipulation, and hiding places) than in those in "impoverished" environments (living with mother and siblings only, no water aeration, less frequent feeding, minimal external stimulation, no hiding places, very shallow water). [**Fig. 20.6 near here**]

<2> Educational Simulations

Although often not stated specifically, crustacean research has contributed to computational simulations that teach principles of cellular and systems neuroscience. For example, the "Swimmy" software (Grisham et al. 2008) uses fish swimming to guide students through the properties of a central pattern generator based on an understanding of these networks and their cellular components from crustacean research (see chapter 5). A computer simulation tool that highlights crustaceans is AnimatLab (Cofer et al. 2010, AnimatLab 2011). This is an interesting project that could be very useful for student neuromechanical models of behavioral systems. Example models are presented for crayfish escape behavior and walking-leg movements, and there is potential for student development of other systems.

<2> Equipment and Software

Our work developing crustacean neurobiology preparations for teaching neuroscience also motivated us to design inexpensive hardware specifically for the student laboratory. These

ranged from build-it-yourself extracellular amplifiers (Land et al. 2001), nerve stimulation circuits (Land et al. 2004), and suction electrodes (Johnson et al. 2007), to inexpensive manipulators (Krans et al. 2006). In addition, we designed free software for data acquisition and analysis that highlights analysis of crayfish nerve 3 activity, synaptic physiology, and MRO responses (Lott et al. 2009).

<2> Commercially Sponsored Material

Some suppliers of research equipment have produced lab handouts for faculty to use crustaceans in laboratory teaching. These are usually adapted for a specific manufacturer's equipment, but are still useful as lab exercise guides. The descriptions usually have limited background and lack the depth of exploration found in published lab exercises. For example, iWorx offers crayfish exercises (iWorx 2011a), similar to those in the *Crawdad* project, that examine (1) variability in resting potential of abdominal fast extensor muscles and the effects of changing external concentration of Na^+ and K^+; (2) matching of motor nerve APs and muscle synaptic potentials in crayfish superficial flexor muscle, with motor nerve stimulation to determine thresholds for initial and maximal EPSP generation; and (3) stimulus-response properties and adaptation of abdominal MROs. In addition, their newsletter (iWorx 2011b) offers exercises for examining effects of temperature changes and pharmacological agents on ECG and muscle tension in crayfish heart, and the pharmacology of crayfish hindgut contractions. ADInstruments offers downloads of working guides designed to complement the *Crawdad* lab exercises using ADInstruments equipment (AD Instruments 2011a). There is also a "Peer Submitted Lab Chart Experiment" section (AD Instruments 2011b) including some exercises described above (Leksrisawat et al. 2010, Baierlein et al. 2011, Cooper et al. 2011, Robinson et al. 2011), and an

exercise examining heart rate in the transparent shrimp *Palaemonetes kadiakensis* in response to temperature changes and nicotine.

Although not designed for teaching, the handouts produced by the Grass Instrument Company (now Grass Technologies, a subsidiary of Astro-Med, Inc.) are educational. They describe live neurophysiological demonstrations at annual meetings of the Society for Neuroscience between 1969 and 1993. These delightful handouts highlight a variety of preparations including five dedicated to crustaceans: (1) ECG recording from the American lobster and the effect of temperature on heart and respiration rates (1973); (2) crayfish ERG recording, with brief descriptions of the abdominal stretch receptor and crayfish escape response (1976); (3) claw asymmetry in fiddler crabs using EMG recordings from the major claw, and ECG recordings from freely moving animals (1982); (4) conduction velocity of axons in the crayfish ventral nerve cord (1985); and (5) responses of crayfish abdominal MROs (1990). These handouts were often prepared with the help of crustacean neurobiologists. The methods are not detailed enough for student directions, but the background information is often rich with extensive bibliographies. They are a good guide for an experienced neurophysiologist to develop a class exercise. The full set of handouts is now available online (Grass Technologies 2011).

<1> Behavioral Exercises with Crustaceans

We draw attention to a few behavioral lab exercises because they easily lead to discussion of neural mechanisms. For example, the older Harvard lab manuals have brief descriptions of behavioral exercises that could be further developed in a neuroethological context. These include photo- and geotaxis in *Daphnia*; social, environmental, and physiological conditions controlling leg autotomy in crabs; control of eye and leg posture by statocyst balance organs in crabs; visual

acuity and flicker fusion responses to rotating striped drums in crabs; and the measurement of diurnal rhythms in crabs (Welsh and Smith 1949, 1960, Welsh et al. 1968). Environmental control of pigment dispersion for body coloration in shrimp is described by O'Halloran (1990; see also Oakley and Schafer 1978); students consider whether body coloration is under humoral or neural control. To examine hormonal control of antagonistic behavior, Mead (2008) describes a lab exercise for non-biology majors, with an evaluative component, in which students compare male crayfish fighting behavior between controls, sham-operated animals, and animals with their androgenic glands removed. Students favorably reported that the exercise was interesting, they learned to quantify behavior, they better understood the link between hormones and aggression, and they were motivated to do more science. An exercise examining the visual contribution to dominance hierarchy establishment is described in a video article by Mercier and May (2010). Crayfish responses to their own reflections and to other crayfish in adjacent tanks are observed and discussed in the context of the sensory control of social behavior.

<1> Insects and Other Arthropods

We mention a small selection of insect neurobiological exercises because these also demonstrate basic principles of nervous system physiology in small nervous systems. Insects and crustaceans can often substitute for each other in student lab exercises. For example, an exercise in Welsh et al. (1968) examines chemosensory hair stimulation in insects and suggests that crayfish can be substituted. The classic student preparation examining coding of stimulus intensity and sensory adaptation in the cockroach leg appears in the early Harvard lab manuals. Updated versions of this exercise are found in Oakley and Schafer (1978) and Linder and Palka (1992), and a simplified version of this exercise is being developed for high school students (Gage and

Marzullo 2010). Descriptions of responses of cockroach interneurons to cercal sensory stimulation recorded from the ventral cord are found in Welsh and Smith (1949, 1960), Welsh et al. (1968), and Oakley and Schafer (1978). A series of linked lab exercises describe recordings of sensory stimulation of the cockroach leg and antennae, and sensory interneuronal activity in the ventral nerve cord (Ramos et al. 2007). Other recent sensory lab exercises using insects examine the physiology of taste reception in flies (Pollack 2005a), central processing of wind information in crickets (Pollack 2005b), ERG recordings from eyes of flies (Krans et al. 2006), and proprioceptive coding of wing movements in flying locusts (Gray and Robertson 2005). The generation of rhythmic motor patterns is examined in locust flight (Dawson and Meldrum 2005; see also Welsh and Smith 1949) and the tobacco hornworm (Trimmer 2005). Articles describing experimental protocols on synaptic physiology of the NMJ of *Drosophila* (Zhang and Stewart 2010) and recording of the giant fiber system of flies (Allen and Godenschwege 2010) facilitate the use of these classic preparations for the student teaching laboratory. Finally, recent advances in optogenetics have made fruit fly preparations more practical for the undergraduate student teaching lab by controlling neural activity through temperature and light stimuli (Berni et al. 2010, Pulver et al. 2011).

<1> Future Directions

Many of the crustacean teaching preparations described in early lab manuals could be further developed and updated for the student neuroscience laboratory. For example, neural control of rhythmicity of heart and hindgut could be more widely used models for efferent control of rhythmic activity, while re-connecting crustacean neuromuscular physiology with claw movement and/or muscle tension would emphasize the functional importance of motor

innervation strategies and synaptic plasticity in crustaceans and insects. Further development of the crayfish ventral photoreceptor, ERG, and leg proprioception will provide alterative models for sensory physiology. The swimmeret neural network is one of the most accessible models for study of central pattern generation and could be intellectually packaged to reach a broad audience of students. Refining these older exercises presents more modern options for demonstrating core principles of signal transmission in the nervous system. Exercises on coordination and interaction of segmental motor networks and on sensory processing could introduce students to systems neuroscience and complex integration by neural networks. Examples are coordination of nerve 3 postural motor activity and central pattern generator activity for pleopod movement (see chapter 5) across crayfish tail segments, and visual integration in crayfish optic ganglia (Glantz and Miller 2002; Glantz, this volume). Almost all published lab exercises using crustaceans do not have student evaluative components (but see Mead 2008 above). Future development of teaching exercises should include an assessment of their educational effectiveness.

As other disciplines, such as engineering and computational biology, continue to take advantage of small systems neurobiology for teaching, new teaching synergies will develop. For example, DiCecco et al. (2007) describe the "Cricket Car," made by engineering students, which translates cricket EMG recordings related to ultrasound/collision avoidance into steering directions for a remote-controlled car. In addition, the AnimatLab project (Cofer et al. 2010) combines small systems neurobiology with computational strategies for simulations of neuromechanical models. Finally, molecular analysis techniques applied to important nervous system molecules such as amine receptors in crustaceans are now transferable to student laboratories (McCoole et al. 2012; Christie, pers. comm.). Bioinformatics based exercises

(Grisham 2009) could also take advantage of advances in genomics and proteomics to inspire the development of crustacean teaching exercises for the 21st century.

<1> Conclusions

Crustacean neurobiology has contributed immensely to the teaching laboratory toolbox for hands-on learning of basic principles of nervous system physiology. A relatively large selection of crustacean teaching preparations were represented in early published lab manuals. Presently, however, it appears that only a few preparations are used widely for studies of the resting potential, synaptic transmission, and sensory physiology. Many older preparations not commonly in use could be modernized for the millennial student, and there are yet more crustacean research preparations that could be developed for general student studies of nervous system principles. The impact of crustacean neurobiology on the understanding of nervous system physiology will grow as students and faculty from varied disciplines apply their specific tools and paradigms to neuroscience questions.

<1> Acknowledgments

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Tina Pollard of Astro-Med, Inc. for providing us with the handouts from Grass Instrument Company and making them now easily available online (Grass Technologies 2011).

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Table Legend:

Table 20.1.

Survey of selected student exercises highlighting crustaceans in laboratory teaching.

Figure Legends:

Fig. 20.1.

Crayfish leg muscle preparations. (A) Motor nerve for the leg extensor muscle, stained with the fluorescent vital dye 4-Di-2-ASP. Examples of tonic (varicosities) and phasic (thin, less prominent synapses) neurons are labeled. (B) Diagram of dactyl opener and extensor muscle preparations. (C) Mixed tonic and phasic (upper trace) and tonic-only (lower trace) excitatory post-synaptic potentials (EPSPs) from leg extensor muscle fibers during stimulation of tonic and phasic motor neurons. Tonic EPSPs show facilitation, while phasic EPSPs are of greater amplitude. A and C adapted with permission from Wu and Cooper (2011); B courtesy of Ronald Hoy.

Fig. 20.2.

The crayfish superficial flexor preparation. (A) Ventral view of a crayfish and cross-section (at the indicated line) showing the main muscle groups. (B) Ventral view of a crayfish tail segment with cuticle removed, stained with methylene blue. Ventral nerve cord, vnc; third and fourth segment ganglia (g3, g4); ganglionic nerves (n1, n2, n3); superficial flexor muscle (sf) and its attachment point (ma); pleopod stumps (pl); sternites cut along midline (s3,s4). (C) Extracellular

recording of motor neuron action potentials from nerve 3. Top trace, activity after telson stimulation; middle trace, activity after pleopod (swimmeret) stimulation; bottom trace, spontaneous activity without stimulation. Distinct action potential amplitudes indicate firing from individual axons of different diameters. Adapted with permission from Wyttenbach et al. (1999).

Fig. 20.3.

Spontaneous and evoked activity at the superficial flexor neuromuscular junction. (A) Top: Recording setup for matching presynaptic action potentials with muscle EPSPs. Bottom: Traces show selective and polyneuronal innervation of the muscle fiber and summation of synaptic potentials. (B) Top: Recording set up for evoking and recording EPSPs in response to paired stimulation of nerve 3 stimulation at varying intervals. Bottom: Dependence of synaptic facilitation strength on interval, showing student data fit with an exponential equation. Inset: single trace showing calculation of the facilitation index as the ratio of the amplitudes of two EPSPs elicited by paired 20 ms stimulations of the same motor neuron. Adapted with permission from Wyttenbach et al. (1999).

Fig. 20.4.

Simultaneous conduction-velocity measurements from multiple motor neurons of different axon diameters. (A) Cross-section of nerve 3 from a crayfish abdominal ganglion. Six motor neurons (*) of different diameters are visible. Tissue stained with toluidine blue. (B) Dual extracellular recordings from nerve 3 indicating faster conduction of large action potentials than smaller ones. (C) Student conduction-velocity calculations from the time intervals of 6 amplitude classes of

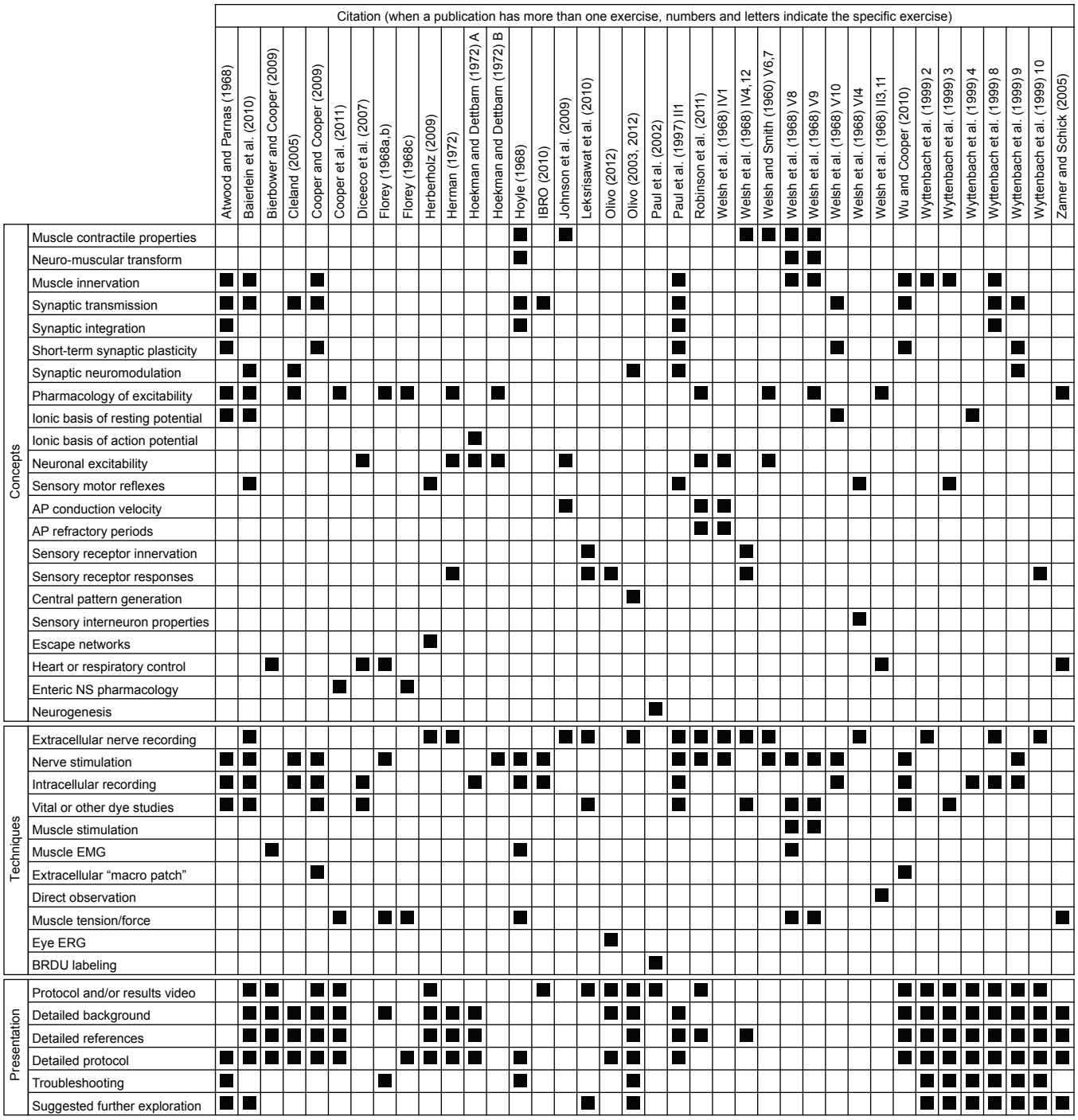
APs spontaneously firing and recorded with two extracellular electrodes a known distance apart. The largest diameter axons in A have the fastest conduction time. (D) Student data on the effect of temperature on the conduction velocity of neuron 2 in C. A adapted with permission from Wyttenbach et al. (1999); B courtesy of Steve Hauptman; C and D from student data gathered at Cornell University.

Fig. 20.5.

Stimulus-response properties of the slowly adapting stretch receptor (MRO_1) in crayfish. (A) Diagram showing how flexing the tail stretches the superficial extensor muscle and stimulates the stretch receptor. (B) Extracellular recording of MRO_1 response to a maintained stretch, showing adaptation of action potential firing. (C) Stimulus-response curve for MRO_1. (D) Instantaneous spike rate of MRO_1 plotted against time for maintained stretches of different amounts. Adaptation curve for each stretch fit with an exponential equation (time constant ~ 4s). Figure adapted with permission from Wyttenbach et al. (1999).

Fig. 20.6.

Neurogenesis in crayfish brains. Confocal images of juvenile crayfish brains from enriched (A) and impoverished (B) environments. Bright areas in A (indicated by arrow heads) demonstrate neurogenesis in a crayfish from an enriched environment. Adapted with permission from Paul et al. (2002).


Citation (when a publication has more than one exercise, numbers and letters indicate the specific exercise)
Atwood and Parnas (1968)
Baierlein et al. (2010)
Bierbower and Cooper (2009)
Cleland (2005)
Cooper and Cooper (2009)
Cooper et al. (2011)
Diceeco et al. (2007)
Florey (1968a,b)
Florey (1968c)
Herberholz (2009)
Herman (1972)
Hoekman and Dettbarn (1972) A
Hoekman and Dettbarn (1972) B
Hoyle (1968)
IBRO (2010)
Johnson et al. (2009)
Leksrisawat et al. (2010)
Olivo (2012)
Olivo (2003, 2012)
Paul et al. (2002)
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Robinson et al. (2011)
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Welsh et al. (1968) IV4,12
Welsh and Smith (1960) V6,7
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Welsh et al. (1968) V10
Welsh et al. (1968) VI4
Welsh et al. (1968) II3,11
Wu and Cooper (2010)
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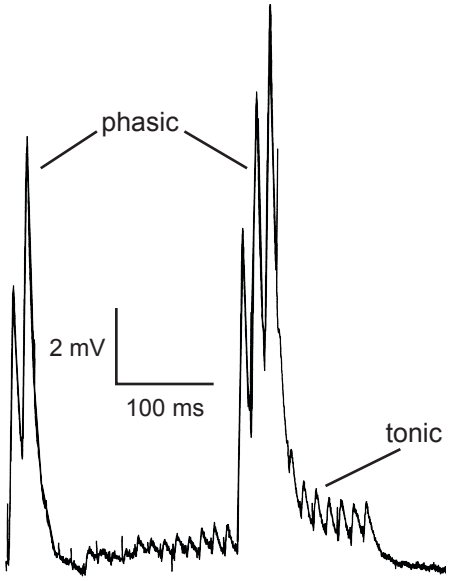
Wyttenbach et al. (1999) 8
Wyttenbach et al. (1999) 9
Wyttenbach et al. (1999) 10
Zamer and Schick (2005)
Concepts
Muscle contractile properties
Neuro-muscular transform
Muscle innervation
Synaptic transmission
Synaptic integration
Short-term synaptic plasticity
Synaptic neuromodulation
Pharmacology of excitability
Ionic basis of resting potential
Ionic basis of action potential
Neuronal excitability
Sensory motor reflexes
AP conduction velocity
AP refractory periods
Sensory receptor innervation
Sensory receptor responses
Central pattern generation
Sensory interneuron properties
Escape networks
Heart or respiratory control
Enteric NS pharmacology
Neurogenesis
Techniques
Extracellular nerve recording
Nerve stimulation
Intracellular recording
Vital or other dye studies
Muscle stimulation
Muscle EMG
Extracellular “macro patch”
Direct observation
Muscle tension/force
Eye ERG
BRDU labeling
Presentation
Protocol and/or results video
Detailed background
Detailed references
Detailed protocol
Troubleshooting
Suggested further exploration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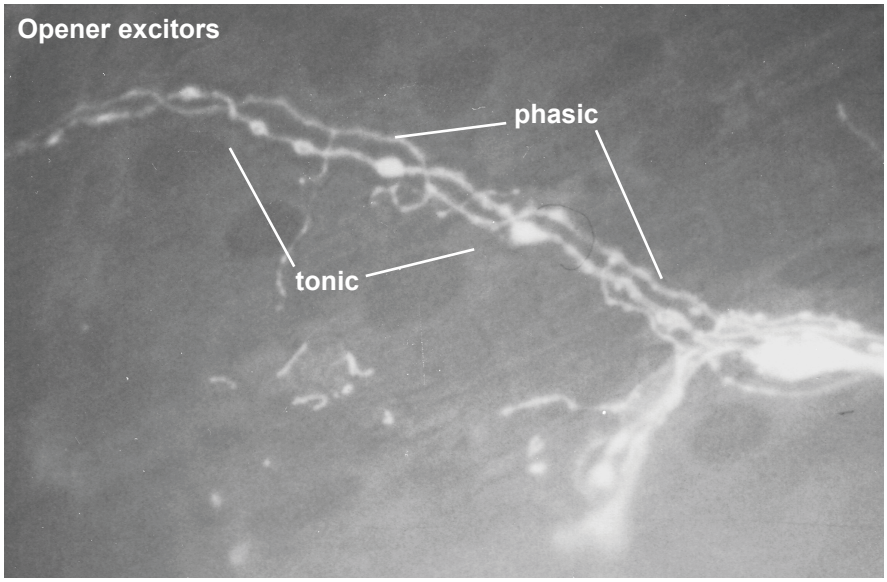
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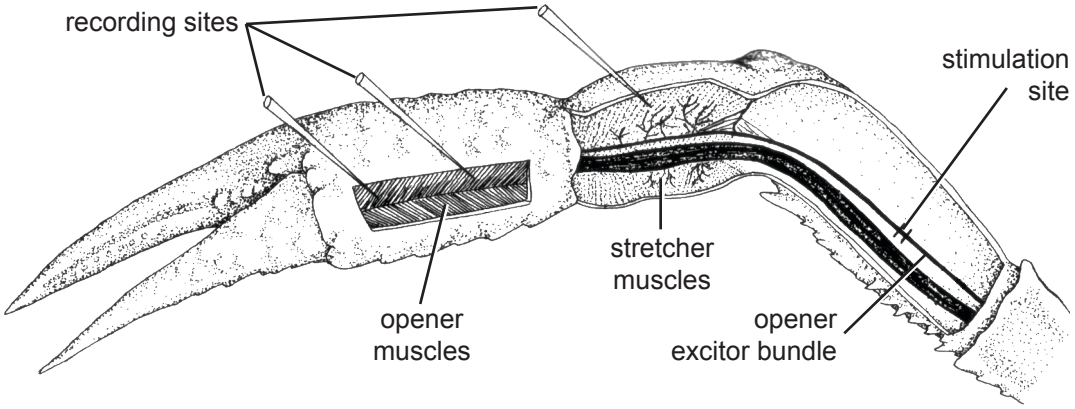
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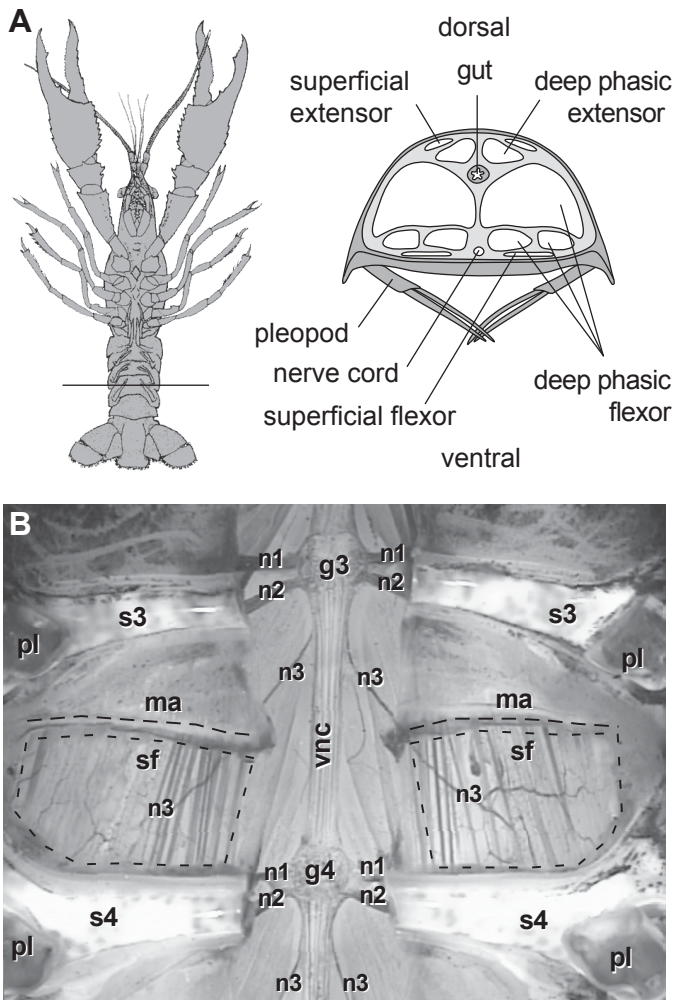
opener excitor bundle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gut
superficial extensor
deep phasic extensor
pleopod
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g3
n1
n2
s3
s3
pl
pl
n3
n3
ma
ma
vnc
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sf
n3
n3
n1
n2
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n3
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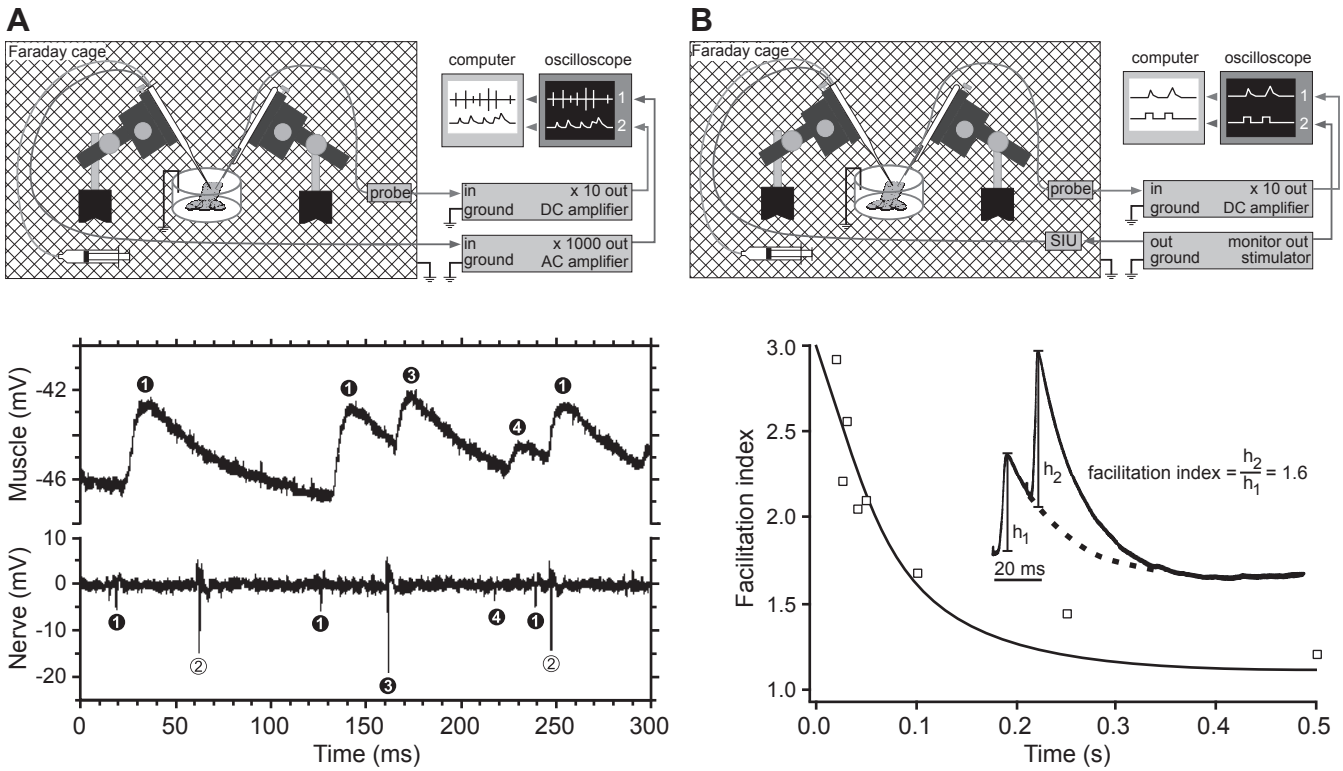
100 ms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Faraday cage
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oscilloscope
probe
in
ground
x 10 out
DC amplifier
in
ground
x 1000 out
AC amplifier
B
Faraday cage
computer
oscilloscope
probe
in
ground
x 10 out
DC amplifier
SIU
out
ground
monitor out
stimulator
Muscle (mV)
-42
-46
Nerve (mV)
10
0
-10
-20
0
50
100
150
200
250
300
Time (ms)
Facilitation index
3.0
2.5
2.0
1.5
1.0
0.0
0.1
0.2
0.3
0.4
0.5
Time (s)
h2
h1
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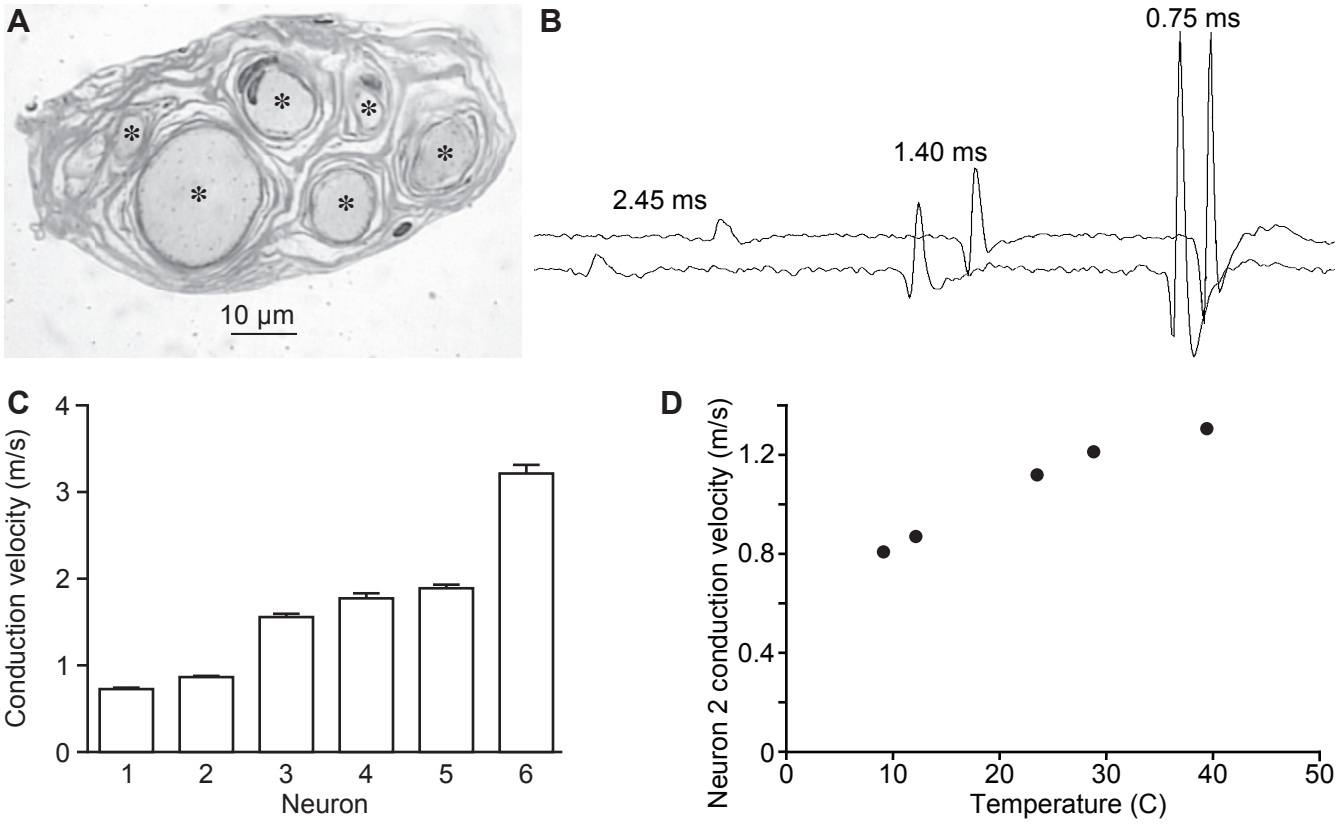
facilitation index = h2/h1 = 1.6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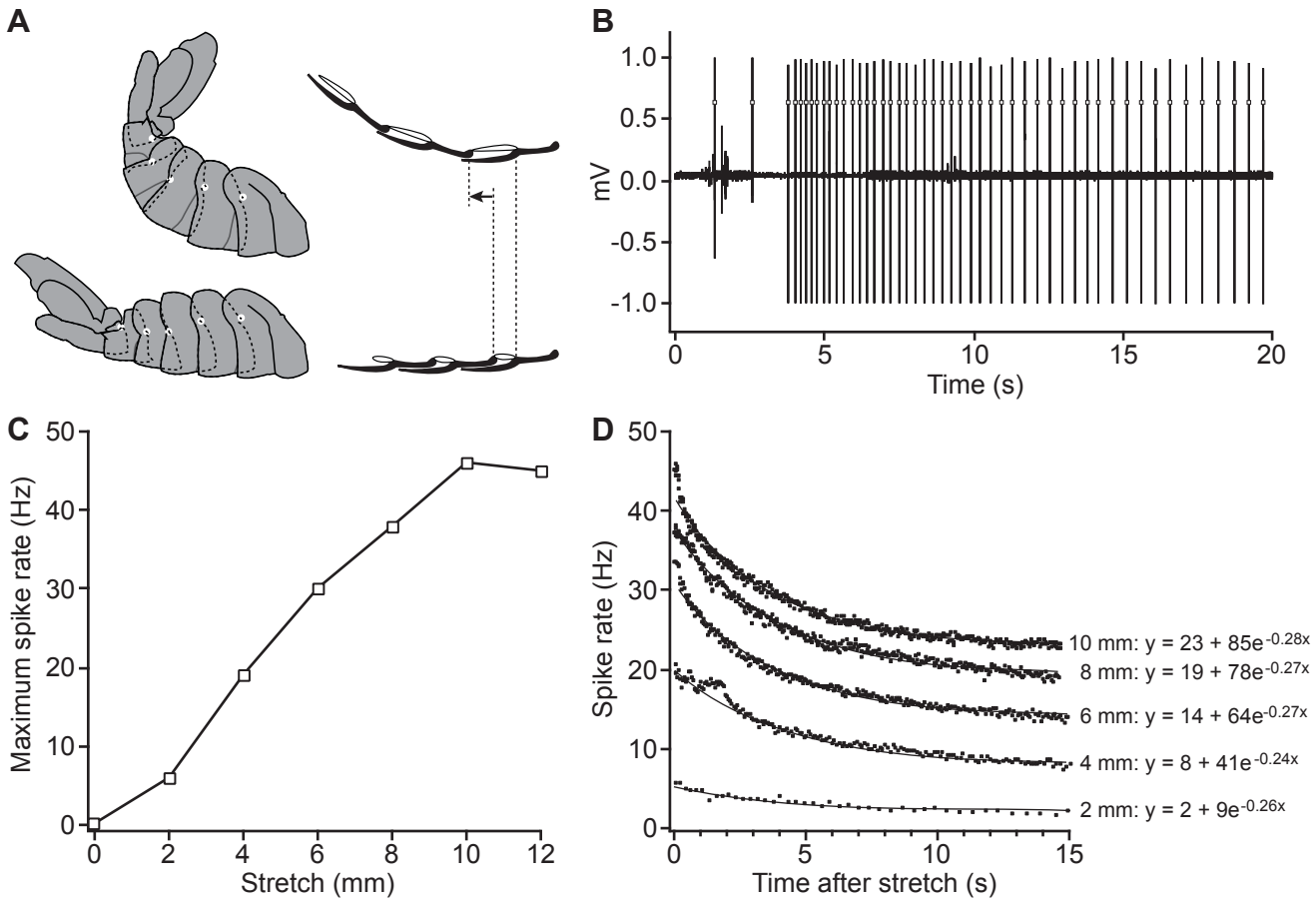
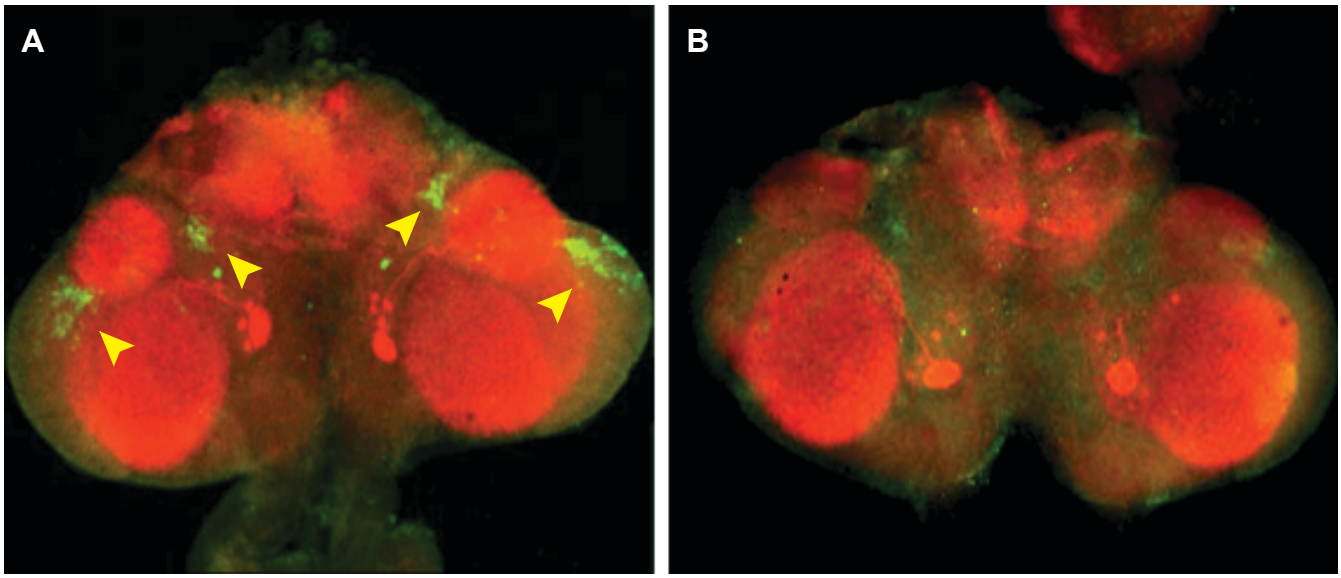
B
0.75 ms
1.40 ms
2.45 ms
C
Conduction velocity (m/s)
0
1
2
3
4
1
2
3
4
5
6
Neuron
D
Neuron 2 conduction velocity (m/s)
0
0.4
0.8
1.2
0
10
20
30
40
50
Temperature (C)


A
B
1.0
0.5
0.0
-0.5
-1.0
mV
0
5
10
15
20
Time (s)
C
Maximum spike rate (Hz)
50
40
30
20
10
0
0
2
4
6
8
10
12
Stretch (mm)
D
Spike rate (Hz)
50
40
30
20
10
0
0
5
10
15
Time after stretch (s)
10 mm: y = 23 + 85e-0.28x
8 mm: y = 19 + 78e-0.27x
6 mm: y = 14 + 64e-0.27x
4 mm: y = 8 + 41e-0.24x
2 mm: y = 2 + 9e-0.26x


A
B