Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Research 48 (2004) 221–227
Neuroscience Research
www.elsevier.com/locate/neures

Technical note

Modulation of sensory–CNS–motor circuits by serotonin, octopamine, and dopamine in semi-intact *Drosophila* larva

Sameera Dasari, Robin L. Cooper*

Department of Biology, University of Kentucky, 101 Rose Street, Lexington, KY 40506-0225, USA

Received 20 August 2003; accepted 7 October 2003

Abstract

We have introduced an in-situ preparation to induce motor unit activity by stimulating a sensory–CNS circuit, using the third instar larvae of *Drosophila melanogaster*. Discrete identifiable motor units that are well defined in anatomic and physiologic function can be recruited selectively and driven depending on the sensory stimulus intensity, duration, and frequency. Since the peripheral nervous system is bilaterally symmetric to coordinate bilateral symmetric segmental musculature patterns, fictive forms of locomotion are able to be induced. Monitoring the excitatory postsynaptic potentials (EPSP) on the prominent ventral longitudinal body wall muscles, such as m6 and m12, provides additional insight into how the selective motor units might be recruited within intact animals. We also introduce the actions of the neuromodulators (serotonin, octopamine (OA) and dopamine (DA)) on the inducible patterns of activity within the sensory–motor circuit. The powerful genetic manipulation in *Drosophila* opens many avenues for further investigations into the circuitry and cellular aspects of pattern generation and developmental issues of circuitry formation and maintenance in the model organism.
© 2003 Elsevier Ireland Ltd and The Japan Neuroscience Society. All rights reserved.

Keywords: Neurotransmission; Neuromuscular junction; Behavior; Development; Locomotion; Central pattern generator

1. Introduction

Sensory input early in life sculpts central circuits which can become relatively hard wired after defined critical periods. This was most elegantly shown in the 1960's experimentally for the visual system in cats and monkeys (Hubel and Wiesel, 1963a,b, 1968, 1970) and is clinically relevant to humans. Other parts of the brain also show similar dependences on sensory activity in development. The formation of cortical circuits is of interest since this controls thought processes and forms of learning (Pallas, 2001). Refined experimentation of sensory attributes defining CNS and motor units have been possible in relatively less complex organisms. A striking example is in the development of the asymmetric claws of lobsters (Lang et al., 1978) where Govind and colleagues demonstrated that juvenile lobsters depend on sensory stimulation for the asymmetry to occur (Govind and Pearce, 1986). When lobsters (*Homarus americanus*) are not allowed to manipulate objects in their claws they will develop two cutter claws, where as if one claw is exercised a crusher claw will develop over subsequent molts for the side that had prior enhanced sensory stimulation. Not only is the muscle phenotype, biochemistry, and cuticle differentiated but the number of sensory neurons and the central neuropile in the thoracic ganglion are modified during development of the asymmetry (Cooper and Govind, 1991; Govind and Pearce, 1985; Govind et al., 1988).

In the genetically favorable invertebrate *Drosophila*, Suster and Bate (2002) produced embryos with reduced sensory function which results in abnormal peristalsis of embryonic movements, which suggests sensory activity is developmentally important in shaping central control of motor output within invertebrates. However, the problem still challenging the field is in understanding the integration of sensory input that controls muscular movements in a coordinated fashion. Recent studies in pharmacological treatments of spinal cord injuries in cats and in humans have revealed that recovery of locomotion is enhanced by using selective agonists and antagonist of neurotransmitters involved in sensory–CNS–motor circuits (Chau et al., 2002; Rossignol, 2000; Rossignol et al., 2001, 2002). These recent studies are a breakthrough in manipulating selective sensory

* Corresponding author. Tel.: +1-859-257-5950; fax: +1-859-257-1717.
E-mail address: RLCOOP1@pop.uky.edu (R.L. Cooper).

0168-0102/$ – see front matter © 2003 Elsevier Ireland Ltd and The Japan Neuroscience Society. All rights reserved.
doi:10.1016/j.neures.2003.10.005

systems and higher order function in controlling motor output.

The ability to combine a genetically favorable system and pharmacological studies is opening new horizons in regulation of development in neural circuits. In addition, neuromodulators provide a rapid way in which animals can tune up or down activity within a neural circuit and may be responsible for rapid changes in behavior, as recently examined for aggressive behavior in *Drosophila* (Baier et al., 2002). We assessed three common neuromodulators of interest in arthropod neurobiology: serotonin (5-HT), octopamine (OA), and dopamine (DA). Voltage dependent potassium channels and heart rate are modulated by 5-HT in *Drosophila* (Johnson et al., 1997; Zornik et al., 1999). DA is known to alter sexual behavior, habituation (Neckameyer, 1998a,b) and increase activity in adult flies (Friggi-Grelin et al., 2003) but depress synaptic transmission at the NMJ in larval *Drosophila* (Cooper and Neckameyer, 1999). Behaviors in bees are also affected by DA (Taylor et al., 1992). OA expression is related to stress responses in *Drosophila* (Hirashima et al., 2000) and OA receptors are present in mushroom bodies in *Drosophila* CNS (Han et al., 1998). These past studies indicate that there is a precedence of 5-HT, DA, and OA to have central effects in the *Drosophila* brain (Blenau and Baumann, 2001; Monastirioti, 1999). The purpose of these studies is present an in situ preparation of larval *Drosophila*, with intact sensory–CNS–motor circuits, to serve as a model system for investigating actions of neuromodualtors on developing central circuits.

2. Methods

Many of the procedures used here have been previously described in detail (Ball et al., 2003; Cooper and Neckameyer, 1999; Li and Cooper, 2001; Li et al., 2001, 2002). The staining of the nerve terminals with an antibody to HRP was described previously (Li et al., 2002). In brief, the following procedures and condition were used with the modifications emphasized.

2.1. Stock and staging of larvae

The common 'wild-type' laboratory strain of *Drosophila melanogaster*, Canton S, was used in these studies. The methods used to stage fly larvae have been described previously (Campos-Ortega and Hartenstein, 1985; Li et al., 2002). Larvae at the beginning of the "wandering" phase of the third instar were used in these experiments.

2.2. Dissection and physiological conditions

Dissections included removal of the heart and viscera which left a filleted larvae containing only a body wall, body wall muscles and the neural circuitry for the sensory, CNS and body wall (i.e., skeletal) motor units as described earlier (Cooper et al., 1995). The HL3 saline was prepared in the lab from component reagents (Sigma) and contained: 1.0 mM $CaCl_2{\cdot}2H_2O$, 70 mM NaCl, 5 mM KCl, 10 mM $NaHCO_3$, 5 mM trehalose, 115 mM sucrose, and 5 mM BES (*N*,*N*-bis[2-Hydoxyethyl]-2-aminoethanesulfonic acid) (Stewart et al., 1994).

2.3. Electrophysiology

The recording arrangement was essentially the same as previously described (Neckameyer and Cooper, 1998; Stewart et al., 1994). Intracellular recordings in muscles were made with 30–60 MΩ resistance, 3 M KCl-filled microelectrodes. The amplitudes of the excitatory postsynaptic potentials (EPSP) elicited by Is and Ib motor nerve terminals in the various segments of muscles m6 and m12 were monitored. Intracellular responses were recorded with a 1 × LU head stage and an Axoclamp 2A amplifier. Stimulation of segmental nerve roots was provided by suction electrodes (Cooper and Neckameyer, 1999). The stimulator (S-88, Grass) output was passed through a stimulus isolation unit in order to alter polarity and gain (SIU5, Grass). Electrical signals were recorded on-line to a PowerMac 9500 and G4 Mac via a MacLab/4s interface. All events were measured and calibrated with the MacLab Scope software 3.5.4 version. All experiments were performed at room temperature (19–22 °C).

3. Results

In filleted third instar larvae, each segmental nerve root and ventral body wall musculature is readily observed (Fig. 1A). Various identified muscles with a rather simplistic innervation profiles can be used to monitor motor neuron activity (Fig. 1B). In these studies, we utilized muscle 6 (m6) and muscle 12 (m12) because of the well characterized innervation and synaptic properties of the Is and Ib motor nerve terminals (Fig. 1C) (Atwood et al., 1993; Kurdyak et al., 1994; Li et al., 2002). Each segmental nerve root can be stimulated to drive sensory input into the larval brain as well as stimulating motor neurons to the segmental muscles that particular root is associated. By transecting the root and only stimulating the distal aspect of the root, the motor neurons are devoid of CNS activity and defined patterns of stimulation can be given. Likewise, the proximal root can either be left intact or transected to drive sensory patterns to the CNS for a particular segment or segments when multiple roots are utilized. Here we used single intact segmental roots to drive central circuits and record motor unit activity in contra- and ipsi-lateral segments to the segment being stimulated (Fig. 1B).

Since the innervation to m6 and m12 is well defined, one can assess which specific motor neurons are being recruited as a result of sensory stimulation by monitoring the EPSPs induced in these particular muscles. The responses

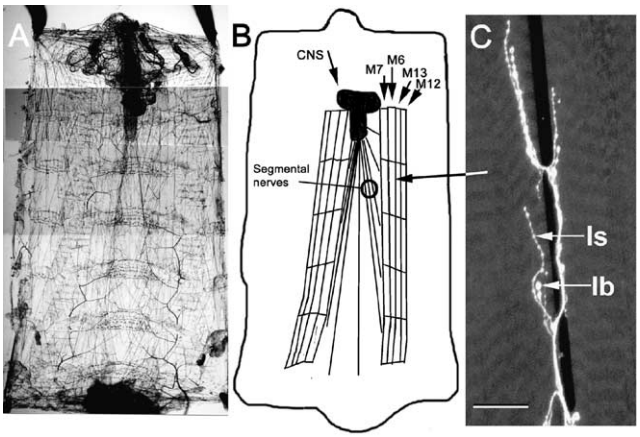

Fig. 1. Schematic diagram of the *Drosophila* larva preparation. (A) The preparation is pinned at the four corners to keep the preparation taut. The ventral abdominal muscles, m6 and m12, were used in this study. (B) The segmental nerves can be stimulated by placing the nerve into the lumen of a suction electrode and recruiting various subsets of sensory neurons. The segmental roots can be severed from the body wall to selectively stimulate sensory nerves orthodromicaly. (C) The terminals of Ib and Is on m6 and m7 are readily observed after treatment with fluorescently tagged anti-HRP antibody. Scale: 750 μm A & B, 90 μm C.

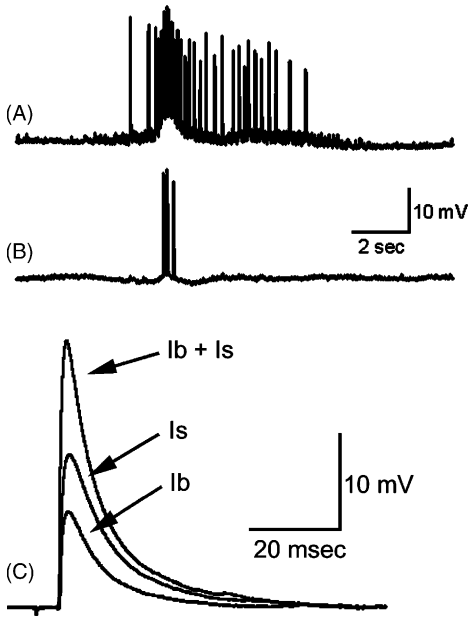

Fig. 2. Representative traces of induced responses recorded in muscle 6 in various segments. The third segmental nerve on the right side of the larva was stimulated at a given voltage and frequency while responses were monitored in the m6 (A) and m12 (B) muscles on the contra-lateral side to the stimulated nerve root. In segment 3, contra-lateral to the segmental root being stimulated, EPSP responses in two different muscles m6 and m12 reveal that selective motor neurons can be recruited. The motor neuron RP3 innervates both m6 and m12 while the motor neuron 6/7b innervates m6 but not m12. Sometimes the Ib is selectively recruited since only a response in m6 is observed. (C) Elicited responses in m6 is readily possible with a intracellular recording as a consequence of stimulating the transected segmental root. Representative individual responses from the Ib and Is motor axons as well as the composite Ib and Is response are shown from late third instars.

that can be evoked in m6 in the various segments when stimulating the third segmental nerve on the right side is shown in Fig. 2A. When monitoring two muscles simultaneously, selective motor neurons that are recruited which innervate both m6 and m12 or motor units which exclude m12 are able to be observed (Fig. 2B). In addition, since the Ib and Is motor nerve terminals that innervate m6 show different morphology and physiological responses they can be discerned individually or when they are recruited in unison. The terminals of the Is axon contain small varicosities along its length and give rise to large EPSPs in the muscle, where as the Ib axon has big varicosities on its terminals (Figs. 1C and 2C), but produces smaller EPSPs (Atwood et al., 1993; Kurdyak et al., 1994; Stewart et al., 1994). The induced depolarizations on these muscles are graded and are non-spiking.

To examine if recruitment of sensory axons, interneurons and motor neurons is dependent on stimulation, three stimulation conditions were used. First, we examined the response of the motor units to stimulus duration. An increase in the duration of a train of stimuli enhanced activity of motor units (Fig. 3A, 40 Hz with 10 stimuli; B, 40 Hz with 15 stimuli). In addition, increasing the frequency of stimulation recruited motor units rapidly as compared to lower stimulus frequencies (Fig. 3A, 40 Hz with 10 stimuli; C, 60 Hz with 10 stimuli). The amount of motor activity is also dependent on the intensity of stimulation (Fig. 3D, 40 Hz with 10 stimuli low stimulus voltage). In five out of five preparations, the higher the stimulation frequency (40–60 Hz), the longer duration of the stimulation (10–20 pulses at 40 Hz), and the higher the stimulation intensity (increased by 1 V to the stimulating electrode) all resulted in an increase in the average activity of the motor neuron. The percent change from 40 Hz with a 10 pulse train is used for comparison (Fig. 3E). For this analysis, five periods of 500 ms duration, every 10 s, were obtained and an average number of EPSPs was determined. Increasing the stimulation duration had the greatest effect in enhancing motor unit activity. It should be noted if the stimulation intensity is too large there a failure to evoke action potentials can occur. Thus, some sensory neurons may drop out of as stimulation increases to very large voltages.

To determine the effects of 5-HT, OA, and DA in altering the sensory to motor neuron central circuit a segmental root was stimulated while the evoked responses in the contra-lateral m6 were monitored prior and during exposure to neuromodulators. The neuromodulators were applied by rapidly exchanging the entire bathing media with the a saline containing the desired concentration. A single preparation was used for a given manipulation. Since the degree of recruiting motor neurons varied in each preparation a percent difference in the firing frequency of the motor units was quantified (Fig. 4A). OA at 10 μM resulted in massive waves of muscle contraction making it difficult to maintain an intracellular recording ($n = 6$). Thus, a lower concentration of 1 μM was used for OA as compared to 5-HT and DA. In all

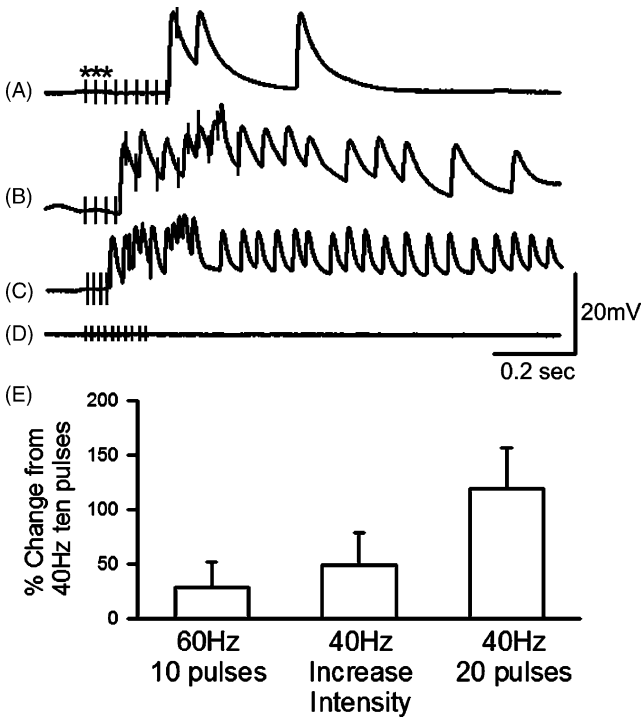

Fig. 3. Recruitment of motor units is dependent on the duration of the stimulation (A, 40 Hz, 10 pulses; B, 40 Hz, 15 pulses), frequency of stimulation (A, 40 Hz, 10 pulses; C, 60 Hz, 10 pulses), and intensity (C, 60 Hz, 10 pulses high stimulus voltage; D, 60 Hz, 10 pulses low stimulus voltage). Represented are EPSPs recorded in m6 induced by stimulating the contra-lateral segmental root. At subthreshold (D) stimulation of sensory afferents no inducible responses are observed. However, recruitment occurs with an increased stimulation intensity (C). Stars in top trace indicate stimulus artifacts for the first three within the stimulus train. (E) An average percent change from 40 Hz with a 10 pulse train is used for comparison to a higher the stimulation frequency (60 Hz), a higher the stimulation intensity (increased by 1 V to the stimulating electrode), and a longer duration of the stimulation (20 pulses at 40 Hz) (at least $n = 5$ for each condition).

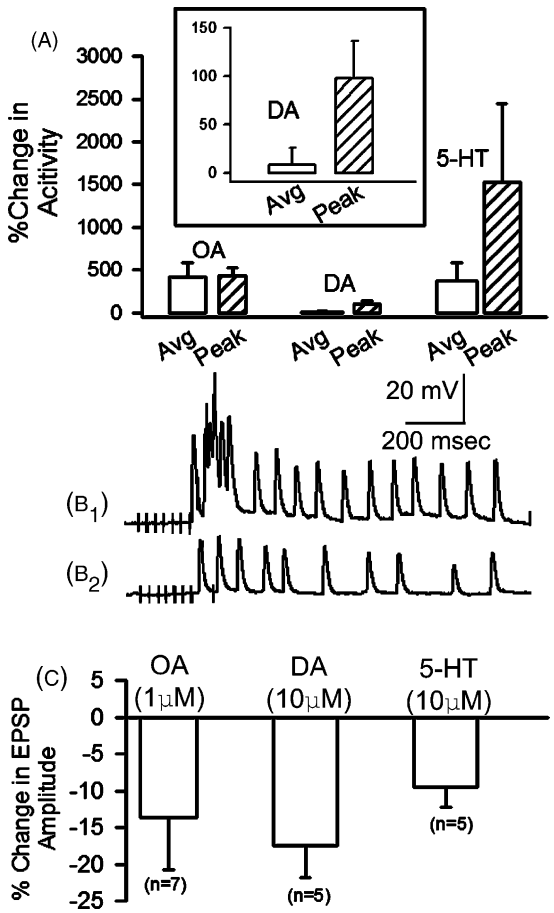

Fig. 4. The influence of neuromodulators in altering the sensory to motor neuron central circuit was examined. (A) A percent difference in the firing frequency of the motor units to m6 was determined before and during exposure to a either serotonin (5-HT,10 μM), octopamine (OA, 1 μM), or dopamine (DA, 10 μM). Five independent preparations were examined for each neuromodulator. Since biphasic responses were observed for 5-HT, a peak enhancement in the firing frequency was measured within 1 min. The peak response and an average response for 2 min were used for analysis. The inset shows an enlarged view of the bar chart for the DA responses. A typical biphasic response induced by 5-HT is depicted by comparing B_1 (upon initial exposure) to B_2 (1 min and 26 s later). (C) Direct assessment of OA (1 μM), DA (10 μM), and 5-HT (10 μM) on the amplitude of evoked combined Is and Ib EPSPs at the neuromuscular junction on m6 revealed that both OA and DA depressed synaptic transmission.

cases, OA enhanced the firing frequency of the motor units. 5-HT (10 μM) showed biphasic effects in altering the frequency of evoked motor unit response. Initially an enhancement in the frequency was observed but within a 1–2 min a decrease in the frequency of the evoked responses occurred. The frequency in the evoked responses was measured for the peak excitatory effect within the first minute and the frequency after 2 min. The results are shown for five preparations (Fig. 4A). Only a small excitatory effect was observed for DA (10 μM), however like for 5-HT, a transitory effect was observed (See enlarged inset). Five preparations were used for each compound and in each case the direction of change was the same ($P < 0.05$, $n = 5$, non-parametric rank sum Wilcoxon test). To illustrate the biphasic response induced by 5-HT the first stimulus train and resulting EPSPs after exposure is shown (Fig. 4B_1) along with a stimulus train 1 min and 26 s later (Fig. 4B_2).

In examining the direct effects of the neuromodulators at the NMJ, a transected segmental nerve was stimulated distally to evoke a combined response from the Ib and Is terminals on m6. A percent change in the amplitude of the composite EPSPs revealed that both OA and DA reduced the amplitude ($P < 0.05$, non-parametric rank sum Wilcoxon test) where as 5-HT had no significant effect on the response (Fig. 4C).

4. Discussion

The topic of rhythmic control of locomotion is an age old question since Sherrington's time (Sherrington, 1898). Significant breakthroughs have occurred over the years,

however the regulation and neural integration of locomotion remains a significant hurdle for the field. In this report, we demonstrate that the model organism, *D. melanogaster* offers a unique advantage to begin to address pattern generation involved in locomotion as well as the role various sensory inputs have that drive circuits. In addition, the role of neuromodulators which is now proving to be advantageous to alter locomotive patterns in spinal injury models in mammals can also be assessed in larval *Drosophila*. The powerful genetic manipulation of the organism opens many avenues for further investigations into the circuitry and cellular aspects of sensory integration. The goal of this technical report is to present one with a preparation to address physiological effects in development and maintenance of central circuitry that could possibly be correlated with behavior.

The projections of sensory neurons in the larva can influence later development of novel sensory neurons in the adult, thus pharmacological manipulation or altered activity profiles in the larva can be examined in shaping the adult CNS of holomotabolus insects. Targeting particularly gene mutations in *Drosophila* towards specific sensory neurons or even all sensory neuronal function by inducible tetanus toxin light chain expression (Suster and Bate, 2002) within neurons will allow refined and gross manipulations of the circuitry for assessment of function and adaptation. As with *Caenorhabditis elegans* (Francis et al., 2003), genetic alterations in the expression of proteins involved in synaptic transmission result in behavioral patterns that can be quantified in larval and adult *Drosophila* (Neckameyer and Cooper, 1998; Li et al., 2001).

In our initial investigations, we were interested in monitoring fictive locomotion from recordings of the segmental nerves in filleted and pinned larvae (Fig. 1). Rhythmic patterns do appear, but the patterns are not reliable between preparations. In addition, when bursts of activity are recorded, the frequency profiles run down rather quickly making it difficult for long-term assessment of fictive locomotion patterns. Hence, we turned to an alternative approach of driving the motor units by sensory nerve stimulation and then assessing the role activity of sensory inputs and neuromodulators on the circuit. A similar approach has been used in the semi-intact leech preparation where electrical stimulation of sensory roots produces a escape swim circuit (Weeks, 1981). The fictive swimming can also be induced by exposure of the ventral nerve cord to 5-HT (Willard, 1981). Like wise, locomotor activity in the isolated spinal cord of the lamprey can be induced by bath application of NMDA (Svensson et al., 2003). The stomatogastric ganglion (STG) of crustaceans also serves as a nice invertebrate model for investigating actions of neuromodulators on motor patterns. It has been shown in the STG that neural circuits and the networks are modulated by biogenic amines and there is both convergence and divergence in their action (Marder and Thirumalai, 2002).

Our particular interests focus on the influences of hormones and neuromodulators in altering central circuitry, particularly the ones already known to have a role in altering synaptic growth and plasticity at the neuromuscular junction (Cooper and Neckameyer, 1999; Li and Cooper, 2001; Li et al., 2001; Neckameyer and Cooper, 1998; Ruffner et al., 1999). It is well established that hormones such as ecdysone and juvenile hormone alter neural development and differentiation in insects (Garen et al., 1977; Pak and Gilbert, 1987; Truman, 1996). The surge of ecdysone in the pupal stage of *Drosophila* likely plays a key role in inducing gross alterations in neural circuitry (Kraft et al., 1998; Thummel, 1996; Truman and Reiss, 1988) and motor unit function (Li and Cooper, 2001; Li et al., 2001). Likewise, other hormones or cocktails of other hormones need to be investigated for their developmental roles, since it has been demonstrated that the sequence of neuromodulator exposure and cocktails produce differential effects on synaptic modulation in other arthropods (i.e., the crustaceans) (Djokaj et al., 2001).

Since in the intact organism, compensatory mechanisms may override experimentally induced genetic, hormonal or environmental alterations, one can now turn to whole CNS and body musculature culture of larval *Drosophila* to address specific questions (Ball et al., 2003). However, many compounding variables need to be considered, such as the loss of normal movements and appropriate feedback responses in culture conditions. The physiological saline based on the composition of larval hemolymph, HL3, preserves synaptic transmission as well as muscular function and integrity (Stewart et al., 1994). Slight modifications of the HL3 saline are used for culturing the preparation (Ball et al., 2003), but perhaps the recently developed HL6 saline (Macleod et al., 2002) should be examined. With the physiological method presented, genetic or pharmacological manipulation of neuromodulators over a long-term, in the whole animal or in culture, can be readily assessed. However, the challenge is now to determine where the neuromodulators are acting (i.e., sensory, interneurons, and/or motor neurons) and what receptor subtypes exists.

Acknowledgements

Funding was provided in part by NSF grants IBN-9808631 (RLC), NSF-ILI-DUE 9850907 (RLC), NSF-IBN-0131459 (RLC) and a G. Ribble Fellowship in the School of Biological Sciences at the University of Kentucky (SD).

References

Atwood, H.L., Govind, C.K., Wu, C.F., 1993. Differential ultrastructure of synaptic terminals on ventral longitudinal abdominal muscles in *Drosophila* larvae. J. Neurobiol. 24, 1008–1024.

Baier, A., Wittek, B., Brembs, B., 2002. *Drosophila* as a new model organism for the neurobiology of aggression? J. Exp. Biol. 205, 1233–1240.

Ball, R., Xing, B., Bonner, P., Shearer, J., Cooper, R.L., 2003. Long-term maintenance of neuromuscular junction activity in cultured *Drosophila* larvae. Comp. Biochem. Physiol. A 134, 247–255.

Blenau, W., Baumann, A., 2001. Molecular and pharmacological properties of insect biogenic amine receptors: lessons from *Drosophila melanogaster* and *Apis mellifera*. Arch. Insect Biochem. Physiol. 48, 13–38.

Campos-Ortega, J.A., Hartenstein, V. (Eds.), 1985. In: The Embryonic Development of *Drosophila melanogaster*. Spring-Verlag, Berlin.

Chau, C., Giroux, N., Barbeau, H., Jordan, L., Rossignol, S., 2002. Effects of intrathecal glutamatergic drugs on locomotion I. NMDA in short-term spinal cats. J. Neurophysiol. 88, 3032–3045.

Cooper, R.L., Govind, C.K., 1991. Axon composition of the proprioceptive PD nerve during growth and regeneration of lobster claws. J. Exp. Zool. 260, 181–193.

Cooper, R.L., Neckameyer, W.S., 1999. Dopaminergic neuromodulation of motor neuron activity and neuromuscular function in *Drosophila melanogaster*. Comp. Biochem. Physiol. B 122, 199–210.

Cooper, R.L., Stewart, B.A., Wojtowicz, J.M., Wang, S., Atwood, H.L., 1995. Quantal measurement and analysis methods compared for crayfish and *Drosophila* neuromuscular junctions and rat hippocampus. J. Neurosci. Methods 61, 67–78.

Djokaj, S., Cooper, R.L., Rathmayer, W., 2001. Effects of octopamine, serotonin, and cocktails of the two modulators on synaptic transmission at crustacean neuromuscular junctions. J. Comp. Physiol. A 187, 145–154.

Francis, M.M., Mellem, J.E., Maricq, A.V., 2003. Bridging the gap between genes and behaviour: recent advances in electrophysiological analysis of neural function in *Caenorhabditis elegans*. Trends Neurosci. 26, 90–99.

Friggi-Grelin, F., Coulom, H., Meller, M., Gomez, D., Hirsh, J., Birman, S., 2003. Targeted gene expression in *Drosophila* dopaminergic cells using regulatory sequences from tyrosine hydroxylase. J. Neurobiol. 54, 618–627.

Garen, A., Kauvar, L., Lepesant, J.A., 1977. Roles of ecdysone in *Drosophila* development. Proc. Natl. Acad. Sci. 74, 5099–5103.

Govind, C.K., Pearce, J., 1985. Lateralization in number and size of sensory axons to the dimorphic chelipeds of crustaceans. J. Neurobiol. 16, 111–125.

Govind, C.K., Pearce, J., 1986. Differential reflex activity determines claw and closer muscle asymmetry in developing lobsters. Science 233, 354–356.

Govind, C.K., Pearce, J., Potter, D.J., 1988. Neural attrition following limb loss and regeneration in juvenile lobsters. J. Neurobiol. 19, 667–680.

Han, K.A., Millar, N.S., Davis, R.L., 1998. A novel octopamine receptor with preferential expression in *Drosophila* mushroom bodies. J. Neurosci. 18, 3650–3658.

Hirashima, A., Sukhanova, M.Jh., Rauschenbach, I.Yu., 2000. Genetic control of biogenic-amine systems in *Drosophila* under normal and stress conditions. Biochem. Genet. 38, 167–180.

Hubel, D.H., Wiesel, T.N., 1963a. Receptive fields of cells in striate cortex of very young, visually inexperienced kittens. J. Neurophysiol. 26, 994–1002.

Hubel, D.H., Wiesel, T.N., 1963b. Shape and arrangement of columns in cat striate cortex. J. Physiol. 165, 559–568.

Hubel, D.H., Wiesel, T.N., 1968. Receptive fields and functional architecture of monkey striate cortex. J. Physiol. 195, 215–243.

Hubel, D.H., Wiesel, T.N., 1970. The period of susceptibility to the physiological effects of unilateral eye closure in kittens. J. Physiol. 206, 419–436.

Johnson, E., Ringo, J., Dowse, H., 1997. Modulation of *Drosophila* heart beat by neurotransmitters. J. Comp. Physiol. B 167, 89–97.

Kraft, R., Levine, R.B., Restifo, L.L., 1998. The steroid hormone 20-hydroxyecdysone enhances neurite growth of *Drosophila* mushroom body neurons isolated during metamorphosis. J. Neurosci. 18, 8886–8899.

Kurdyak, P., Atwood, H.L., Stewart, B.A., Wu, C.F., 1994. Differential physiology and morphology of motor axons to ventral longitudinal muscle in larval *Drosophila*. J. Comp. Neurol. 350, 463–472.

Lang, F., Govind, C.K., Costello, W.J., 1978. Experimental transformation of muscle fiber properties in lobster. Science 201, 1037–1039.

Li, H., Cooper, R.L., 2001. Effects of the ecdysoneless mutant on synaptic efficacy and structure at the neuromuscular junction in *Drosophila* larvae during normal and prolonged development. Neurosciences 106, 193–200.

Li, H., Harrison, D., Jones, G., Jones, D., Cooper, R.L., 2001. Alterations in development, behavior, and physiology in *Drosophila* larva that have reduced ecdysone production. J. Neurophysiol. 85, 98–104.

Li, H., Peng, X., Cooper, R.L., 2002. Development of *Drosophila* larval neuromuscular junctions: maintaining synaptic strength. Neurosciences 115, 505–513.

Macleod, G.T., Hegström-Wojtowicz, M., Charloton, M.P., Atwood, H.L., 2002. Fast calcium signals in *Drosophila* motor neuron terminals. J. Neurophysiol. 88, 2659–2663.

Marder, E., Thirumalai, V., 2002. Cellular, synaptic and network effects of neuromodulation. Neural Netw. 15, 479–493.

Monastirioti, M., 1999. Biogenic amine systems in the fruit fly *Drosophila melanogaster*. Microsc. Res. Tech. 45, 106–121.

Neckameyer, W.S., 1998a. Dopamine and mushroom bodies in *Drosophila melanogaster*: experience-dependent and -independent aspects of sexual behavior. Learn. Mem. 5, 157–165.

Neckameyer, W.S., 1998b. Dopamine modulates female sexual receptivity in *Drosophila melanogaster*. J. Neurogenet. 12, 101–114.

Neckameyer, W.S., Cooper, R.L., 1998. GABA transporters in *Drosophila melanogaster*: developmental expression, behavior, and physiology. Invert. Neurosci. 3, 279–294.

Pak, M.D., Gilbert, L.I., 1987. A developmental analysis of ecdysteroids during the metamorphosis of *Drosophila melanogaster*. J. Liq. Chromatogr. 10, 2591–2611.

Pallas, S.L., 2001. Intrinsic and extrinsic factors that shape neocortical specification. Trends Neurosci. 24, 417–423.

Rossignol, S., 2000. Locomotion and its recovery after spinal injury. Curr. Opin. Neurobiol. 10, 708–716.

Rossignol, S., Giroux, N., Chau, C., Marcoux, J., Brustein, J., Reader, T.A., 2001. Pharmacological aids to locomotor training after spinal injury in the cat. J. Physiol. 533, 65–74.

Rossignol, S., Bouyer, L., Barthelemy, D., Langlet, C., Leblond, H., 2002. Recovery of locomotion in the cat following spinal cord lesions. Brain Res.—Brain Res. Rev. 40, 257–266.

Ruffner, M.E., Cromarty, S.I., Cooper, R.L., 1999. Depression of synaptic efficacy in high- and low-output *Drosophila* neuromuscular junctions by the molting hormone (20-Hydroxyecdysone) during intermolt. J. Neurophysiol. 81, 788–794.

Sherrington, C.S., 1898. Decerebrate rigidity, and reflex coordination of movements. J. Physiol. (Lond.) 22, 319–332.

Stewart, B.A., Atwood, H.L., Renger, J.J., Wang, J., Wu, C.F., 1994. Improved stability of *Drosophila* larval neuromuscular preparation in haemolymph-like physiological solutions. J. Comp. Physiol. A 175, 179–191.

Suster, M.L., Bate, M., 2002. Embryonic assembly of a central pattern generator without sensory input. Nature 416, 174–178.

Svensson, E., Woolley, J., Wikström, M., Grillner, S., 2003. Endogenous dopaminergic modulation of the lamprey spinal locomotor network. Brain Res. 970, 1–8.

Taylor, D.J., Robinson, G.E., Logan, B.J., Laverty, R., Mercer, A.R., 1992. Changes in brain amine levels associated with morphological and behavioural development of the worker honeybee. J. Comp. Physiol. 170A, 715–721.

Thummel, C.S., 1996. Flies on steroids–*Drosophila* metamorphosis and the mechanisms of steroid hormone action. Trends Genet. 12, 306–310.

Truman, J.W., 1996. Steroid receptors and nervous system metamorphosis in insects. Dev. Neurosci. 18, 87–101.

Truman, J.W., Reiss, S.W., 1988. Hormonal regulation of the shape of identified motoneurons in the moth *Manduca Sexta*. J. Neurosci. 8, 763–775.

Weeks, J.C., 1981. Neuronal basis of leech swimming: separation of swim initiation, pattern generation and intersegmental coordination by selective lesions. J. Neurophysiol. 45, 698–723.

Willard, A.L., 1981. Effects of serotonin on the generation of the motor program for swimming by the medicinal leech. J. Neurosci. 1, 936–944.

Zornik, E., Paislet, K., Nichols, R., 1999. Neural transmitters and peptide modulate *Drosophila* heart rate. Peptides 20, 45–51.