A genetic manipulation of motor neuron excitability does not alter locomotor output in *Drosophila* larvae

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Abstract

Motor activity, like that producing locomotion, is generated by networks of neurons. At the last output level of these networks are the motor neurons, which send signals to the muscles, causing them to contract. Current research in motor control is focused on finding out how motor neurons contribute to shaping the timing of motor behaviors. Are motor neurons just passive relayers of the signals they receive? Or, do motor neurons shape the signals before passing them on to the muscles, thereby influencing the timing of the behavior? It is now well accepted that motor neurons have active, intrinsic membrane properties - there are ion channels in the cell membrane that allow motor neurons to respond to input in non-linear and diverse ways. However, few direct tests of the role of motor neuron intrinsic properties in shaping motor behavior have been carried out, and many questions remain about the role of specific ion channel genes in motor neuron function. In this study, two potassium channel transgenes were expressed in *Drosophila* larvae, causing motor neurons to fire at lower levels of current stimulation and at higher frequencies, thereby increasing excitability. Mosaic animals were created in which some identified motor neurons expressed the transgenes while others did not. Motor output underlying crawling was compared in muscles innervated by control and experimental neurons in the same animals. Counterintuitively, no effect of the transgenic manipulation on motor output was seen. Future experiments are outlined to determine how the larval nervous system produces normal motor output in the face of altered motor neuron excitability.

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Introduction

To produce essential motor behaviors like breathing and walking, muscles must contract in the same order, for roughly the same duration, each time a breath or step is taken. There is a pattern of activity, called a motor pattern, that must be reliably produced by the nervous system (for reviews on motor pattern generation see *Büschges et al.*, 2011; *Grillner*, 2003; *Harris-Warrick*, 2010; *Kiehn*, 2006; *Marder & Bucher*, 2001; *Selverston*, 2010). However, the system also has to be flexible enough to respond to changing internal and external conditions (*Blitz & Nusbaum*, 2011; *Harris-Warrick*, 2011). There are open questions about how the nervous system controls rhythmic movements, permitting reliability and flexibility. What determines the timing of the motor pattern? Under what conditions can the timing be altered, and how?

Rhythmic motor behaviors are controlled by networks of neurons which communicate electrically and chemically (*Büschges et al.*, 2011; *Grillner*, 2003; *Harris-Warrick*, 2010; *Kiehn*, 2006; *Marder & Bucher*, 2001; *Selverston*, 2010). Since motor neurons (MNs) represent the direct connection between neurons in those networks and the muscles, it is important to understand how MNs receive, integrate, and generate signals (*Heckman et al.*, 2009; *Kiehn et al.*, 2000; *Perrier & Hounsgaard*, 2000). What happens as signals pass from the MNs to the muscles? Do MNs transmit a temporally similar pattern of activity to the one they received, or do they change the pattern? If the latter, to what extent do MNs contribute to shaping the final timing of motor behavior?

MNs express channels that allow ions such as calcium, potassium, and sodium to cross the membrane, producing currents that change electrical activity (*Harris-Warrick*, 2002; *McLarnon*, 1995). Studies have demonstrated that persistent inward currents (PICs) carried by sodium and calcium can shape MN responsiveness to synaptic input and firing output (*Hultborn et al.*, 2003; *Lee & Heckman*, 1998, 2001; *Perrier & Tresch*, 2005). A recent study combining modeling and experimental approaches showed that MN currents, not necessarily just PICs, can shape the phasing of the motor pattern (*Wright Jr & Calabrese*, 2011). However, the common drawback of many of these studies is that the role of MN currents was not examined during ongoing, spontaneous motor behavior. Open questions remain about the extent to which the intrinsic properties of MNs contribute to the timing of rhythmic motor output and furthermore, which ion channel genes may be involved in MN responsiveness.

The aim of this study was to examine the effects of altering MN intrinsic properties on the timing of a spontaneous rhythmic motor behavior. Two dominant-negative potassium channel transgenes were expressed in *Drosophila* larval MNs using the recombinant line known as 'Electrical Knock-In' (EKI). The EKI manipulation reduces both transient and sustained potassium currents and increases the excitability of larval (*Hartwig et al.*, 2008) and adult *Duch et al.* (2008) MNs. EKI was expressed in two identified MNs, MN1-Ib and MNISN-Is, which display different levels of excitability and contribute in distinct ways to

rhythmic locomotor activity (*Schaefer et al.*, 2010). Mosaic animals were generated using the FLP/FRT system (*Golic & Lindquist*, 1989; *Ryder & Russell*, 2003) in which some MNs expressed the manipulation while others did not, permitting the comparison of control and experimental conditions in the same animal and the relative effects of changing excitability in MN1-Ib or MNISN-Is. Despite changes in MN excitability, the activity of muscles receiving signals from manipulated MNs was no different from controls. This counterintuitive result raises important questions about how networks maintain locomotor behaviors in the face of perturbations.

Material & Methods

Fly lines and genetics

Drosophila melanogaster were reared at 25 °C under 12-hour light-dark cycles on standard yeast-sugar-cornmeal media. Wandering third-instar larvae were used for all experiments. To alter MN activity, UAS-*ether-a-go-go* *Broughton et al.* (2004) and UAS-*Shaker* *Mosca et al.* (2005) dominant-negative transgenes were expressed using a recombinant line (+;UAS-eagDN932,USDT-207/Cyo), also known as 'Electrical Knock-In' (EKI) (*Hartwig et al.*, 2008). Larval MNs expressing EKI have reduced transient and sustained potassium currents, fire action potentials at lower levels of current injection, show a decreased latency to first spike, and fire at higher frequencies than control neurons (*Hartwig et al.*, 2008). EKI expression in adult *Drosophila* MNs converts them from single to repetitive spikers (*Duch et al.*, 2008). Other studies have also used EKI as a tool to increase neuron firing (*Hindle et al.*, 2013; *Timmerman et al.*, 2013; *Vonhoff et al.*, 2013).

Expression of EKI in MN1-Ib and MNISN-Is (*Hoang & Chiba*, 2001) was driven by RN2-GAL4 (*Fujioka et al.*, 2003) (w-;UAS::mRFP;RN2::FLP,Tub<FRT<GAL4,UAS::mRFP) (*Zwart et al.*, 2013). A FLP/FRT recombinase cassette was included in the driver line to create mosaic animals (*Golic & Lindquist*, 1989; *Ryder & Russell*, 2003) in which MN1-Ib and/or MNISN-Is neurons in some nervous system hemisegments expressed EKI, while the same identified MNs in other hemisegments did not. Thus, control and experimental cells could be compared in the same animal. MNs expressing EKI were identified by co-expression of a red fluorescent protein (RFP) tag attached to the promoter and a green fluorescent protein (GFP) tag attached to the EKI construct *Mosca et al.* (2005).

Larval preparation

Larvae were dissected using the off-midline preparation (Fig. 1), as described previously (*McKiernan*, 2013). Briefly, a cut was made from the tail to the head near muscle 4, and larvae were opened and pinned out flat. Organs and trachea were removed to expose the muscles

and nervous system. This preparation minimizes damage to the dorsal-most muscle 1, innervated by MN1-Ib and MNISN-Is (*Hoang & Chiba*, 2001). Larvae dissected in this way generate spontaneous crawling-related motor activity comparable to larvae dissected with the more common dorsal-midline preparation, although at a faster rate (*McKiernan*, 2013).

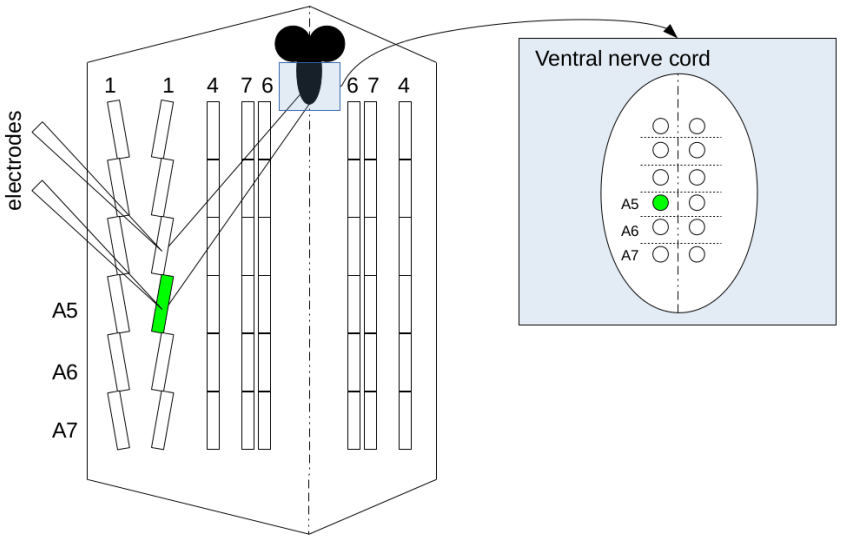

Figure 1: Schematic of the larval preparation. The larva is organized into multiple segments (abdominal segments A5-A7 labeled). Each hemisegment contains a repeated set of 30 muscles (rectangles; 1,4,6,7 labeled). This organization is mirrored across the midline (dashed vertical line). Motor neurons are found in the ventral nerve cord (zoom at right) and screened for GFP (green) to determine if they express EKI. Dual intracellular recordings are made from muscles innervated by EKI-expressing MNs (green) or wildtype MNs. For clarity, not all muscles, nerves, or neurons are shown.

Electrophysiology

Larval preparations were bathed and recorded in HL3.1 saline (*Feng et al.*, 2004) containing (in mM): 70 NaCl, 5 KCl, 1.5 $CaCl_2$, 4 $MgCl_2$, 10 $NaHCO_3$, 5 Trehalose, 115 Sucrose, 5 HEPES, pH 7.1-7.3. All chemicals were obtained from Sigma (St. Louis, Missouri). Dual intracellular recordings were made at $21 - 23\,^{\circ}C$ from dorsal muscle 1 in abdominal segments 2-6, as described previously (*McKiernan*, 2013). Sharp electrodes were pulled from thin-walled borosilicate glass using a filament puller (Sutter Instrument Co., P-87 Flaming/Brown) to a 30-50 MΩ resistance. This produced a long and flexible electrode tip,

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which could move with the muscle during spontaneous waves of contractions. Electrodes were filled with 3 M potassium chloride for recording. Recordings were amplified using a Axoclamp 2B amplifier (Molecular Devices) in bridge mode and digitized at a sampling rate of 10 kHz by Digidata 1320A (Axon Instruments). Recordings were stored using PClamp 8.2 (Molecular Devices) and were imported into Spike2 (Cambridge Electronic Design) for processing.

Experimental design

Forward locomotion in *Drosophila* larvae is produced by peristaltic waves of muscle contractions that travel from the back to the front of the animal and can be recorded in the dissected preparation (*Barclay & Atwood*, 2002; *Cattaert & Birman*, 2001; *Cooper & Neckameyer*, 1999; *Fox et al.*, 2006; *McKiernan*, 2013; *Song et al.*, 2007; *Ueda & Wu*, 2006). The body of a *Drosophila* larva is comprised of multiple segments, and in each hemisegment is a repeated set of 30 muscles (*Hoang & Chiba*, 2001; *Keshishian et al.*, 1996). This organization is mirrored across the midline. The central nervous system, where the MNs are located, has the same repeated and mirrored organization, with the same group of identified neurons found in each segment. Since the relationship between MN action potentials and muscle excitatory junctional potentials is one-to-one (*Choi et al.*, 2004), muscle activity can be recorded as a proxy for the activity of the MNs. One of the muscles involved in locomotion is dorsal muscle 1 (M1), which is innervated by two MNs known as MN1-Ib and MNISN-Is (*Hoang & Chiba*, 2001). When M1s in neighboring abdominal segments are recorded, the activity is very similar, only with a short temporal delay. Thus, two samples of M1 activity and the MNs that innervate it, can be obtained from the same animal. Tests were conducted to establish that there is no gap-junctional coupling between M1s in adjacent segments (Supplemental Fig. 1), as reported for ventral muscles (*Gho*, 1994). Therefore, the activity recorded from each muscle should only arise from its innervating neurons. If one muscle receives signals from a MN expressing EKI and the other from a control MN, their activity can be compared to see how it differs due to the genetic manipulation.

Data Analysis

Preparations were observed through an Olympus BX51WI microscope, and the occurrence of peristaltic waves recorded manually and/or marked with electronic timestamps to restrict analysis only to electrical activity underlying forward locomotion (for more details on inclusion criteria see *McKiernan*, 2013). Burst start and end times were marked manually using cursors in Spike2 and exported to .csv files. Analysis code was written in Python (version 2.7.6) to extract burst durations (time from the start to the end of a burst), cycle durations (time from the start of one burst to start of the next), duty cycles (burst duration divided by cycle duration), and quiescence intervals (time from the end of one burst to the start of

the next) from the recordings. Although intraburst firing frequency was of interest due to the effects of the EKI manipulation on MN firing (*Hartwig et al.*, 2008), recordings showed it could not be effectively analyzed due to problems with event detection and separation of units coming from individual MNs (for more information see Supplemental Fig. 2). All animals exhibited multiple bursts, though not necessarily the same number. As such, pooling bursts would constitute pseudoreplication and give more weight to some animals in the sample than others. To avoid this, the distributions of each measure for the control and experimental conditions were first calculated in single animals. Then, distributions from multiple animals were averaged to generate group distributions in which each animal was represented only once for each condition. The minimum, maximum, and quartile (Q_{25}, Q_{50}, Q_{75}) values for each measure are reported to give a complete description of the averaged group distributions. To statistically test for differences between control and experimental conditions, the Wilcoxon Signed-Rank test for paired samples was used with an alpha of 0.05. The Python SciPy library was used for all analysis (*Jones et al.*, 2001). Graphs were generated using Python Matplotlib (*Hunter*, 2007). All other figures were created using GIMP 2.8.

Results

EKI expression in MN1-Ib

MN1-Ib innervates muscle 1 (M1) with Type I glutamatergic terminals, forming big ('b' designation) synaptic boutons (*Choi et al.*, 2004; *Hoang & Chiba*, 2001). Whole-cell patch clamp recordings during spontaneous locomotor activity have shown that MN1-Ib fires sooner and with a greater number of action potentials during a single burst than the other MN innervating M1, MNISN-Is (*Schaefer et al.*, 2010). Thus, MN1-Ib may be the primary contributor to locomotor activity recorded from M1.

Recordings were analyzed from four larvae in which EKI was expressed in MN1-Ib in one hemisegment while adjacent hemisegments were innervated by wildtype (WT) MNs (Fig. 2). (Recordings from two larvae were excluded due to a lack of rhythmic activity.) Histograms comparing burst duration, cycle duration, duty cycle, and quiescence interval for WT versus EKI segments are shown in Fig. 3. Minimum, maximum, and quartile values can be found for comparison in Table 1. Although in some larvae, measures were significantly different between WT and EKI recordings, no consistent effect was observed. For example, in one animal, quiescence interval was shorter in the WT than the EKI segment, while in another animal, the opposite was seen. In two other larvae, the recorded muscles showed no difference in quiescence interval. This suggests any differences were due to variability in measures across segments, rather than induced by EKI. Comparing the averaged group distributions revealed no significant differences between WT and EKI recordings on any measure ($p>0.05$).

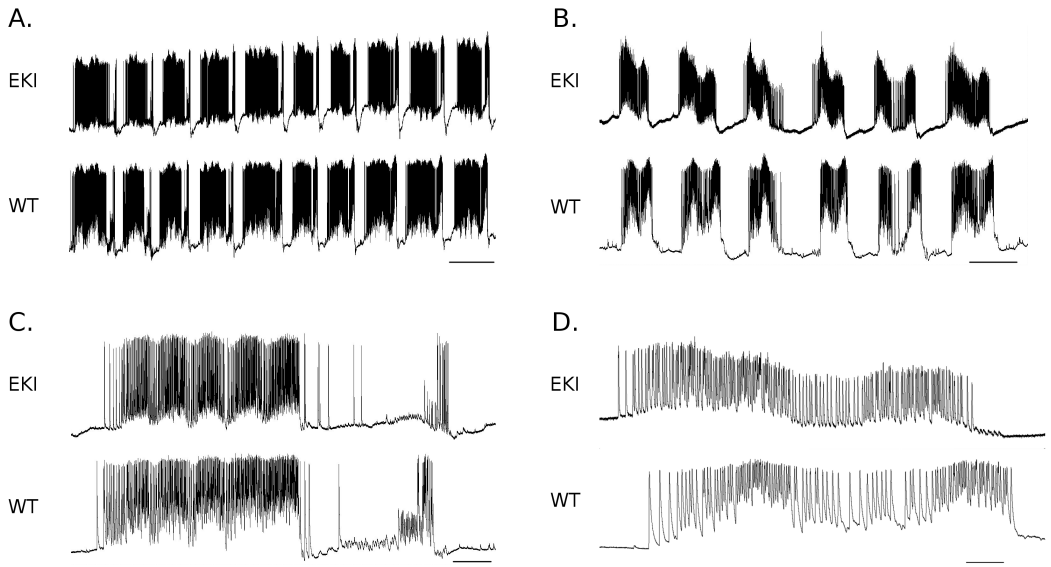

Figure 2: Simultaneous intracellular recordings from muscles innervated by WT MNs or MN1-Ib expressing EKI. *A.* Recording from one larva. M1 in A3 was innervated by MN1-Ib expressing EKI (top trace), while M1 in A4 was innervated by WT MNs (bottom trace). Scale bar is 20s. *B.* Recording from second larva. M1 in A4 (top) was innervated by MN1-Ib expressing EKI, while M1 in A5 was innervated by WT MNs (bottom). Scale bar is 6s. *C, D.* Single bursts from recordings in A and B, respectively. Scale bar is 2s in C and 0.5s in D.

EKI expression in MNISN-Is

MNISN-Is innervates muscles 1-4, 9, 10, and 18-20 via the intersegmental nerve (ISN) with Type I glutamatergic terminals ending in small ('s') boutons (*Hoang & Chiba*, 2001; *Choi et al.*, 2004). Whole-cell patch clamp recordings have shown that MNISN-Is activates later and fires less than MN1-Ib during locomotor activity and may not be recruited during every cycle (*Schaefer et al.*, 2010). However, at least in ventral muscles, Type Is boutons are associated with larger excitatory junctional currents (EJCs) and potentials (EJPs) than Type Ib boutons, and have been likened to the "phasic" or "fast" motor axons found in crustaceans (*Atwood et al.*, 1993; *Kurdyak et al.*, 1994). The multiple innervation provided by Type Is MNs could also be important for coordinating the activity of muscle groups (*Choi et al.*, 2004).

Recordings were analyzed from seven larvae in which EKI was expressed in MNISN-Is in one hemisegment while adjacent hemisegments were innervated by WT MNs (Fig. 4). (Recordings from seven other animals were not included in the analysis due to a lack of rhythmic activity, or electrode instability in one of the channels.) Histograms comparing

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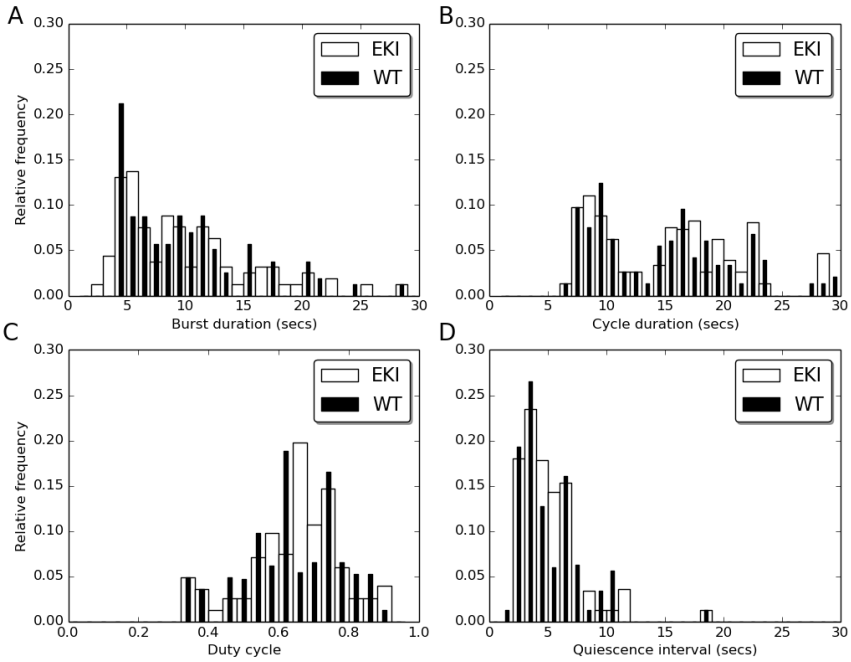

Figure 3: Quantification of motor activity. Histograms of burst durations (A), cycle durations (B), duty cycles (C), and quiescence intervals (D) as calculated from recordings of muscles innervated by WT MNs (black) and MN1-Ib expressing EKI (white). N=4. Bins: A,B,C = 1s; D = 0.04.

burst duration, cycle duration, duty cycle, and quiescence interval for WT versus EKI segments are shown in Fig. 5. Minimum, maximum, and quartile values can be found for comparison in Table 2. As with MN1-Ib, in some larvae, select measures were significantly different between WT and EKI recordings. However, no consistent effect was observed, again suggesting these differences were due to inherent variability across segments. Comparing the averaged group distributions revealed no significant differences between WT and EKI recordings on any measure ($p>0.05$).

EKI expression in MN1-Ib and MINISN-Is

Since M1 is innervated by both MN1-Ib and MNISN-Is (*Hoang & Chiba*, 2001; *Choi et al.*, 2004), it is possible the effect of EKI expression in one MN could be compensated for by the other. To test this, recordings were analyzed from two larvae in which both MN1-Ib and MNISN-Is innervating a given segment expressed EKI, while MNs in adjacent segments were WT (Fig. 6). (A recording from one larva was excluded due to a lack of rhythmic activity.)

measure	n	min	Q_{25}	Q_{50}	Q_{75}	max
WT						
burst duration	4	4.02	5.87	8.93	12.01	28.52
cycle duration	4	6.99	9.24	15.04	18.85	31.34
duty cycle	4	0.34	0.56	0.64	0.73	0.91
quiescence interval	4	1.73	3.16	4.25	6.51	18.40
EKI in MN1-Ib						
burst duration	4	2.78	5.14	8.66	12.64	28.81
cycle duration	4	6.89	9.00	15.55	19.51	31.72
duty cycle	4	0.33	0.57	0.66	0.73	0.91
quiescence interval	4	2.26	3.14	4.46	6.06	18.82

Table 1: Bursting measures in M1s innervated by WT MNs or MN1-Ib expressing EKI

Histograms comparing burst duration, cycle duration, duty cycle, and quiescence interval for WT versus EKI segments are shown in Fig. 7. Minimum, maximum, and quartile values can be found for comparison in Table 2. Neither of the two larvae showed a significant difference in burst duration or cycle duration between muscles innervated by WT or EKI MNs. In one larva, quiescence intervals were longer and duty cycles were smaller in muscles innervated by EKI versus WT MNs ($p < 0.05$). However, no differences in either duty cycle or quiescence interval were seen between muscles recorded in the second larva. Comparing the averaged group distributions revealed no significant differences between WT and EKI recordings on any measure ($p>0.05$).

Discussion

This study used transgenic mosaics to study the role of MN intrinsic properties in shaping the timing of a rhythmic motor behavior. The most obvious advantage of this approach is that animals act as their own controls, reducing the confounding effects of extraneous variables and permitting smaller sample sizes. Furthermore, the model system (*Drosophila* larva) allowed for examining the effects of changing MN excitability on spontaneous motor

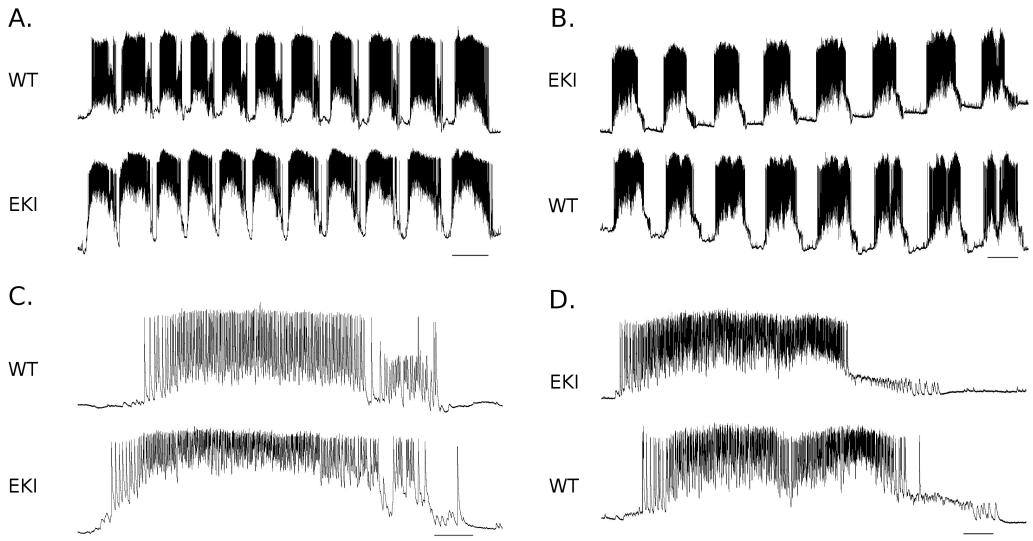

Figure 4: Simultaneous intracellular recordings from muscles innervated by WT MNs or MNISN-Is expressing EKI. *A.* Recording from one larva in which M1 in segment A5 was innervated by MNISN-Is expressing EKI (bottom trace), while M1 in A4 was innervated by WT MNs (top trace). Scale bar is 10s. *B.* Recording from second larva in which M1 in A6 (top) was innervated by MNISN-Is expressing EKI, while M1 in A5 was innervated by WT MNs (bottom). Scale bar is 10s. *C, D.* Single bursts from recordings in A and B, respectively. Scale bar is 1s in both.

behavior. Surprisingly, although previous studies have shown that EKI expression alters the firing properties of larval MNs (*Hartwig et al.*, 2008), no difference was seen in the patterned activity of muscles innervated by EKI-expressing MNs relative to controls.

Importance of MN intrinsic properties

Although the results reported herein did not show an effect of changing MN excitability on motor output, this does not mean MN intrinsic properties are not important for motor control. Several key studies conducted in the late 1970s and 1980s showed that MNs have active membrane properties (voltage-gated ion currents) that can influence their responsiveness to input and firing output (*Hounsgaard et al.*, 1984, 1988b; *Schwindt & Crill*, 1977, 1980). More recent studies have confirmed these results and specifically emphasized the role of persistent currents in MN recruitment, amplification of synaptic input, and repetitive and prolonged firing behaviors in MNs (*Lee & Heckman*, 1998, 2001; *Li et al.*, 2004; *Manuel et al.*, 2012; *Perrier & Tresch*, 2005). Several MN ion currents are altered by the presence of neuromodulators such as serotonin and norepinephrine (*Hounsgaard & Kiehn*, 1985; *Hounsgaard et al.*,

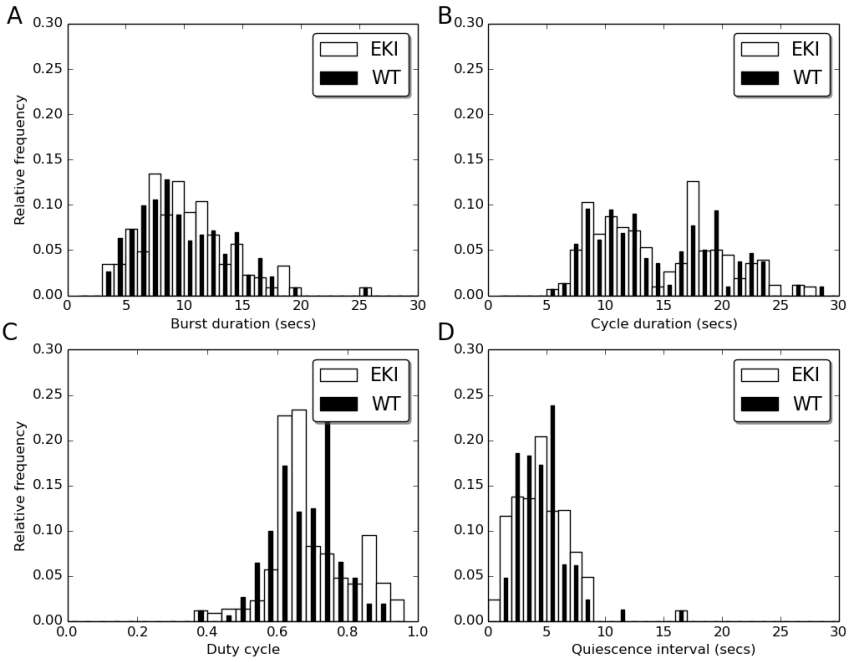

Figure 5: Quantification of motor activity. Histograms of burst durations (A), cycle durations (B), duty cycles (C), and quiescence intervals (D) as calculated from recordings of muscles innervated by WT MNs (black) and MNISN-Is expressing EKI (white). N = 7. Bins: A,B,C = 1s; D = 0.04.

1988a; *Hounsgaard & Kiehn*, 1989; *Perrier & Hounsgaard*, 2003; *Lindsay & Feldman*, 1993; *Zhang & Harris-Warrick*, 1995); (for reviews see *Heckman et al.*, 2009; *Perrier et al.*, 2013). Recordings in cat have shown that changes in the firing patterns of serotonergic neurons correspond to the onset and offset of specific motor behaviors (*Fornal et al.*, 1996; *Veasey et al.*, 1995). These results provide correlative, though not direct, evidence that MN excitability may be modulated to meet changing motor demands. In the crustacean stomatogastric (STG) system, dopamine-induced changes in MN potassium currents alter digestive motor behavior (*Kiehn & Harris-Warrick*, 1992). Correlations have been found between potassium ion channel expression in MNs and the period of the STG motor pattern (*Goaillard et al.*, 2009). However, it is important to recognize that in the STG, MNs themselves participate in generating the motor rhythm (*Marder & Calabrese*, 1996). In many other systems, MNs do not generate the rhythm, in which case the effects of changing MN intrinsic properties in these systems may be distinct. Recent work studying heartbeat in the leech - a system in which the rhythm-generating network is composed of interneurons - used a combination of experimental and computational approaches to show that MN currents contribute to motor

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measure	n	min	Q_{25}	Q_{50}	Q_{75}	max
WT						
burst duration	7	3.55	6.91	9.10	12.77	25.47
cycle duration	7	6.42	10.48	13.35	18.99	28.00
duty cycle	7	0.36	0.60	0.68	0.74	0.91
quiescence interval	7	1.34	3.01	4.46	5.56	16.95
EKI in MNISN-Is						
burst duration	7	3.68	7.46	9.49	12.12	25.47
cycle duration	7	6.18	10.44	13.44	18.83	27.63
duty cycle	7	0.37	0.62	0.66	0.77	0.96
quiescence interval	7	0.40	2.69	4.30	6.09	16.40

Table 2: Bursting measures in M1s innervated by WT MNs or MNISN-Is expressing EKI

pattern phasing (*Wright Jr & Calabrese*, 2011).

A number of studies have shown that ion channel expression in *Drosophila* larvae and adults is important for producing different types of excitability and response properties in MNs (*Chang et al.*, 2013; *Duch et al.*, 2008; *McKiernan*, 2013; *Ryglewski & Duch*, 2009; *Ryglewski et al.*, 2012, 2014; *Srinivasan et al.*, 2012a,b; *Wolfram et al.*, 2012). Recordings from larval MN1-Ib (also referred to as aCC) neurons in abdominal or thoracic segments of the ventral ganglion show differences in the size of transient and sustained potassium currents, leading to differences in delay to first spike and firing frequency (*Srinivasan et al.*, 2012b). Decreasing expression of *eag*, which contributes to transient and sustained potassium currents, causes hyperexcitable responses to current injection and increases in amplitude and frequency of EPSPs during rhythmic oscillations recorded from MN1-Ib (*Srinivasan et al.*, 2012a). In a mutant larval model of amyotrophic lateral sclerosis (ALS), expression of the calcium channel gene *cacophony* in MNs was sufficient to recover normal crawling behavior (*Chang et al.*, 2013). Manipulating expression of the calcium-dependent potassium channel gene *slowpoke* in larvae suggests that MNs may contribute to the frequency of crawling activity (*McKiernan*, 2013). In adult *Drosophila*, EKI expression in MNs increases the probability of induced flight behavior in response to a wind stimulus and the duration of flight activity (*Duch et al.*, 2008).

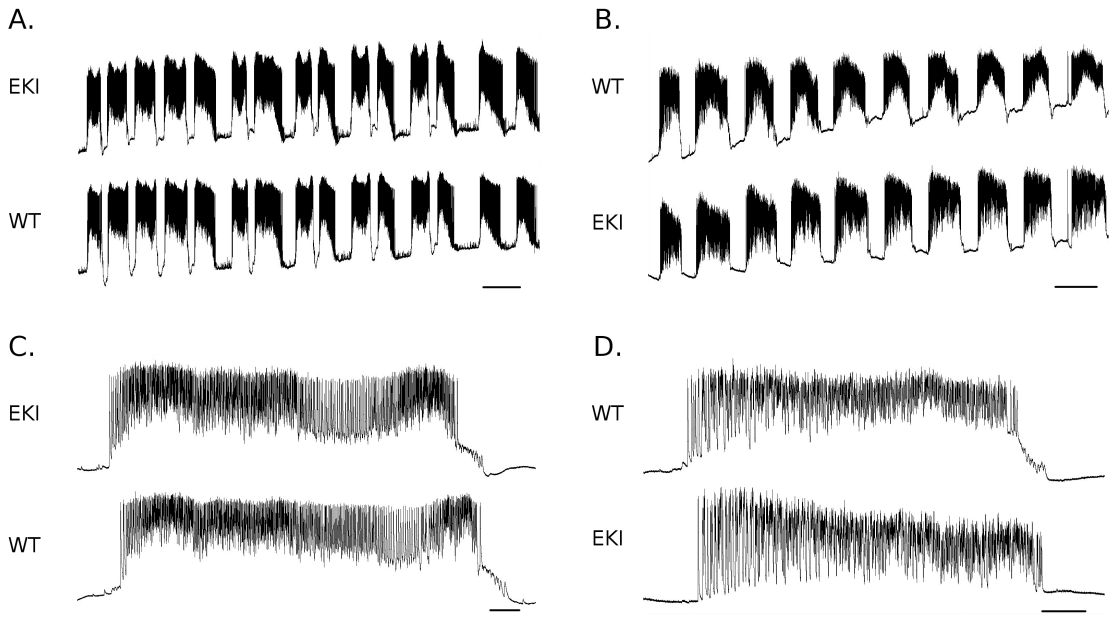

Figure 6: Simultaneous intracellular recordings from muscles innervated by WT MNs or both MN1-Ib and MNISN-Is expressing EKI. *A.* Recording from one larva in which M1 in segment A5 was innervated by MNISN-Is expressing EKI (top trace), while M1 in A4 was innervated by WT MNs (bottom trace). Scale bar is 20s. *B.* Recording from second larva in which M1 in A5 (bottom) was innervated by MNISN-Is expressing EKI, while M1 in A6 was innervated by WT MNs (top). Scale bar is 10s. *C, D.* Single bursts from recordings in A and B, respectively. Scale bar is 1s in both.

Why was an effect of EKI not seen?

There are several possible explanations for why an effect of the EKI manipulation was not seen in this study. First, while there was not an effect on the timing of muscle contractions, there could have been an effect on the force of contractions. Force could be altered by an increase in MN firing frequency, as is observed in EKI-expressing MNs under current injection (*Hartwig et al.*, 2008). However, such an effect, if present, was below the threshold for detection in this preparation due to issues with analyzing intraburst firing frequency. EJPs recorded from muscle 1 arise from two different MNs, MN1-Ib and MNSIN-Is. These MNs are recruited at different times, fire at different frequencies, and the latter may not spike during every locomotor cycle (*Schaefer et al.*, 2010). Dual recordings from muscles 1 and 2, innervated by 1 common (MNISN-Is) and 1 non-common MN (MN1-Ib or MN2-Ib, respectively) (*Hoang & Chiba*, 2001), showed that unit separation during the majority of a motor burst was not possible (Supplemental Fig. 2). Thus, a rigorous analysis of the firing

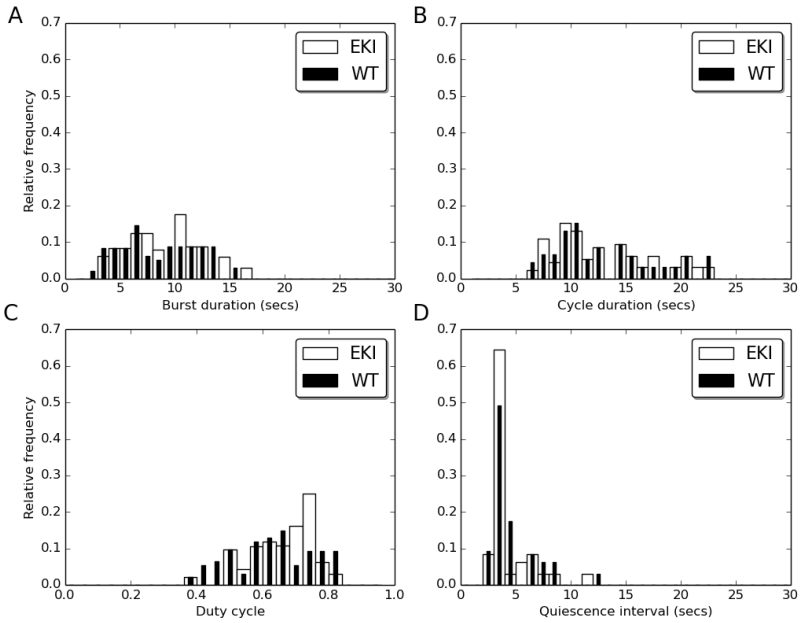

Figure 7: Quantification of motor activity. Histograms of burst durations (A), cycle durations (B), duty cycles (C), and quiescence intervals (D) as calculated from recordings of muscles innervated by WT MNs (black) or MN1-Ib and MNISN-Is expressing EKI (white). N=2. Bins: A,B,C = 1s; D = 0.04.

frequency of control and transgenic MNs could not be extracted from muscle recordings. Such analysis would require patching on to individual MNs during motor behavior.

Second, although larval MNs expressing EKI show altered responses to square-pulse current injections, their responses to synaptic input may not be different from controls. As a simple first approach, MNs expressing EKI could be stimulated with ramps instead of square-pulse currents. The non-instantaneous change in membrane potential initiated by ramp stimulation allows for gradual activation and inactivation of ion currents and can reveal distinct neuron response properties (*Estacion & Waxman*, 2013; *Guan et al.*, 2007; *Izhikevich*, 2007; *Magistretti & Alonso*, 1999; *Li et al.*, 2002). MNs could also be stimulated with different types of stochastic current inputs (e.g. white noise) to determine their responsiveness (*Bryant & Segundo*, 1976; *Destexhe et al.*, 2001; *Tateno et al.*, 2004). An even better but more difficult approach is to record genetically manipulated MNs as they receive endogenous motor-related input, as done recently by others in WT MNs (*Schaefer et al.*, 2010). If the response to synaptic input is not altered in EKI neurons, this would explain why motor output was unchanged. In this case, it would be interesting to investigate

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which ionic currents might compensate for the change in potassium channel expression. Ion channel homeostasis has previously been reported in *Drosophila* (*Bergquist et al.*, 2010; *Lee et al.*, 2008; *Peng & Wu*, 2007) and other animals (*MacLean et al.*, 2003; *Swensen & Bean*, 2005); (for review see *Marder & Goaillard*, 2006).

If, however, the response of EKI-expressing MNs to synaptic input is altered, then this would suggest that the network is somehow compensating for changes in MN excitability such that normal motor output is maintained, as seen in other systems (*Maffei & Fontanini*, 2009). The embryonic predecessors of MN1-Ib and MNISN-Is, aCC and RP2, adjust their excitability in response to changes in synaptic input (*Baines*, 2003). It is possible the reverse happens - neurons in the motor network may adjust their output in response to changes in MN excitability. However, the upstream synaptic partners of larval MNs will need to be identified before this could be tested directly. Although candidate interneurons involved in locomotor pattern generation have been identified (*Suster et al.*, 2004; *Iyengar et al.*, 2011), their connections with one another and with MNs have not yet been established.

The presence of an *eag* dominant negative transgene in the recombinant EKI line could be important for compensation. Eag channels can activate calcium/calmodulin-dependent protein kinase II (CaMKII) (*Sun et al.*, 2004), a kinase shown to regulate neuronal excitability and motor behavior in *Drosophila* (*Park et al.*, 2002; *Yao & Wu*, 2001), as well as other animals (*Nelson et al.*, 2005) (for review see *Liu & Murray*, 2012). Work in *Xenopus* oocytes has shown that eag channels can regulate intracellular signaling pathways that induce cell proliferation - an effect not observed in response to Shaker channel transfection (*Hegle et al.*, 2006). Expression of an activity-dependent spliced form of the eag protein alters the structure of cultured cells (*Sun et al.*, 2009). In mice, *eag*-related gene (ERG) expression is regulated in response to changes in neural activity (*Hagendorf et al.*, 2009). All of these studies suggest that *eag* could act as a 'sensor of excitability' (*Srinivasan et al.*, 2012a), inducing compensation in EKI-expressing larvae through a variety of possible downstream mechanisms. Recording from mosaic larvae expressing the *eag* or *Shaker* dominant negative transgenes individually could help to tease apart the role(s) of each gene in locomotor behavior.

Possible role of sensory feedback in compensation

In many animals, sensory feedback is important for regulating the timing of motor behaviors (*Hiebert et al.*, 1996; *Sinkjær et al.*, 2000; *Borgmann et al.*, 2009; *Ausborn et al.*, 2007); (for reviews see *Grillner*, 2003; *Pearson*, 2000; *Büschges et al.*, 2011). In *Drosophila*, studies have reported that input from multi-dendritic (MD) sensory neurons found in the larval body wall supports wave progression from one segment to the next (*Cheng et al.*, 2010; *Hughes & Thomas*, 2007; *Song et al.*, 2007). Labeling of MD neurons has shown that some project into the same area of the neuropile where the MN dendrites are located, indicating that MNs may receive direct input from MD neurons (*Grueber et al.*, 2007). If this is the

measure	n	min	Q_{25}	Q_{50}	Q_{75}	max
WT						
burst duration	2	2.80	5.51	8.00	11.29	15.02
cycle duration	2	6.34	9.36	11.17	15.90	22.99
duty cycle	2	0.39	0.56	0.64	0.75	0.82
quiescence interval	2	2.49	3.32	3.89	4.46	12.90
EKI						
burst duration	2	3.28	6.00	8.26	11.25	16.15
cycle duration	2	6.68	9.40	11.18	15.89	22.77
duty cycle	2	0.36	0.59	0.68	0.74	0.81
quiescence interval	2	2.45	3.36	3.59	4.27	11.81

Table 3: Bursting measures in M1s innervated by WT MNs or MN1-Ib and MNISN-Is expressing EKI

case, increased firing of MNs induced by the expression of EKI, and thus changes in the contractions of target muscles, could be detected by MD cells and relayed to MNs. Since in this study EKI was expressed throughout development, it is possible sensory feedback during embryonic stages, when peristaltic contractions first begin (*Crisp et al.*, 2008), could allow the system to recalibrate and produce normal motor output. Alternatively, it may be sufficient to have cycle-by-cycle sensory feedback during the larval stages to compensate for altered motor neuron excitability. Acute expression of EKI during the third larval instar, for example using temperature-sensitive GAL80 in concert with the UAS/GAL4 system (*McGuire et al.*, 2004), would help determine whether expression during earlier developmental stages is necessary for compensation to occur.

What can transgenic mosaics tell us about motor behavior?

The results of this study bring into question whether using mosaics to study locomotion in larval *Drosophila* is the best approach. The original idea was to be able to compare the activity of control and experimental cells in the same animal to determine the effects of manipulating MN excitability. However, given that the locomotor activity being examined

consists of peristaltic waves that progress from one segment to the next, it is questionable that one could see normal activity in one segment and altered activity in an adjacent one. The work on sensory feedback in this system suggests that termination of bursting in one segment is important for the initiation of bursting in an adjacent segment (*Hughes & Thomas*, 2007; *Song et al.*, 2007). If the wave is progressing from back to front and activity in a posterior segment is altered due to innervation by a manipulated MN, the activity in the next segment, although innervated by control MNs, could in turn be altered. Even if the control muscle is posterior to the manipulated muscle, activity could still be altered as the wave slows or fails to complete and initiate a new cycle. Alternatively, sensory feedback from the recorded or surrounding muscles could help to maintain the proper bursting pattern even in the manipulated muscle. In other words, it is possible that either activity will be altered in multiple segments, or that compensation will occur and normal activity will be maintained in all segments. Related to this, the fact that only rhythmically active animals were included in the analysis constitutes a form of selection bias. If the dominant negative transgenes express with variable strength across animals, then it is possible that larvae with stronger expression would not show rhythmic activity, leading to a very different conclusion regarding the effect of the EKI manipulation. For this reason, it was important to report the percentage of recordings that were excluded from each sample, which varied from 33-50%. Since this percentage includes some rhythmically active animals that were excluded due to electrode instability in the recordings, the percentage of 'non-crawlers' tended toward the low end of the range for all samples. However, to determine whether EKI expression strength is important, quantification of potassium current reduction could be compared in 'crawlers' and 'non-crawlers'.

Mosaics could be used to study the role of intrinsic MN properties in isolated CNS preparations in which sensory input and muscle coupling would not play a role. However, previous studies have shown that the motor pattern is irregular in such preparations (*Fox et al.*, 2006), which could make evaluating the effects of any manipulation more difficult. Furthermore, an isolated preparation would defeat attempts to link gene expression to ongoing motor behavior.

Mathematical modeling could answer open questions

A mathematical modeling approach could help answer several lingering questions from this study. Model MNs could be constructed in which ion channel expression is alterable (*Herrera-Valdez et al.*, 2013) to mimic the effects of the EKI manipulation. The output of these model neurons could then be characterized under different patterns of rhythmic synaptic input or even network connectivity. A hybrid system could also be constructed in which real WT and EKI-expressing MNs receive different patterns of input from model neurons, similar to recent work in other systems (*Wright Jr & Calabrese*, 2011).

Conclusions

There is still a lot we do not understand about the interactions between MNs and other cells in the network during the generation of rhythmic motor behaviors. This study used a characterized transgenic manipulation to increase MN excitability. However, this manipulation failed to produce changes in motor output. This work raises several important questions regarding reliability and flexibility in motor networks. A number of future experiments are needed to increase our understanding of the role of MN intrinsic properties in motor pattern generation and, more specifically, our understanding of how networks may compensate for altered MN excitability to maintain proper locomotion.

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References

Atwood, H., Govind, C., and Wu, C. Differential ultrastructure of synaptic terminals on ventral longitudinal abdominal muscles in *Drosophila* larvae. *Developmental Neurobiology*, 24(8):1008–1024, 1993.

Ausborn, J., Stein, W., and Wolf, H. Frequency control of motor patterning by negative sensory feedback. *Journal of Neuroscience*, 27(35):9319–9328, 2007.

Baines, R. Postsynaptic protein kinase A reduces neuronal excitability in response to increased synaptic excitation in the *Drosophila* CNS. *Journal of Neuroscience*, 23(25):8664–8672, 2003.

Barclay, J. and Atwood, H. Impairment of central pattern generation in *Drosophila* cysteine string protein mutants. *Journal of Comparative Physiology A*, 188:71–78, 2002.

Bergquist, S., Dickman, D., and Davis, G. A hierarchy of cell intrinsic and target-derived homeostatic signaling. *Neuron*, 66(2):220–234, 2010.

Blitz, D. and Nusbaum, M. Neural circuit flexibility in a small sensorimotor system. *Current Opinion in Neurobiology*, 21(4):544–552, 2011.

Borgmann, A., Hooper, S., and Büschges, A. Sensory feedback induced by front-leg stepping entrains the activity of central pattern generators in caudal segments of the stick insect walking system. *Journal of Neuroscience*, 29(9):2972–2983, 2009.

Broughton, S., Kitamoto, T., and Greenspan, R. Excitatory and inhibitory switches for courtship in the brain of *Drosophila melanogaster*. *Current Biology*, 14(7):538–547, 2004.

Bryant, H. L. and Segundo, J. P. Spike initiation by transmembrane current: a white-noise analysis. *Journal of Physiology*, 260(2):279–314, 1976.

Büschges, A., Scholz, H., and El Manira, A. New moves in motor control. *Current Biology*, 21(13): R513–R524, 2011.

Cattaert, D. and Birman, S. Blockade of the central generator of locomotor rhythm by noncompetitive NMDA receptor antagonists in *Drosophila* larvae. *Journal of Neurobiology*, 48(1):58–73, 2001.

Chang, J., Hazelett, D., Stewart, J., and Morton, D. Motor neuron expression of the voltage-gated calcium channel cacophony restores locomotion defects in a drosophila, tdp-43 loss of function model of als. *Brain research*, DOI: 10.1016/j.brainres.2013.11.019, 2013.

Cheng, L., Song, W., Looger, L., Jan, L., and Jan, Y. The role of the TRP channel NompC in *Drosophila* larval and adult locomotion. *Neuron*, 67(3):373–380, 2010.

Choi, J., Park, D., and Griffith, L. Electrophysiological and morphological characterization of identified motor neurons in *Drosophila* third instar larva central nervous system. *Journal of Neurophysiology*, 91(5):2353–2365, 2004.

Cooper, R. and Neckameyer, W. Dopaminergic modulation of motor neuron activity and neuromuscular function in *Drosophila melanogaster*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 122(2):199–210, 1999.

Crisp, S., Evers, J. F., Fiala, A., and Bate, M. The development of motor coordination in *Drosophila* embryos. *Development*, 135(22):3707–3717, 2008.

Destexhe, A., Rudolph, M., Fellous, J.-M., and Sejnowski, T. J. Fluctuating synaptic conductances recreate *in vivo* like activity in neocortical neurons. *Neuroscience*, 107(1):13–24, 2001.

Duch, C., Vonhoff, F., and Ryglewski, S. Dendritic elongation and dendritic branching are affected separately by different forms of intrinsic motoneuron excitability. *Journal of Neurophysiology*, 100(5):2525–2536, 2008.

Estacion, M. and Waxman, S. G. The response of nav1. 3 sodium channels to ramp stimuli: multiple components and mechanisms. *Journal of neurophysiology*, 109(2):306–314, 2013.

Feng, Y., Ueda, A., and Wu, C. A modified minimal hemolymph solution, HL3.1, for physiological recordings at the neuromuscular junction of normal and mutant *Drosophila* larvae. *Journal of Neurogenetics*, 18(2):377–402, 2004.

Fornal, C., Metzler, C., Marrosu, F., and Jacobs, B. A subgroup of dorsal raphe serotonergic neurons in the cat is strongly activated during oral-buccal movements. *Brain Research*, 716(1): 123–133, 1996.

Fox, L., Soll, D., and Wu, C. Coordination and modulation of locomotion pattern generators in *Drosophila* larvae: Effects of altered biogenic amine levels by the Tyramine β Hydroxlyase mutation. *Journal of Neuroscience*, 26(5):1486–1498, 2006.

Fujioka, M., Lear, B., Landgraf, M., Yusibova, G., Zhou, J., Riley, K., Patel, N., and Jaynes, J. Even-skipped, acting as a repressor, regulates axonal projections in *Drosophila*. *Development*, 130(22):5385, 2003.

Gho, M. Voltage-clamp analysis of gap junctions between embryonic muscles in *Drosophila*. *Journal of Physiology*, 481(Pt 2):371–383, 1994.

Goaillard, J.-M., Taylor, A., Schulz, D., and Marder, E. Functional consequences of animal-to-animal variation in circuit parameters. *Nature Neuroscience*, 12(11):1424–1430, 2009.

Golic, K. and Lindquist, S. The FLP recombinase of yeast catalyzes site-specific recombination in the *Drosophila* genome. *Cell*, 59(3):499–509, 1989.

Grillner, S. The motor infrastructure: From ion channels to neuronal networks. *Nature Reviews Neuroscience*, 4(7):573–586, 2003.

Grueber, W., Ye, B., Yang, C., Younger, S., Borden, K., Jan, L., and Jan, Y. Projections of *Drosophila* multidendritic neurons in the central nervous system: links with peripheral dendrite morphology. *Development*, 134(1):55–64, 2007.

Guan, D., Lee, J., Higgs, M., Spain, W. J., and Foehring, R. C. Functional roles of kv1 channels in neocortical pyramidal neurons. *Journal of Neurophysiology*, 97(3):1931–1940, 2007.

Hagendorf, S., Fluegge, D., Engelhardt, C., and Spehr, M. Homeostatic control of sensory output in basal vomeronasal neurons: activity-dependent expression of ether-a-go-go-related gene potassium channels. *The Journal of Neuroscience*, 29(1):206–221, 2009.

Harris-Warrick, R. Voltage-sensitive ion channels in rhythmic motor systems. *Current Opinion in Neurobiology*, 12:646–651, 2002.

Harris-Warrick, R. General principles of rhythmogenesis in central pattern networks. *Progress in Brain Research*, 187:213, 2010.

Harris-Warrick, R. Neuromodulation and flexibility in central pattern generator networks. *Current Opinion in Neurobiology*, 21(5):685–692, 2011.

Hartwig, C., Worrell, J., Levine, R., Ramaswami, M., and Sanyal, S. Normal dendritic growth in *Drosophila* motor nuerons requires the AP-1 transcription factor. *Developmental Neurobiology*, 68(10):1225–1242, 2008.

Heckman, C., Mottram, C., Quinlan, K., Theiss, R., and Schuster, J. Motoneuron excitability: the importance of neuromodulatory inputs. *Clinical Neurophysiology*, 120(12):2040–2054, 2009.

Hegle, A. P., Marble, D. D., and Wilson, G. F. A voltage-driven switch for ion-independent signaling by ether-a-go-go k+ channels. *Proceedings of the National Academy of Sciences of the United States of America*, 103(8):2886–2891, 2006.

Herrera-Valdez, M. A., McKiernan, E. C., Berger, S. D., Ryglewski, S., Duch, C., and Crook, S. Relating ion channel expression, bifurcation structure, and diverse firing patterns in a model of an identified motor neuron. *Journal of Computational Neuroscience*, 34(2):211–229, 2013.

Hiebert, G., Whelan, P., Prochazka, A., and Pearson, K. Contribution of hind limb flexor muscle afferents to the timing of phase transitions in the cat step cycle. *Journal of Neurophysiology*, 75 (3):1126–1137, 1996.

Hindle, S., Afsari, F., Stark, M., Middleton, C. A., Evans, G. J., Sweeney, S. T., and Elliott, C. J. Dopaminergic expression of the parkinsonian gene lrrk2-g2019s leads to non-autonomous visual neurodegeneration, accelerated by increased neural demands for energy. *Human molecular genetics*, 22(11):2129–2140, 2013.

Hoang, B. and Chiba, A. Single-cell analysis of *Drosophila* larval neuromuscular synapses. *Developmental Biology*, 229(1):55–70, 2001.

Hounsgaard, J. and Kiehn, O. Ca^{2+} dependent bistability induced by serotonin in spinal motoneurons. *Experimental Brain Research*, 57(2):422–425, 1985.

Hounsgaard, J. and Kiehn, O. Serotonin-induced bistability of turtle motoneurones caused by a nifedipine-sensitive calcium plateau potential. *Journal of Physiology*, 414(1):265–282, 1989.

Hounsgaard, J., Hultborn, H., Jespersen, B., and Kiehn, O. Intrinsic membrane properties causing a bistable behaviour of α-motoneurones. *Experimental Brain Research*, 55(2):391–394, 1984.

Hounsgaard, J., Hultborn, H., Jespersen, B., and Kiehn, O. Bistability of alpha-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *Journal of Physiology*, 405(1):345–367, 1988a.

Hounsgaard, J., Kiehn, O., and Mintz, I. Response properties of motoneurones in a slice preparation of the turtle spinal cord. *Journal of Physiology*, 398(1):575–589, 1988b.

Hughes, C. and Thomas, J. A sensory feedback circuit coordinates muscle activity in *Drosophila*. *Molecular and Cellular Neuroscience*, 35(2):383–396, 2007.

Hultborn, H., Denton, M., Wienecke, J., and Nielsen, J. Variable amplification of synaptic input to cat spinal motoneurones by dendritic persistent inward current. *Journal of Physiology*, 552 (3):945, 2003.

Hunter, J. D. Matplotlib: A 2D graphics environment. *Computing In Science & Engineering*, 9(3): 90–95, 2007.

Iyengar, B., Chou, C., Vandamme, K., Klose, M., Zhao, X., Akhtar-Danesh, N., Campos, A., and Atwood, H. Silencing synaptic communication between random interneurons during *Drosophila* larval locomotion. *Genes, Brain and Behavior*, 10(8):883–900, 2011.

Izhikevich, E. *Dynamical Systems in Neuroscience*. MIT press, 2007.

Jones, E., Oliphant, T., Peterson, P., et al. SciPy: Open source scientific tools for Python, 2001. URL *http://www.scipy.org/*.

Keshishian, H., Broadie, K., Chiba, A., and Bate, M. The *Drosophila* neuromuscular junction: a model system for studying synaptic development and function. *Annual Review of Neuroscience*, 19(1):545–575, 1996.

Kiehn, O. Locomotor circuits in the mammalian spinal cord. *Annual Review of Neuroscience*, 29: 279–306, 2006.

Kiehn, O. and Harris-Warrick, R. 5-HT modulation of hyperpolarization-activated inward current and calcium-dependent outward current in a crustacean motor neuron. *Journal of Neurophysiology*, 68(2):496–508, 1992.

Kiehn, O., O. Kjaerulff, M. T., and Harris-Warrick, R. Contributions of intrinsic motor neurons properties to the production of rhythmic motor output in the mammalian spinal cord. *Brain Research Bulletin*, 53(5):649–659, 2000.

Kurdyak, P., Atwood, H., Stewart, B., and Wu, C. Differential physiology and morphology of motor axons to ventral longitudinal muscles in larval *Drosophila*. *Journal of Comparative Neurology*, 350(3):463–472, 1994.

552 Lee, J., Ueda, A., and Wu, C. Pre- and post-synaptic mechanisms of synaptic strength homeostasis
553 revealed by Slowpoke and Shaker K^+ channel mutations in *Drosophila*. *Neuroscience*, 154(4):
554 1283–1296, 2008.

555 Lee, R. and Heckman, C. Bistability in spinal motoneurons in vivo: systematic variations in
556 persistent inward currents. *Journal of Neurophysiology*, 80(2):583, 1998.

557 Lee, R. and Heckman, C. Essential role of a fast persistent inward current in action potential
558 initiation and control of rhythmic firing. *Journal of Neurophysiology*, 85(1):472–475, 2001.

559 Li, Y., Gorassini, M., and Bennett, D. Role of persistent sodium and calcium currents in motoneuron
560 firing and spasticity in chronic spinal rats. *Journal of Neurophysiology*, 91(2):767–783, 2004.

561 Li, Y., Gao, X.-B., Sakurai, T., and van den Pol, A. N. Hypocretin/orexin excites hypocretin
562 neurons via a local glutamate neurona potential mechanism for orchestrating the hypothalamic
563 arousal system. *Neuron*, 36(6):1169–1181, 2002.

564 Lindsay, A. and Feldman, J. Modulation of respiratory activity of neonatal rat phrenic motoneu-
565 rones by serotonin. *Journal of Physiology*, 461(1):213–233, 1993.

566 Liu, X.-B. and Murray, K. D. Neuronal excitability and calcium/calmodulin-dependent protein
567 kinase type ii: Location, location, location. *Epilepsia*, 53(s1):45–52, 2012.

568 MacLean, J., Zhang, Y., Johnson, B., and Harris-Warrick, R. Activity-independent homeostasis in
569 rhythmically active neurons. *Neuron*, 37(1):109–120, 2003.

570 Maffei, A. and Fontanini, A. Network homeostasis: A matter of coordination. *Current Opinion in*
571 *Neurobiology*, 19(2):168–173, 2009.

572 Magistretti, J. and Alonso, A. Biophysical properties and slow voltage-dependent inactivation
573 of a sustained sodium current in entorhinal cortex layer-ii principal neurons a whole-cell and
574 single-channel study. *Journal of General Physiology*, 114(4):491–509, 1999.

575 Manuel, M., Li, Y., ElBasiouny, S., Murray, K., Griener, A., Heckman, C., and Bennett, D. NMDA
576 induces persistent inward and outward currents that cause rhythmic bursting in adult rodent
577 motoneurons. *Journal of Neurophysiology*, 2012.

578 Marder, E. and Bucher, D. Central pattern generators and the control of rhythmic movements.
579 *Current Biology*, 11:R986–R996, 2001.

580 Marder, E. and Calabrese, R. Principles of rhythmic motor pattern generation. *Physiological*
581 *Reviews*, 76:687–717, 1996.

582 Marder, E. and Goaillard, J. Variability, compensation and homeostasis in neuron and network
583 function. *Nature Reviews Neuroscience*, 7(7):563–574, 2006.

McGuire, S., Mao, Z., and Davis, R. Spatiotemporal gene expression targeting with the TARGET and gene-switch systems in *Drosophila*. *Science STKE*, 2004(220):p16, 2004.

McKiernan, E. C. Effects of manipulating slowpoke calcium-dependent potassium channel expression on rhythmic locomotor activity in *Drosophila* larvae. *PeerJ*, 1:e57, 2013.

McLarnon, J. Potassium currents in motoneurones. *Progress in Neurobiology*, 47(6):513–531, 1995.

Mosca, T., Carrillo, R., White, B., and Keshishian, H. Dissection of synaptic excitability phenotypes by using a dominant-negative shaker k^+ channel subunit. *PNAS*, 102(9):3477–3482, 2005.

Nelson, A. B., Gittis, A. H., and du Lac, S. Decreases in camkii activity trigger persistent potentiation of intrinsic excitability in spontaneously firing vestibular nucleus neurons. *Neuron*, 46(4): 623–631, 2005.

Park, D., Coleman, M. J., Hodge, J. J., Budnik, V., and Griffith, L. C. Regulation of neuronal excitability in drosophila by constitutively active camkii. *Journal of neurobiology*, 52(1):24–42, 2002.

Pearson, K. Neural adaptation in the generation of rhythmic behavior. *Annual Review of Physiology*, 62(1):723–753, 2000.

Peng, I. and Wu, C. *Drosophila* cacophony channels: a major mediator of neuronal Ca^{2+} currents and a trigger for K^+ channel homeostatic regulation. *Journal of Neuroscience*, 27(5):1072, 2007.

Perrier, J.-F. and Hounsgaard, J. 5-HT2 receptors promote plateau potentials in turtle spinal motoneurons by facilitating an L-type calcium current. *Journal of Neurophysiology*, 89(2):954–959, 2003.

Perrier, J.-F. and Hounsgaard, J. Development and regulation of response properties in spinal cord motoneurons. *Brain Research Bulletin*, 53(5):529–535, 2000.

Perrier, J.-F., Rasmussen, H., Christensen, R., and Petersen, A. Modulation of the intrinsic properties of motoneurons by serotonin. *Current Pharmaceutical Design*, 19(24):4371–4384, 2013.

Perrier, J. and Tresch, M. Recruitment of motor neuronal persistent inward currents shapes withdrawal reflexes in the frog. *Journal of Physiology*, 562(2):507–520, 2005.

Ryder, E. and Russell, S. Transposable elements as tools for genomics and genetics in drosophila. *Briefings in Functional Genomics & Proteomics*, 2(1):57–71, 2003.

Ryglewski, S. and Duch, C. Shaker and Shal mediate transient calcium independent potassium current in a *Drosophila* flight motoneuron. *Journal of Neurophysiology*, pages 00693–2009, 2009.

Ryglewski, S., Lance, K., Levine, R. B., and Duch, C. Cav2 channels mediate low and high voltage-activated calcium currents in drosophila motoneurons. *The Journal of physiology*, 590 (4):809–825, 2012.

Ryglewski, S., Kilo, L., and Duch, C. Sequential acquisition of cacophony calcium currents, sodium channels and voltage-dependent potassium currents affects spike shape and dendrite growth during postembryonic maturation of an identified drosophila motoneuron. *European Journal of Neuroscience*, 39(10):1572–1585, 2014.

Schaefer, J. E., Worrell, J. W., and Levine, R. B. Role of intrinsic properties in drosophila motoneuron recruitment during fictive crawling. *Journal of Neurophysiology*, 104(3):1257–1266, 2010.

Schwindt, P. and Crill, W. A persistent negative resistance in cat lumbar motoneurons. *Brain research*, 120(1):173, 1977.

Schwindt, P. and Crill, W. Properties of a persistent inward current in normal and TEA-injected motoneurons. *Journal of Neurophysiology*, 43(6):1700–1724, 1980.

Selverston, A. Invertebrate central pattern generator circuits. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1551):2329–2345, 2010.

Sinkjær, T., Andersen, J., Ladouceur, M., Christensen, L., and Nielsen, J. Major role for sensory feedback in soleus EMG activity in the stance phase of walking in man. *Journal of Physiology*, 523(3):817–827, 2000.

Song, W., Onishi, M., Jan, L., and Jan, Y. N. Peripheral multidendritic sensory neurons are necessary for rhythmic locomotion behavior in *Drosophila* larvae. *Proceedings of the National Academy of Sciences*, 104(12):5199–5204, 2007.

Srinivasan, S., Lance, K., and Levine, R. B. Contribution of eag to excitability and potassium currents in drosophila larval motoneurons. *Journal of neurophysiology*, 107(10):2660–2671, 2012a.

Srinivasan, S., Lance, K., and Levine, R. B. Segmental differences in firing properties and potassium currents in drosophila larval motoneurons. *Journal of neurophysiology*, 107(5):1356–1365, 2012b.

Sun, X. X., Hodge, J. J., Zhou, Y., Nguyen, M., and Griffith, L. C. The eag potassium channel binds and locally activates calcium/calmodulin-dependent protein kinase ii. *Journal of Biological Chemistry*, 279(11):10206–10214, 2004.

Sun, X. X., Bostrom, S. L., and Griffith, L. C. Alternative splicing of the¡ i¿ eag¡/i¿ potassium channel gene in¡ i¿ drosophila¡/i¿ generates a novel signal transduction scaffolding protein. *Molecular and Cellular Neuroscience*, 40(3):338–343, 2009.

Suster, M., Seugnet, L., Bate, M., and Sokolowski, M. Refining GAL4-driven transgene expression in *Drosophila* with a GAL80 enhancer-trap. *Genesis*, 39(4):240–245, 2004.

Swensen, A. and Bean, B. Robustness of burst firing in dissociated purkinje neurons with acute or long-term reductions in sodium conductance. *Journal of Neuroscience*, 25(14):3509–3520, 2005.

650 Tateno, T., Harsch, A., and Robinson, H. Threshold firing frequency–current relationships of
651 neurons in rat somatosensory cortex: type 1 and type 2 dynamics. *Journal of Neurophysiology*,
652 92(4):2283–2294, 2004.

653 Timmerman, C., Suppiah, S., Gurudatta, B. V., Yang, J., Banerjee, C., Sandstrom, D. J., Corces,
654 V. G., and Sanyal, S. The drosophila transcription factor adf-1 (nalyot) regulates dendrite growth
655 by controlling fasii and staufen expression downstream of camkii and neural activity. *The Journal*
656 *of Neuroscience*, 33(29):11916–11931, 2013.

657 Ueda, A. and Wu, C. Distinct frequency-dependent regulation of nerve terminal excitability and
658 synaptic transmission by I_A and I_K potassium channels revealed by *Drosophila Shaker* and *Shab*
659 mutations. *Journal of Neuroscience*, 26(33):6238–6248, 2006.

660 Veasey, S., Fornal, C., Metzler, C., and Jacobs, B. Response of serotonergic caudal raphe neurons
661 in relation to specific motor activities in freely moving cats. *Journal of Neuroscience*, 15(7):
662 5346–5359, 1995.

663 Vonhoff, F., Kuehn, C., Blumenstock, S., Sanyal, S., and Duch, C. Temporal coherency between
664 receptor expression, neural activity and ap-1-dependent transcription regulates drosophila mo-
665 toneuron dendrite development. *Development*, 140(3):606–616, 2013.

666 Wolfram, V., Southall, T. D., Brand, A. H., and Baines, R. A. The lim-homeodomain protein islet
667 dictates motor neuron electrical properties by regulating k¡ sup¿+¡/sup¿ channel expression.
668 *Neuron*, 75(4):663–674, 2012.

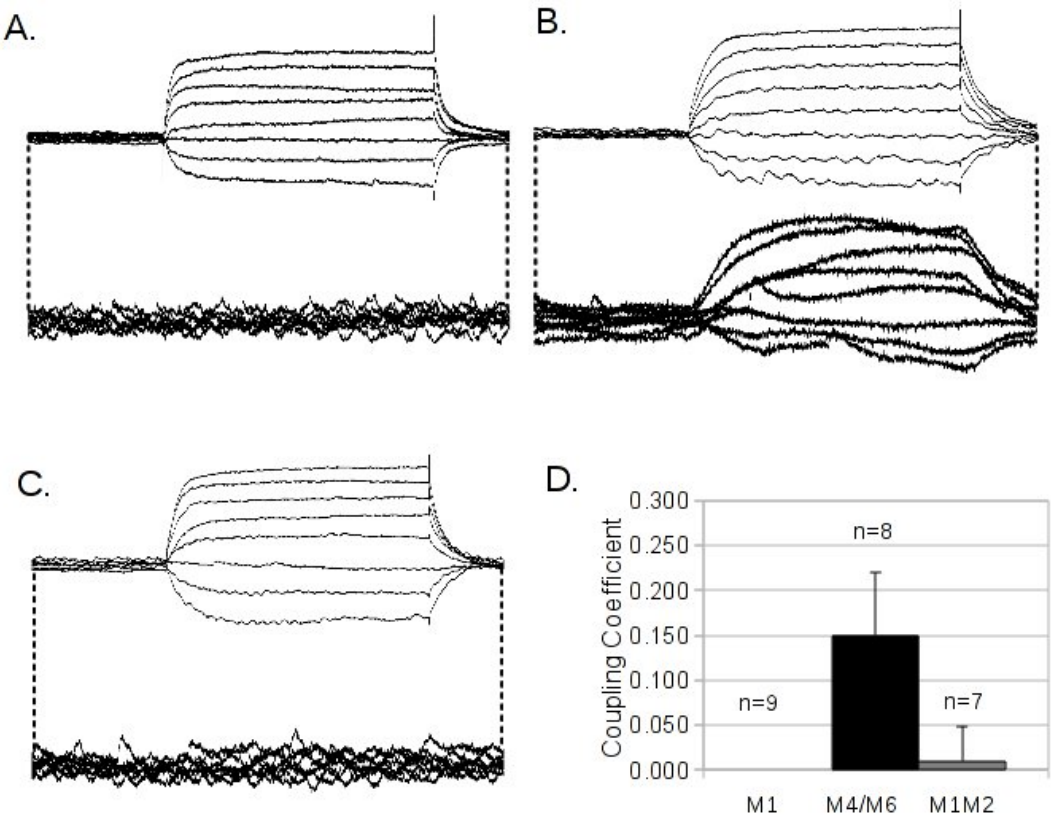
669 Wright Jr, T. and Calabrese, R. Contribution of motoneuron intrinsic properties to fictive motor
670 pattern generation. *Journal of Neurophysiology*, 106(2):538–553, 2011.

671 Yao, W. and Wu, C. Distinct roles of CaMKII and PKA in regulation of firing patterns and K^+
672 currents in *Drosophila* neurons. *Journal of Neurophysiology*, 85(4):1384, 2001.

673 Zhang, B. and Harris-Warrick, R. Calcium-dependent plateau potentials in a crab stomatogas-
674 tric ganglion motor neuron. I. Calcium current and its modulation by serotonin. *Journal of*
675 *Neurophysiology*, 74:1929–1929, 1995.

676 Zwart, M. F., Randlett, O., Evers, J. F., and Landgraf, M. Dendritic growth gated by a steroid
677 hormone receptor underlies increases in activity in the developing drosophila locomotor system.
678 *Proceedings of the National Academy of Sciences*, 110(40):E3878–E3887, 2013.

679 # Supplemental material



Supplemental Fig.1: Electrical coupling of body wall muscles in WT larvae. A. Current was injected into a muscle 1 (M1) segment (top trace) and the response measured in an adjacent M1 segment (bottom trace). Dashed lines indicate that the records were made simultaneously. B. Same as in A, but for two adjacent muscle 6 (M6) segments. Note that a voltage change is measured in the adjacent segment (bottom trace) in response to current injection in the top segment, indicating electrical coupling. C. Same as in A, but for M1 and M2 in the same segment. D: Average coupling coefficient calculated for M1 pairs (n=9), M4 or M6 pairs (n=8), and M1M2 pairs (n=7). Error bars shown are standard deviations.

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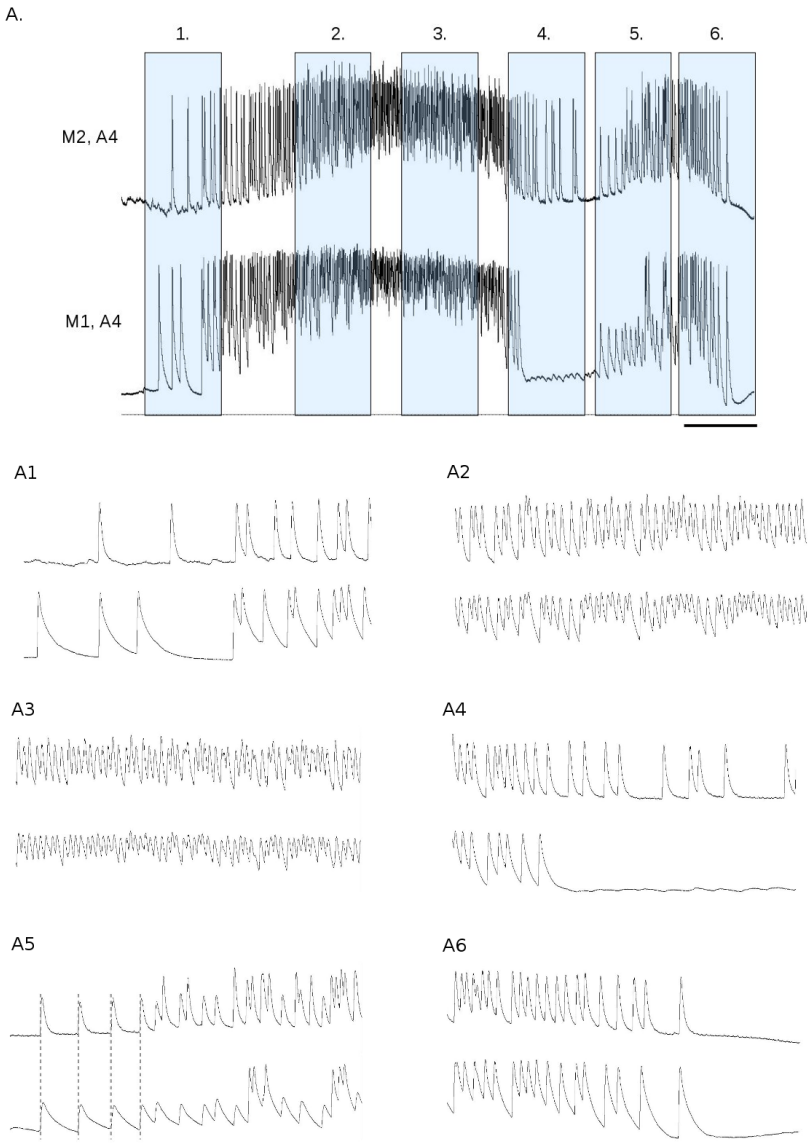

Supplemental Fig.2: Activity of two dorsal muscles receiving common and non-common MN input during rhythmic motor activity. A: Simultaneous intracellular recordings from muscle 2 (top trace) and muscle 1 (bottom trace) in segment A4. Shaded rectangles 1-6 indicate regions of the burst which were examined at higher temporal resolution below. Scale bar is 1 second. A1-A6: Each panel is a 1 second window corresponding to the shaded and numbered regions in A. Dashed lines in A5 indicate coincident EPSPs. M1 and M2 are innervated by 1 common (MNISN-Is) and one non-common MN (MN1-Ib or MN2-Ib, respectively). It was hypothesized that by looking at coincident and non-coincident EPSPs, a unit separation could be performed. However, EPSP summation and compound events throughout the majority of the burst made this not possible.