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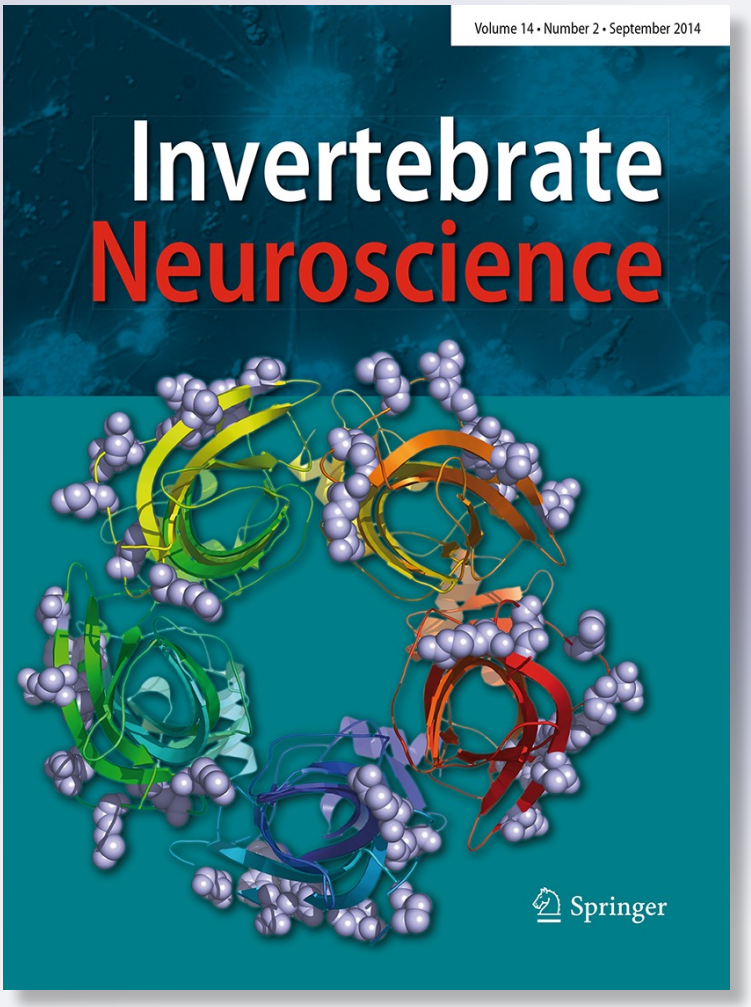

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ORIGINAL PAPER

Differing synaptic strengths between homologous mechanosensory neurons

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Abstract Leeches have four mechanosensory pressure neurons (P cells) in each midbody ganglion. Within a ganglion, P cells show complex electrical and chemical connections that vary between species. In *Hirudo verbana*, stimulating one P cell causes a weak depolarization followed by a strong hyperpolarization in the other P cells; however, stimulating a P cell in *Erpobdella obscura* produces strong depolarizations in the other P cells. In this study, we examined interactions between P cells in the American medicinal leech *Macrobdella decora*. Not only is *Macrobdella* more closely related to *Hirudo* than to *Erpobdella*, but *Hirudo* and *Macrobdella* also have very similar behavioral responses to mechanical stimulation. Despite the phylogenetic relationship and behavioral similarities between the two species, we found that intracellular stimulation of one P cell in *Macrobdella* causes a depolarization in the other P cells, rather than the hyperpolarization seen in *Hirudo*. Experiments performed in a high Mg^{2+}, 0 Ca^{2+} saline solution and a high Mg^{2+}, high Ca^{2+} saline solution suggest that the P cells in *Macrobdella* have a monosynaptic excitatory connection, a polysynaptic inhibitory connection, and a weak electrical coupling, similar to the connections between P cells in *Hirudo*. The difference in net response of P cells between these two species seems to be based on differences in the strengths of the chemical connections. These results demonstrate that even when behavioral patterns are conserved in closely related species, the underlying neural circuitry is not necessarily tightly constrained.

Keywords Mechanosensory · Behavior · Evolution · *Hirudo* · *Macrobdella*

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Introduction

One of the underlying issues in understanding how evolution shapes neural circuits is identifying which features of neural circuits are evolutionarily labile and which features are conserved (Breidbach and Kutsch 1995). In order to identify how neural circuits evolve, a number of studies have examined the functions of homologous neurons in closely related species. These studies have collectively shown that the behavioral output of neural circuits can change due to altering neuromodulatory input (Fenelon et al. 2004; Newcomb and Katz 2007), synaptic strengths (Chiang et al. 2006), neuron morphology (Dacks et al. 2006), and synaptic connections (Baltzley et al. 2010). Predictably, there are several known examples of neural circuitry in which the circuitry and the dependent behavior are conserved in closely related species. For example, homologous neurons in the European medicinal leech *Hirudo verbana* and the American medicinal leech *Macrobdella decora* function to initiate swimming (Nusbaum and Kristan 1986). Likewise, homologous neurons in the nudibranch molluscs *Melibe leonina* and *Dendronotus iris* both function in the swimming central pattern generator (Sakurai et al. 2011). In this study, we describe a neural circuit where synaptic strengths between sensory neurons vary across two species that have conserved behavioral output.

Interactions between sensory neurons provide the first layer of information processing in behavioral reflexes

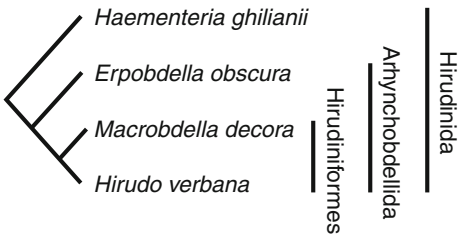


Fig. 1 Phylogenetic relationship of leech species where homologous pressure mechanosensory neurons (P cells) have been identified

(Envoy and Cohen 1969; Grider and Jin 1994). For example, *Hirudo* responds to body wall stimulation by contracting longitudinal muscles at the point of stimulation (Kristan 1982). The pressure mechanosensory neurons (P cells) in *Hirudo* are mutually inhibitory, which may aid in localizing the site of sensory stimulation (Baltzley et al. 2010). In contrast, in the ribbon leech *Erpobdella obscura*, the homologous P cells are mutually excitatory; when stimulated, *Erpobdella* demonstrates a segment-wide response rather than a localized response (Baltzley et al. 2010).

Leeches are ideal for investigating the differences in neural circuitry underlying behavioral patterns because the structure of the leech midbody ganglia is highly conserved across the entire clade (Lent and Frazer 1977; Kramer and Goldman 1981; Nusbaum 1986; Elsas et al. 1995). Because P cells have been identified in a basal leech, *Haementeria ghilianii*, as well as in the highly derived leeches *Hirudo*, *Erpobdella*, and *Macrobdella* (Nicholls and Baylor 1968; Kristan et al. 1982; Kramer and Stent 1985; Kramer et al. 1985; Johansen and Kleinhaus 1990; Baltzley et al. 2010), they are likely to be present in all leeches. In addition to being more closely related to *Hirudo* than to *Erpobdella* (Fig. 1; Siddall et al. 2001; Borda and Siddall 2004), the behavioral responses to skin stimulation displayed by *Macrobdella* are more similar to *Hirudo* than they are to *Erpobdella* (Kristan et al. 1982; Gaudry et al. 2010). These similarities in behavioral responses appear to be correlated with feeding strategy rather than phylogenetic relationships; sanguivores are more similar to each other than they are to predatory leeches, regardless of relatedness (Gaudry et al. 2010). Because of their similar behavioral repertoires, their similar responses to skin stimulation, and their phylogenetic relationship, we predicted that the interactions between the pressure mechanosensory cells in *Macrobdella* would be similar to the interactions between P cells in *Hirudo*.

We identified P cells in *Macrobdella* by their location in the midbody ganglia and their physiological properties. *Hirudo*, *Erpobdella*, and *Macrobdella* have four P cells in each segmental ganglion that innervate overlapping quadrants of the body wall (Nicholls and Baylor 1968; Kristan et al. 1982; Baltzley et al. 2010). Each ventral P cell (P_V) innervates roughly half of the ventral body wall, and each dorsal P cell (P_D) innervates roughly half of the dorsal body wall. We used intracellular electrodes to stimulate one P cell while recording from another P cell to examine their synaptic connections. We found that, unlike the mutual inhibition between P cells seen in *Hirudo*, P cells in *Macrobdella* appeared to be mutually excitatory, similar to the interactions seen in *Erpobdella*. However, unlike in *Erpobdella*, stimulation of one P cell did not elicit action potentials in the other P cells possibly because the interactions between P cells in *Macrobdella* include an inhibitory component that is obscured by the excitatory electrical and chemical connections between the cells.

Methods

Leech care

Hirudo verbana were purchased from Carolina Biological Supply Company (Burlington, North Carolina); historically, these leeches have been misidentified as *Hirudo medicinalis* (Siddall et al. 2007). *Macrobdella* were purchased from the Land of Lakes Bait Shop (Watersmeet, Michigan). All leeches used were adults weighing between 0.7 g and 3.0 g. Because feeding may affect the interactions between sensory cells for several weeks (Baltzley et al. 2010), leeches were not feed for at least a month prior to experimentation. Leeches were kept at 15 °C on a 12 h light/dark cycle in 5 gallon aquaria filled with Instant Ocean artificial seawater (Aquarium Systems, Mentor, Ohio) diluted 1:1,000.

Intracellular recordings

We performed experiments using midbody ganglia 8–12. We anesthetized the leeches before and during dissections using ice-cold leech saline. Unless otherwise stated, ganglia were bathed in normal leech saline containing 115 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1.5 mM $MgCl_2$, 10 mM Glucose, 10 mM Hepes, at pH 7.4 (Nicholls and Baylor 1968; Nusbaum and Kristan 1986). Intracellular recordings were performed in bridge mode using 20–40 MΩ sharp microelectrodes filled with a 2 M potassium acetate solution containing 20 mM KCl. In experiments performed at the University of California, San Diego (La Jolla, CA, USA), electrodes were connected to an Axoclamp 2 amplifier (Axon Instruments, Union City, CA, USA) and recordings were digitized using National Instruments BNC-2090 (National Instruments, Austin, TX, USA). When examining connections between P cells, we acquired recordings and delivered stimulus using LabVIEW 5.0 (National

Instruments). When measuring the responses of P cells to body wall stimulation, we acquired the recordings and delivered stimulus trains using Axograph 4.9 software (Axon Instruments). In experiments performed at Western Oregon University (Monmouth, Oregon), electrodes were connected to an IX2-700 Dual Intracellular Preamp amplifier (Dagan Instruments, Minneapolis, Minnesota) and a PowerLab 26T (ADInstruments, Colorado Springs, Colorado) analog-to-digital converter was used for data acquisition.

P cell response to mechanical stimulation

We tested the responses of putative P cells to body wall stimulation using a semi-intact preparation. We isolated five segments of the body wall and cut the body wall along the dorsal midline. All ganglia except the middle ganglion were removed. A hole was cut in the ventral body wall above the ganglion, and the ganglion was pinned in a Sylgard dish with the body wall facing up. We mechanically stimulated the body wall using Dual-Mode Lever Arm System (Aurora Scientific, Ontario, Canada, Model 300B) to deliver a 100 mN tactile stimulus to the body wall with a 1-mm diameter bead of epoxy on the tip of a needle while recording intracellularly from putative P cells in *Macrobdella* (Baltzley et al. 2010).

Recording from connected P cells

Microelectrodes were inserted into two P cells within a ganglion. One P cell was stimulated to fire a burst of five action potentials by injecting five 20 ms pulses of 2–3 nA at 25 Hz. This protocol causes the P cell to fire action potentials in a pattern that mimics a normal response to body wall stimulation (Marin-Burgin et al. 2006; Baltzley et al. 2010). To determine whether the connections between P cells were monosynaptic or polysynaptic, we used a modified leech saline with 10 mM $MgCl_2$ and 10 mM $CaCl_2$. To test whether connections were chemical or electrical, we used a modified saline with 20 mM $MgCl_2$ and 0 mM $CaCl_2$ (Baylor and Nicholls 1969). In both solutions, the NaCl concentration was adjusted to maintain a constant osmolarity. We also used a 0.1 mM bicuculline methiodide (BMI) solution to test whether there was GABAergic inhibition between the P cells (Baca et al. 2008). In these alternative saline solutions, we first ran the stimulation protocol in normal leech saline, and then used a gravity-driven perfusion system to switch to one of the alternative bath solutions. After running the stimulation protocol in the alternative bath solution, we returned the ganglion to normal saline and ran the stimulation protocol to ensure recovery to the expected response.

Data were analyzed in MATLAB 7.4. We used the average membrane potential of the postsynaptic cell over the 50 ms preceding the stimulation protocol to establish a baseline membrane potential. We calculated the average deviation from baseline during the first 75 ms following stimulation and then from 75 to 250 ms following stimulation. These time intervals capture both the initial depolarization and the subsequent hyperpolarization in *Hirudo* (Marin-Burgin et al. 2006; Baltzley et al. 2010). To determine whether the response of the cell was statistically different from the resting membrane potential, we used the 500-ms segment preceding stimulation as a control. The first 50 ms served as the baseline, and we calculated the average deviation from baseline over the next 75 ms and then the subsequent 175 ms.

Previous research has shown that there is no difference in the postsynaptic response when different combinations of P cells are compared (Baltzley et al. 2010). For example, when a P_D cell is stimulated, the responses in the ipsilateral P_V cell, the contralateral P_V cell, and the contralateral P_D cell are not significantly different. In this study, we compared the responses of cells based on the region of skin that they innervated. We used an ANOVA with post hoc *t* tests to compare connections between ipsilateral P cells (e.g., right P_V to right P_D and right P_D to right P_V), contralateral P cells (e.g., right P_V to left P_V and right P_D to left P_D), and non-adjacent P cells (e.g., right P_V to left P_D and right P_D to left P_V).

To compare the mean deviation from baseline in the postsynaptic P cells in *Hirudo* and *Macrobdella*, we used a repeated measures ANOVA. Post hoc unpaired *t* tests were used to compare *Hirudo* with *Macrobdella*, while paired *t* tests were used for intraspecific comparisons. A repeated measures ANOVA with post hoc paired *t* tests was used to compare the response of P cells when the *Macrobdella* ganglia were bathed in a high Mg^{2+}/high Ca^{2+} solution or in a 0 Ca^{2+} solution. Paired *t* tests were used to compare the response of P cells when ganglia were bathed in BMI. Repeated measures ANOVAs were performed in IBM SPSS Statistics 21 (IBM Corporation, Armonk, New York). All other analyses were performed in Microsoft Excel (Microsoft, Redmond, Washington).

Dye injections

We injected 2.5 % dextran Alexafluor 488 (Molecular Probes, Eugene, OR, USA) into four *Hirudo* and ten *Macrobdella* P cells after recording was complete. We backfilled an electrode tip with the dye and then filled the electrode with 3 M KCl. 2–4 nA pulses at 1 Hz were used to inject dye into the P cell (Baltzley et al. 2010). The ganglia were observed under a CCD fluorescent camera (Princeton Instruments, Trenton, NJ, USA). Four *Macrobdella* ganglia with dye-injected P cells were fixed with 4 % paraformaldehyde, dehydrated, and cleared with

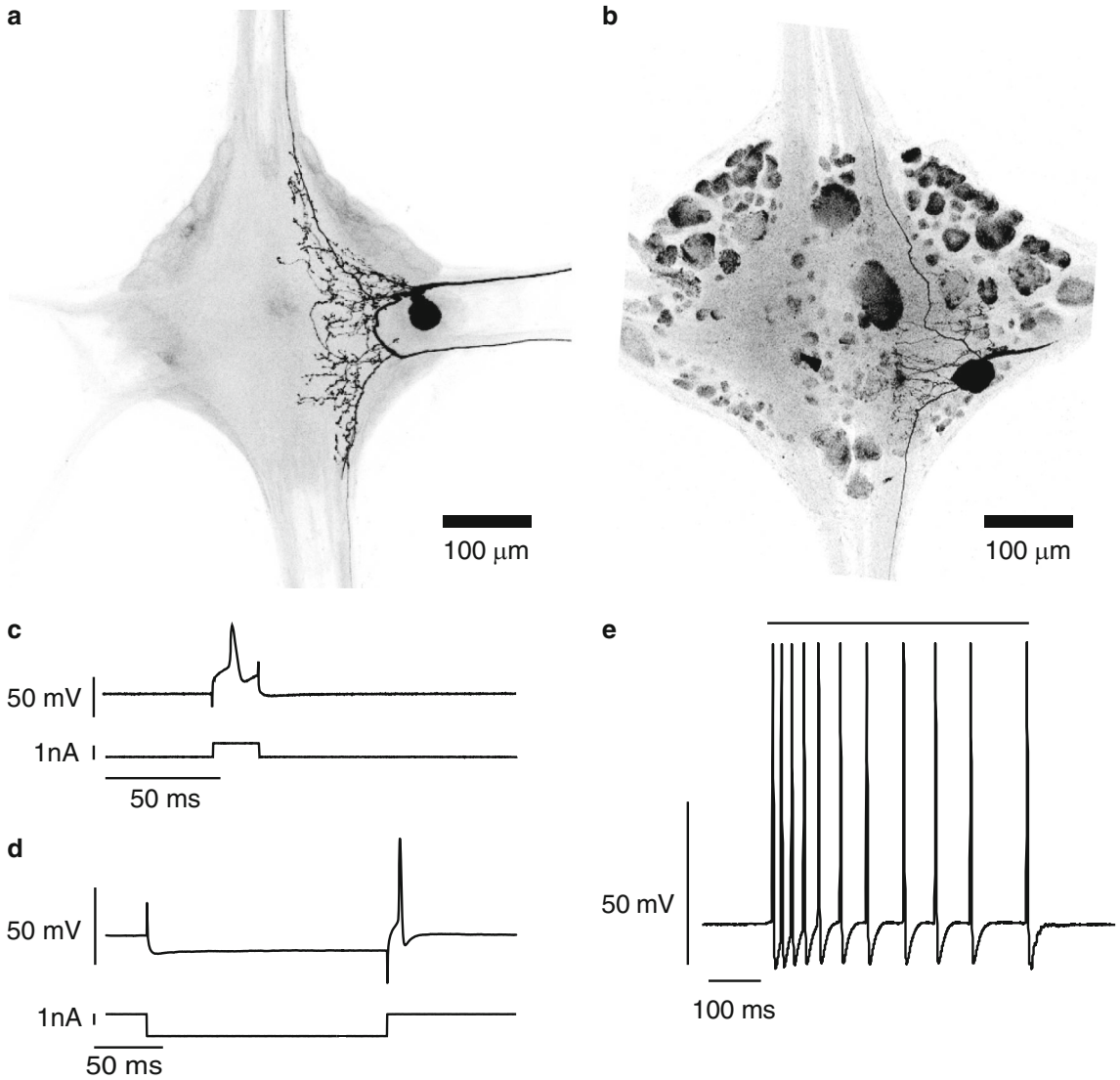


Fig. 2 Anatomical and physiological characteristics of P cells in *Macrobdella*. **a** Dye fill of a P_V cell. **b** Dye fill of a P_D cell. *Brightness* and *contrast* were adjusted in Adobe Photoshop. **c** *Representative traces* showing the response of a P cell to depolarizing current. *Top trace* is a current clamp recording from the P cell, and the *bottom trace* shows the magnitude and timing of the injected current. **d** *Representative traces* showing the response of a P cell to hyperpolarizing current. *Top* and *bottom traces* are same as in (**c**). **e** Response of a P cell to a 500 ms mechanical stimulation of the body wall with a force of 100 mN/mm^2. The *black bar* above the trace represents the timing of the mechanical stimulation

methyl salicylate (Marin-Burgin et al. 2006). Ganglia were imaged on a Bio-Rad Radiance confocal microscope using Bio-Rad LaserSharp2000 software (Bio-Rad Laboratories, Hercules, CA, USA).

Results

The anatomy and physiology of P cells in *Macrobdella* were similar to P cells in *Hirudo* and *Erpobdella* (Nicholls and Baylor 1968; Baltzley et al. 2010). Dye fills of putative P cells in *Macrobdella* showed that P_V cells were located between the lateral roots with neurites running ipsilaterally out the dorsal and ventral roots as well as out the anterior and posterior connectives (Fig. 2a). P_D cells were slightly posterior and closer to the midline, with neurites running down the dorsal root, posterior connective, and anterior connective on the ipsilateral side (Fig. 2b). Injection of a 1 nA depolarizing current for 20 ms initiated one or two action potentials (Fig. 2c). We did not observe spontaneous activity in the P cells and injecting a hyperpolarizing current often caused a sag response, sometimes followed by a rebound action potential (Fig. 2d). In semi-intact preparations, simulation of the body wall with a force of 100 mN/mm^2

Table 1 Average amplitude (±SEM) of the postsynaptic potential in a P cell in response to five action potentials produced by a stimulated P cell

P cell pair	*Hirudo*			*Macrobdella*		
	PSP amplitude (mV)			PSP amplitude (mV)		
	n	First 75 ms	Next 175 ms	*n*	First 75 ms	Next 175 ms
Ipsilateral	17	0.40 ± 0.07	−0.68 ± 0.18	20	1.07 ± 0.27	1.32 ± 0.30
Contralateral	8	0.39 ± 0.11	−0.11 ± 0.19	5	0.35 ± 0.07	0.44 ± 0.09
Non-adjacent	3	0.47 ± 0.16	−0.07 ± 0.15	7	0.45 ± 0.14	0.66 ± 0.12
ANOVA: *p* value		0.92	0.63		0.21	0.18

resulted in the production of 11.1 ± 0.9 (mean ± SEM; $n = 10$) action potentials over 500 ms (Fig. 2e).

When we compared the postsynaptic responses of P cells based on the region of skin that they innervated, we found that in *Hirudo* the responses between P cells were not significantly different in either the first 75 ms or the next 175 ms (Table 1). Likewise, the responses between P cells were not significantly different in *Macrobdella* in either the first 75 ms or the next 175 ms. Because the responses of ipsilateral and contralateral P cells were not statistically different, the data were pooled for the remaining analyses.

Using a repeated measures ANOVA to compare the postsynaptic responses of P cells between species, we found that there was a significant difference between species ($p < 0.001$), there was a significant effect of time after stimulation ($p < 0.001$), and there was a significant interaction between species and time ($p < 0.001$). In *Hirudo*, the average resting potential of P cells was −43.9 ± 1.0 mV. When one P cell was stimulated to fire five action potentials, the postsynaptic P cell showed an initial depolarization during the first 75 ms (0.42 ± 0.05 mV; mean ± SEM) followed by a hyperpolarization over the subsequent 175 ms (−0.25 ± 0.12 mV; Fig. 3a). The average membrane potential in the first 75 ms was greater than the average membrane potential in the subsequent 175 ms in 27 of 28 trials. Additionally, the average membrane potential in the 75–250 ms time period was more negative than the resting membrane potential in 19 of the 28 trials. The initial depolarization was significantly different from the following hyperpolarization and from the baseline recordings (Table 2). This response is similar to previously published results (Baltzley et al. 2010).

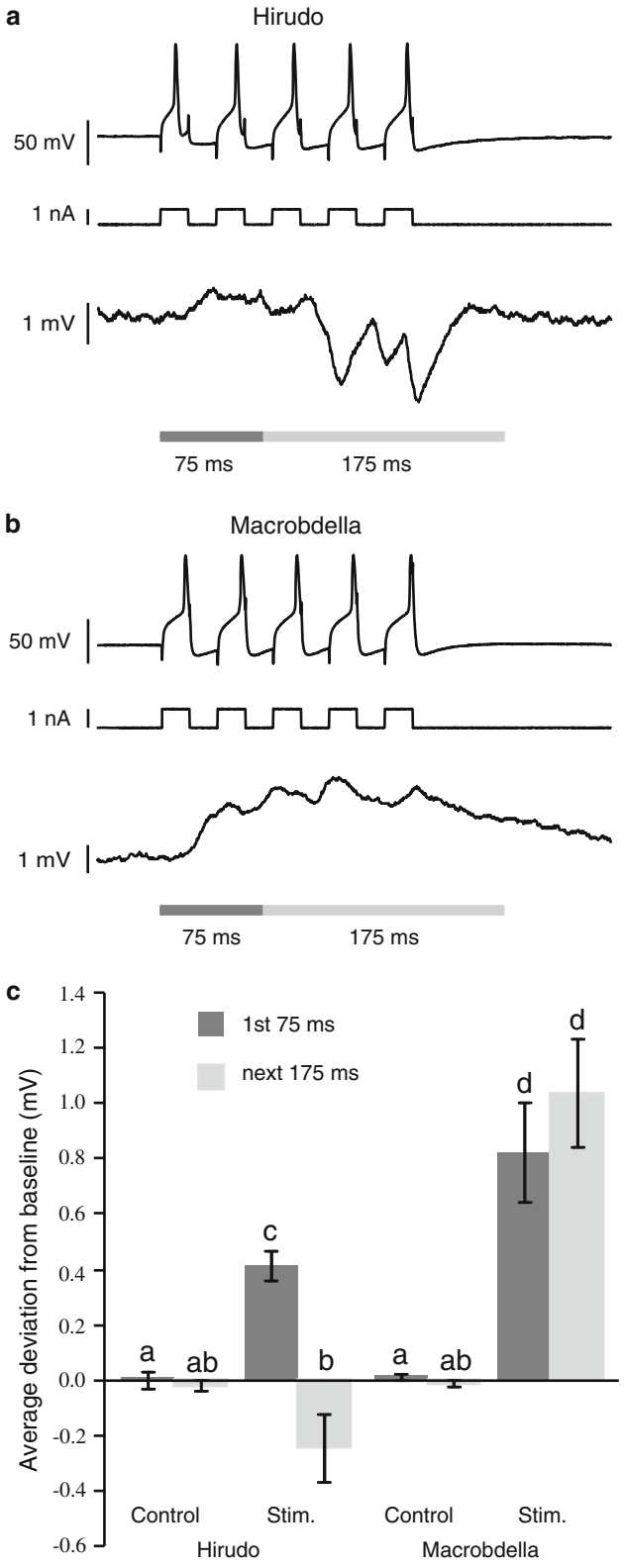
In contrast to *Hirudo*, *Macrobdella* postsynaptic P cells responded to five action potentials with a similar depolarization during both the first 75 ms and the following 175 ms (first 75 ms = 0.82 ± 0.18 mV, next 175 ms = 1.04 ± 0.20 mV; Fig. 3b). The depolarization during both time periods was significantly different from baseline, and in 25 out of 32 trials the depolarization in the first 75 ms was smaller than in the subsequent 175 ms. In none of our trials did we observe a hyperpolarization in the postsynaptic P cell. The average deviation in *Macrobdella* was also significantly different from the response in *Hirudo* both during the first 75 ms and during the subsequent 175 ms. The average resting potential of P cells in *Macrobdella* was −42.9 ± 1.3 mV, which was not significantly different from the average resting membrane potential of P cells in *Hirudo* (*t* test, $p = 0.54$).

In *Macrobdella*, when bathed in a high Mg^{2+}/high Ca^{2+} solution to block polysynaptic connections, the depolarization during the first 75 ms did not change (normal saline: 0.94 ± 0.20 mV; high Mg^{2+}/high Ca^{2+}: 1.12 ± 0.19 mV; $n = 5$; paired *t* test: $p = 0.11$); however, the depolarization during the subsequent 175 ms increased from 1.34 ± 0.22 to 2.44 ± 0.38 mV ($p = 0.03$; Fig. 4). When placed in a 0 Ca^{2+} solution to block all synaptic activity, the magnitude of the depolarization decreased during both the first 75 ms (normal saline: 1.11 ± 0.11 mV; 0 Ca^{2+}: 0.34 ± 0.07 mV; paired *t* test: $p < 0.001$) and the subsequent 175 ms (normal saline: 1.59 ± 0.21 mV; 0 Ca^{2+}: 0.41 ± 0.09 mV; paired *t* test: $p < 0.01$). Additionally, hyperpolarizing one P cell led to a small hyperpolarization in the other P cells, with a coupling strength of 0.22 ± 0.06 mV/nA (mean ± SEM; $n = 3$).

In *Hirudo*, when we bathed the ganglia in a 0.1 M BMI solution, the amplitude of PSPs during the first 75 ms appeared to increase, but was not significantly different (normal saline: 0.41 ± 0.16 mV; BMI: 1.29 ± 0.47 mV; $n = 3$; paired *t* test, $p = 0.21$). However, during the next 175 ms, the average hyperpolarization changed from −0.24 ± 0.21 to 2.15 ± 0.57 (paired *t* test, $p = 0.02$). Qualitatively similar results were seen in *Macrobdella*, where the average depolarization appeared increased from 0.58 ± 0.32 to 0.94 ± 0.48 mV in the presence of BMI in the first 75 ms (paired *t* test, $p = 0.45$) and from 0.62 ± 0.07 to 1.07 ± 0.45 mV in the next 175 ms (paired *t* test, $p = 0.22$), but the apparent changes in PSP amplitude were not statistically significant.

Discussion

Pressure mechanosensory cells in *Macrobdella* and *Hirudo* have conserved anatomical and physiological properties (Fig. 2; Kristan et al. 1982). Due to the similar behavioral



◀**Fig. 3** P cell to P cell connections in *Hirudo* and *Macrobdella*. **a** Representative response of a P cell to stimulation of another P cell in *Hirudo*. The *top trace* shows the stimulated P cell, the *middle trace* shows the stimulation protocol, and the *bottom trace* shows the response in the non-stimulated P cell. **b** Representative response of a P cell to stimulation of another P cell in *Macrobdella*. *Traces* are the same as in (**a**). **c** The average deviation from baseline in the non-stimulated P cells over the first 75 ms and the subsequent 175 ms. The control period began 500 ms before the stimulation protocol. *Error bars* represent SEM. *Bars* with similar *letters* were not significantly different

Table 2 Summary of post hoc *t* tests comparing the postsynaptic potentials of P cells across species, between treatment conditions, and between the first 75 ms and the next 175 ms after stimulation of the presynaptic cell

Species	Treatment	Time period (ms)	*p* value
Hirudo	Stimulus versus baseline	0–75	<0.0001
Hirudo	Stimulus versus baseline	75–250	0.07
Hirudo	Stimulus	0–75 versus 75–250	<0.0001
Macrobdella	Stimulus versus baseline	0–75	<0.0005
Macrobdella	Stimulus versus baseline	75–250	<0.0001
Macrobdella	Stimulus	0–75 versus 75–250	0.42
Hirudo versus *Macrobdella*	Stimulus	0–75	0.04
Hirudo versus *Macrobdella*	Stimulus	75–250	<0.0001

In the 'stimulus' treatment, the presynaptic cell fired five action potentials. In the 'baseline' treatment, the presynaptic cell was not stimulated to fire action potentials

responses of *Hirudo* and *Macrobdella* (Kristan et al. 1982; Gaudry et al. 2010) as well as their phylogenetic relationship (Fig. 1; Siddall et al. 2001; Borda and Siddall 2004), we expected that the chemical and synaptic connections between the cells would be conserved. Contrary to this prediction, we found that while the P cells in *Hirudo* are mutually inhibitory, when one P cell in *Macrobdella* is stimulated the other P cells are depolarized (Fig. 3). However, when bathed in a high divalent cation solution, the depolarization seen in *Macrobdella* increased, indicating that there is polysynaptic inhibition that is masked by monosynaptic excitation (Fig. 4). When bathed in a 0 Ca^{2+} solution, a small depolarization remained which suggests there is a weak electrical connection between the cells. Additionally, when one P cell is hyperpolarized, a weak hyperpolarization is seen in the other P cells.

Previous research indicates that P cells in *Hirudo* have a weak electrical connection, an excitatory monosynaptic connection, and a polysynaptic inhibitory connection (Baltzley et al. 2010). We have now provided evidence that

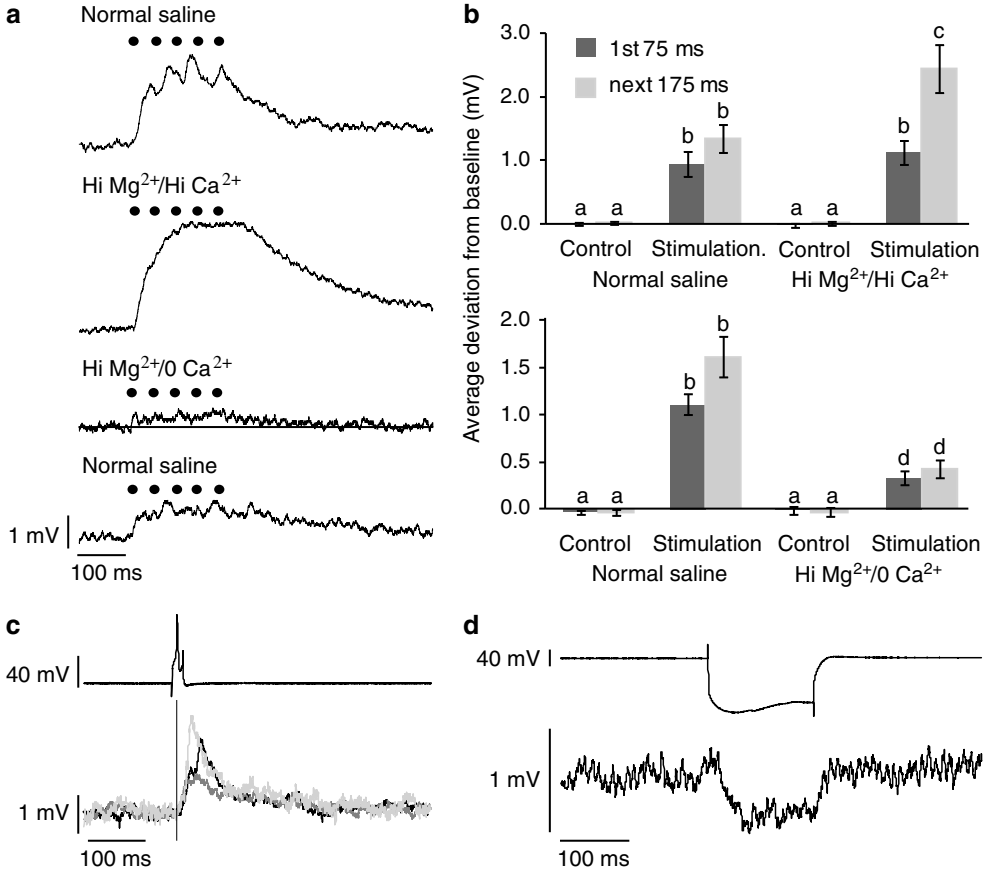


Fig. 4 Characterization of P cell to P cell connections in *Macrobdella*. **a** *Representative trace* of the response of a P cell to intracellular stimulation of another P cell when bathed in alternative saline solutions. *Top trace* is normal saline. The *second trace* is a high divalent cation saline solution. The *third trace* is a 0 Ca^{2+} saline solution. The *bottom trace* is a washout in normal saline. *Black dots* represent the peak of action potentials in the stimulated P cell. **b** The average deviation from baseline in the non-stimulated P cells over the first 75 ms and the subsequent 175 ms. The control period began 500 ms before the stimulation protocol. *Error bars* represent SEM. *Bars* with similar *letters* were not significantly different. **c** Overlay of postsynaptic responses of P cells to one action potential generated by intracellular stimulation of another P cell in normal saline. *Top trace* is from one of the stimulated P cells. The *bottom trace* is the postsynaptic P cells. The *vertical black line* represents the peak of the action potential in the stimulated P cells. **d** Response of a P cell to −2 nA injected into another P cell

the inhibitory synapse is modulated by GABA by showing that the inhibition is blocked by BMI (Fig. 5). In *Macrobdella*, we found that BMI appeared to have a similar effect on the postsynaptic response of the P cells, but the effect was not statistically significant. There are several plausible explanations for this result, including the possibilities that our sample size was too small to detect the effect of the BMI solution or, alternatively, that BMI does not block GABAergic inhibition in *Macrobdella*.

We conclude that the differences between the postsynaptic responses of P cells in *Hirudo* and *Macrobdella* is most likely due to differences in the relative strengths of the chemical synapses. We cannot discount the possibility that the strength of the chemical synapses is the same, but the reversal potential of the inhibitory postsynaptic potential is different in the two species. Regardless of which mechanism is responsible for the differences between species, despite the relatively large depolarization seen in *Macrobdella* P cells, stimulating one P cell does not appear to cause action potentials in other P cells. In *Erpobdella*, the third species where interactions between P cells have been examined, stimulating one P cell to fire five action potentials caused action potentials to occur in the other P cells in roughly 70 % of experimental trials (Baltzley et al. 2010). Importantly, *Erpobdella* does not appear to have any inhibitory connection between the P cells (Baltzley et al. 2010). It is possible that the inhibition seen in *Macrobdella* is responsible for preventing the P cell from reaching action potential threshold, even though the cell is depolarized.

Ultimately, understanding the interactions between neurons is important for understanding how sensory input is manifested as behavioral output. When the body wall of *Hirudo* or *Macrobdella* is stimulated, the animals respond

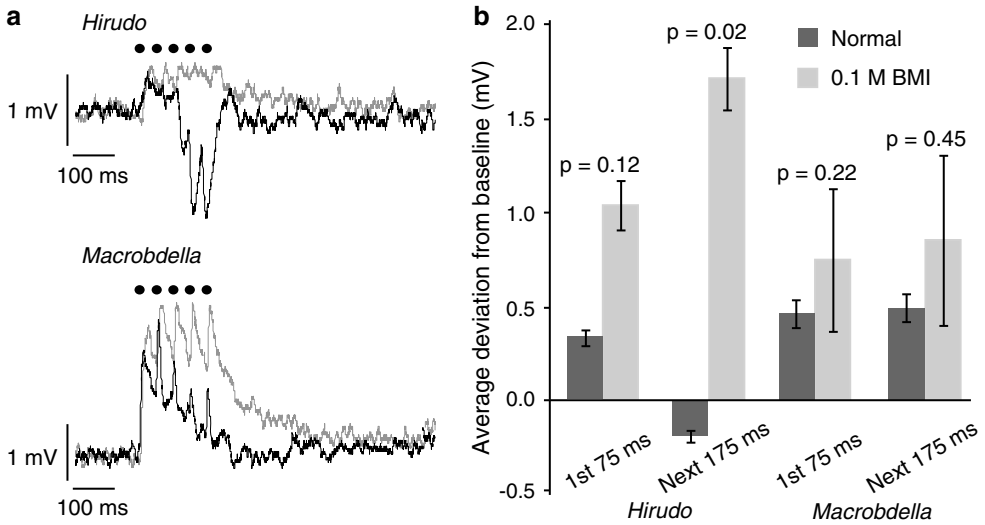


Fig. 5 Characterization of P cell to P cell connections in the presence of 0.1 M bicuculline methiodide (BMI). **a** *Representative trace* of the response of a P cell to intracellular stimulation of another P cell in *Hirudo* (*top traces*) and *Macrobdella* (*bottom traces*). The *black traces* were recorded in normal saline, and the *gray traces* were recorded in 0.1 M BMI. *Black dots* represent the peak of action potentials in the stimulated P cell. **b** The average deviation from baseline of the postsynaptic potential in normal saline and in 0.1 M BMI. *p* values are from paired *t* tests. *Error bars* represent SEM

with a stereotyped behavior called 'local bending' where longitudinal muscles are contracted in the body wall area innervated by the stimulated P cell (Lockery and Kristan 1990a, b). The inhibition between P cells may help localize the site of sensory input, similar to the inhibition seen between sensory neurons in a variety of sensory systems (Fahey and Burkhardt 2003; Nobili et al. 1998; Hill and Blagburn 1998). In contrast, when the body wall of *Erpobdella* is stimulated, the animal contracts longitudinal muscles across the entire segment (Baltzley et al. 2010). This behavioral response is consistent with the mutual excitation of P cells seen in *Erpobdella*.

It seems counterintuitive that two species with conserved behavioral responses would have different synaptic strengths; however, similar results have been found in other species. For example, the nudibranch molluscs *Melibe* and *Dendronotus* have a conserved swimming behavior, and while one pair of homologous central pattern generator interneurons is conserved, a second pair of homologous neurons is part of the central pattern generator in one species, but not the other (Sakurai et al. 2011). While swimming in *Melibe* and *Dendronotus* and the behavioral responses to mechanical stimulation in *Hirudo* and *Macrobdella* are examples of conserved behavior in closely related species, the converse scenario, where different species converge on a similar behavioral pattern, is not uncommon (Berman 1985; Kawasaki 1993; Katz et al. 2001, 2011). For different animals to independently evolve similar behavioral patterns within different nervous systems, there must be multiple successful strategies to produce the same outcome. Therefore, if the same behavioral output can be generated using a variety of cellular parameters, it should not be surprising that the cellular parameters underlying homologous behavioral patterns in closely related species are not tightly constrained evolutionarily.

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Conflict of interest None.

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