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Electrophysiological recording from parasitic nematode muscle

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Abstract

Infection of man and animals with parasitic nematodes is recognized as a significant global problem (McLeod in *Int J Parasitol* 25(11):1363–1367, 1994; Hotez et al. in *N Engl J Med* 357(10):1018–1027, 2007). At present control of these infections relies primarily on chemotherapy. There are a limited number of classes of anthelmintic compounds and the majority of these act on ion-channels of the parasite (Martin et al. in *Parasitology* 113:S137–S156, 1996). In this report, we describe electrophysiological recording techniques as applied to parasitic nematodes. The aim of this report is: (1) to promote the study of ion channels in nematodes to help further the understanding of antinematodal drug action; (2) to describe our recording equipment and experimental protocols; and (3) provide some examples of the information to be gleaned from this approach and how it can increase our understanding of these important pathogens.

Keywords

Anthelmintic; Ion-channel; Patch-clamp; Current-clamp; Voltage-clamp; Electrophysiology

Introduction

Nematode infections are a significant problem in both human (Hotez et al. 2007) and veterinary medicine (McLeod 1994). Chemotherapy is widely used for treating these infections. The range of drugs available for treatment is limited and repeated large scale use has led to the development of drug resistance in numerous parasite species (Kaplan 2004). It is anticipated that the problem of drug resistance will get worse particularly since only one new class of anthelmintic has come to market recently (emodepside). A Consortium on Anthelmintic Resistance SNPS (CARS) has been set up to monitor drug resistance and advance molecular methods for detecting resistance (<http://consortium.mine.nu/cars/pmwiki.php/Main/HomePage>).

The majority of anthelmintic compounds act on the neuromuscular system of the worm, for review see Robertson and Martin (2007). As with any excitable system, ion-channels are central to nematode neuromuscular signaling and function. Here we review the methods we have used to study ion-channels on nematode muscle that are either potential or actual target sites of new and existing compounds. The current anthelmintics that act on nematode ion-channels include: the avermectins/mylbemycins which act on glutamate-gated chloride channels and/or GABA channels; the nicotinic anthelmintics (pyrantel, etc.) gate non-selective cation channels (nicotinic acetylcholine receptors). However, our understanding of the receptors activated during the therapeutic response is incomplete. In addition, there are many other ion-channels

(peptide-gated, potassium and calcium selective channels) that may have critical roles for neuromuscular function in the nematode.

Here we describe the electrophysiological methods we have used to examine nematode ion channels. These techniques are widely used by biologists to study channels in almost every living system and are not specific to our approach. We give details of methods of how we use them to study parasitic nematode ion channels. Our aim is to encourage others to study this important but overlooked field. This report is not intended as an introduction to electrophysiology. It is intended to highlight the small alterations in methodology required to adapt these classical electrophysiological techniques to study currents and channels in parasitic nematodes.

Methods

Nematode tissue

Successful electrophysiological studies require a regular supply of live, viable parasite tissue (Fig. 1). This, in itself, is a common limiting experimental step: many parasitic species cannot easily be maintained for long periods in vitro. We have found *Ascaris suum* can be obtained from the local abattoir, although the ease of collection (related to the incidence of infection in the local swine population) appears to be somewhat seasonal. Adult worms remain viable for 4–7 days when kept at 30–35°C in Locke's solution (mM): NaCl 155; KCl 5; CaCl₂ 2; NaHCO₃ 1.5; D-glucose 5. It is possible, though significantly more labor and cost intensive, to maintain experimental infections of different parasite species; the *Oesophagostomum dentatum* life-cycle can be successfully maintained by passage through pigs (the native host) and will also yield useful adult worms on euthanasia of the hog. An additional benefit of using laboratory infections is the possibility of maintaining specific isolates, e.g. drug resistant isolates, that have less genetic diversity than sampling the wild population. Obtaining viable material from other parasite species (e.g. human pathogens) can be more problematic and may necessitate the studies to be carried out on non-adult life cycle stages or even expression of the ion channel of interest in a heterologous system, e.g. *Xenopus laevis* oocytes.

Dissection

Ascaris are large worms and the dissection needed to expose the muscle cells for recording is simple. A ~1 cm section of the worm is cut from the anterior region of the parasite. The resulting tube is then cut along one of the lateral lines and pinned onto a sylgard lined recording chamber cuticle side down. The gut is easily removed using fine forceps to expose muscle bags. With smaller nematodes the same approach can be applied but this time using the whole length of the worm. For adult *O. dentatum*, the entire worm (~1 cm) is pinned into the chamber (head and tail only) and then cut along a lateral line using a scalpel. The gut and reproductive tissue can then be removed and the preparation pinned out further to reveal the somatic muscle cells. A similar approach has been developed for electrophysiological recording from the muscle cells in *Caenorhabditis elegans* (Richmond and Jorgensen 1999). For *C. elegans*, the small size of the worms means that the pins have been replaced by cyano-acrylate glue, but the principles of sticking the worm down, cutting it open, removing the gut and reproductive tissue and producing a “flap” or “filleted worm” remain the same. Thus, electrophysiological techniques have been applied to worms from > 30 cm to less than 1 mm in size. It should be noted, however, that as the worm size decreases the technical difficulty of the dissection increases substantially.

Two electrode current-clamp

The large size of many nematode cells makes them amenable for study using classical two-electrode recording techniques. The electrophysiology “rig” used for both current-clamp and

voltage-clamp experiments is essentially identical (Fig. 2). The electronic components are: a current/voltage amplifier (Axoclamp 2A or 2B); a digitizer to convert the amplified signals from analog to digital format (digidata 1320A/1322A) and a computer for running the data acquisition software. The computer software (Clampex v8 or v9, Axon Instruments) not only acquires the data but can be used to control the perfusion system and command the amplifier to inject current or voltage through either electrode. The tissue is perfused by a system controlled by six valves, a computer and a Warner VC-6 valve controller. The incoming perfusate is warmed to the desired temperature by a Warner SH-27B inline heater controlled by a Warner TC-324B heater controller. The preparation is viewed using a Stereo zoom dissecting microscope (Bausch & Lomb) and a fiber optic light source. The tissue is mounted in a sylgard lined perspex chamber (custom made) surrounded by a water jacket to maintain temperature. The water jacket is perfused with warm water using a heated water pump (Isotemp 301b, Fisher Scientific). Microelectrodes are mounted on the amplifier headstages and maneuvered into position using a Leica micromanipulator.

For current-clamp experiments we pull microelectrodes using standard walled borosilicate glass with filament, o.d. 1.5 mm, i.d. 0.86 mm (G150F-6, Warner Instruments). Microelectrodes are fabricated using a Flaming/Brown horizontal electrode puller (Model P-97, Sutter Instruments) and are typically pulled to a resistance of 20–30 M Ω . The filament allows easy backfilling of the electrodes with the relevant solution, typically for current-clamp this is 3 M potassium acetate. The recording chamber is mounted on a nitrogen supported anti-vibration table (TMC Corp.) to minimize mechanical noise. A Faraday cage (TMC Corp.) surrounds the recording chamber to reduce electrical noise.

Microelectrodes are positioned directly over the cell to be recorded from. The muscle cell is carefully impaled with both electrodes. Typically resting membrane potentials are in the range –25 to –40 mV for somatic muscle cells in *Ascaris*. The current injecting protocol is then applied through one microelectrode (I_m , Fig. 2d); our standard protocol is 0.5 s pulses of –40 nA current at a frequency of 0.25 Hz. Another microelectrode (V_m , Fig. 2d) can then be used to monitor the membrane potential and also the input conductance of the cell (typically 1–3 μ S). The signal is filtered at 0.3 kHz, digitized and stored on the computer hard drive for later analysis. The effect of perfused drugs can then be monitored. It is possible to record for >1 h from a single cell in a healthy preparation. Our basic recording solution, *Ascaris* Perienteric Fluid (APF) consists of NaCl (23 mM), Na-acetate (110 mM), KCl (24 mM), CaCl₂ (6 mM), MgCl₂ (5 mM), glucose (11 mM), HEPES (5 mM), pH 7.6, adjusted with NaOH, and can be modified when necessary to determine the ionic basis of drug effects.

Two electrode voltage-clamp

The electrophysiology “rig” for two-electrode voltage clamp is identical to that used for current-clamp experiments. However, there are some small but significant changes required to perform successful voltage-clamp experiments. Firstly, in electrode manufacture, the large size of the *Ascaris* muscle cell means that space clamp is quite poor. The injection of the large currents required to effect the desired voltage change requires a lower resistance current injecting electrode. Typically for voltage-clamp experiments we use a current injecting electrode (I_m) with a resistance of 2–5 M Ω . This is easily achieved by carefully breaking the tip of a standard current-clamp electrode using a piece of tissue paper. The voltage sensing electrode (V_m) is a standard current-clamp electrode.

Secondly, in voltage-clamp experiments, it is desirable to investigate the current flow through specific ion channel types or currents carried by individual ion species, e.g. outward K currents or inward Ca currents. To this end it is desirable to eliminate, as much as possible, currents carried by other ions and channels. Traditionally, this is achieved by either elimination/substitution of ions (other than the ion of interest) from recording solutions or by

pharmacological block of other channel types present. For example, to record voltage activated inward calcium currents we have added cesium to the pipette filling solution (intracellular Cs blocks potassium currents, electrode fill solution is 1.5 M Cesium acetate +1.5 M potassium acetate) and 4-amino pyridine (4-AP) to the bathing solution (4-AP is a selective blocker of K channels). Conversely, we have found that voltage-activated outward potassium currents are more easily studied when calcium is substituted for magnesium in the bathing medium, thus eliminating voltage activated inward calcium currents. It is also possible to isolate a current of interest by varying the voltage changes applied to the cell.

Isolating and optimizing the current to be studied is often the most demanding and time consuming aspect of these experiments. Unfortunately, parasitic nematodes are not the most widely studied group of organisms and drugs that affect ion channels in other preparations have been found to be inactive or significantly less active on *Ascaris* muscle cells. For example, the calcium channel blocker verapamil is frequently used to eliminate certain types of calcium current in vertebrate preparations, thus facilitating the study of other current types. Unfortunately, in *Ascaris* verapamil has no significant effect on voltage gated inward currents.

Single-channel patch-clamp

The majority of parasitic nematode cell types we have worked with are too large to render whole cell patch-clamp recording a viable option; so we use two-electrode techniques. Whole cell patch recording has been successfully developed for investigating the muscle cells of *C. elegans* (Richmond and Jorgensen 1999) and is not described further here. It should be noted, however, that this approach may be suitable for the study of smaller nematode cells where impalement with two sharp electrodes is not possible.

It is possible to use the patch-clamp technique to measure the properties of individual ion channel molecules, this “single-channel” patch recording technique is relatively straightforward using parasitic nematode muscle cells. The principle of this technique is the electrical isolation of a small “patch” of membrane containing one (or very few) ion channel molecules. Then conventional voltage protocols are applied to the membrane patch and the opening and closing of the single channel molecule can be measured.

The principles behind this technique are straightforward but in practice this is probably the most technically demanding compared to the other approaches outlined in this review. For single-channel recording from nematode muscle we use an anti-vibration table and Faraday cage (TMC Corp.) as in the current-clamp “rigs”. The amplifier is an Axopatch 200B (Axon Instruments) connected to a PC (Dell) via a digitizer (Digidata 1320A/1322A) and controlled by Clampex (v8 or v9) data acquisition software (Axon Instruments). Nematode muscle cells or muscle cell derived vesicles are held in a recording chamber (Warner Instruments) and viewed through a Nikon TE2000 inverted light microscope at $\times 400$ magnification. Vesicles are easily viewed under normal light but small *C. elegans* muscle cells are best viewed using DIC optics. The amplifier headstage and microelectrode are positioned using a Narishige (MHW-3, Narishige Inc.) hydraulic micromanipulator.

Microelectrodes for patch clamp studies are pulled from thin walled glass capillaries, o.d. 1.5 mm, i.d. 1.16 mm with no filament (G85150T-3, Warner Instruments) using a two stage vertical electrode puller (models PP-830 or PC-10, Narishige Inc.). Electrodes are coated close to the tip with Sylgard to improve frequency responses and fire polished (MF-900 micro-forge, Narishige Instruments) to the desired resistance, typically 2–5 M Ω .

A major requirement for successful patch-clamp experiments is the formation of a high resistance seal (>1 G Ω , a giga seal) between the glass microelectrode and the cell membrane. Giga seal formation requires clean debris free membranes, which are reasonably common in

cells in tissue culture but less so in intact tissue. *Ascaris* and other nematodes have a large amount of collagen overlying the muscle cell preventing giga seal formation. This must be removed by enzyme treatment using collagenase (type 1A, Sigma). Collagenase treatment removes the collagen matrix and allows access of the patch pipette to clean muscle cell membranes. One result of collagenase treatment is the “budding” off of clean membrane vesicles from the bag region of the muscle cells. By applying the patch clamp technique Martin et al. (1990) discovered that these membrane vesicles contain functional ion-channels. We have successfully applied this method to record ion channels from vesicles originating from *O. dentatum* muscle cells (Robertson et al. 1999). Details of vesicle preparation and recording protocols are given below.

Ascaris were dissected and a muscle flap was prepared and pinned cuticle side down onto a plastic dish lined with Sylgard. The muscle flap preparation was washed with maintenance solution to remove fragments of the gut. Maintenance solution is (in mM): 35 NaCl, 105 sodium acetate, 2.0 KCl, 2.0 MgCl₂, 10 HEPES, 3.0 D-glucose, 2.0 ascorbic acid, 1.0 EGTA, pH 7.2 with NaOH. The maintenance solution was then replaced with collagenase solution. Collagenase solution is maintenance solution without EGTA and with 1 mg/ml collagenase Type 1A added (Sigma). After collagenase treatment for 4–8 min at 37°C, the muscle preparation was washed (5–10 times) and incubated in maintenance solution at 37°C for 20–40 min. Small membranous vesicles, 10–50 µm in diameter, grew out from the membrane of the muscle cells. These membranous vesicles are transferred to a recording chamber using a glass Pasteur pipette. For *O. dentatum* the vesicle preparation protocol is unchanged, however, the yield of vesicles is significantly less due to the smaller size of the parasite. We have found that vesicle yield and quality can vary significantly between batches of worms and worms of different size. As a guide, smaller worms require less collagenase treatment than larger ones. Prolonged collagenase treatment yields an abundance of vesicles but they are more fragile and rapidly become unusable. Shorter collagenase treatment yields fewer vesicles but they are generally more robust. For worms as small as *C. elegans* the collagen matrix is significantly less of a problem and collagenase treatments of 0.5 mg/ml for 5–10 s are adequate to clean the muscle cell membrane and allow seal formation directly from the body wall muscle cells. Finally, we have found that collagenase from different suppliers or even different batches from the same supplier can affect the quality of vesicles produced.

Vesicles are placed in the recording chamber and patch experiments are carried out in the isolated inside out patch configuration. Achieving the outside-out patch configuration is considerably more difficult when using membrane vesicles as they tend to implode when rupturing the patch membrane. The recording conditions for studying nAChR channels are given below. Voltage protocols and solution recipes can be altered depending on the ion-channel to be studied.

The pipette was filled with pipette solution containing (mM): CsCl, 140; MgCl₂, 2; HEPES, 10; EGTA, 1; pH 7.2 with CsOH. The pipette solution also contained the agonist (levamisole, acetylcholine, etc.) at the desired concentration. The bathing solution was (mM): CsCl, 35; Cs acetate, 105; MgCl₂, 2; HEPES, 10; EGTA, 1; pH 7.2 with CsOH. As in other voltage clamp experiments, it is desirable to isolate the specific ion-channel of interest. To this end the bathing solutions contained symmetrical Cs as it permeates the nAChR but blocks potassium channels. The chloride concentration was asymmetrical to identify contaminating chloride channels by their non-zero reversal potentials on later analysis. Calcium is absent from the solutions to prevent contamination of the recordings with Ca-dependent chloride channel openings. Typically for ligand-gated ion channels we record for approximately 1 min at several different holding potentials between –100 and +100 mV (normally, –100, –75, –50, +50, +75 and +100 mV). Membrane breakdown is common at both –100 and +100 mV. In some preparations, we have found that addition of 0.5 mM dithiothreitol helps to stabilize the membrane at more

extreme potentials (Robertson et al. 1999). Recordings are viewed in real time by filtering at 2.5 kHz (8-pole Besel filter, custom made) and viewing on a digital storage oscilloscope (Hitachi VC-6025). The recordings are also filtered by the amplifier (5 KHz, Besel filter) digitized and stored on the PC for later analysis.

As with all recordings made using the above methods the data generated is suitable for analysis using standard methods. In the case of nAChR single-channel currents we normally calculate the single-channel conductance, mean open-time, mean closed-times and the probability of the channel being in the open state (P_{open}). Other more complex single-channel analysis is possible but beyond the scope of this manuscript.

Results

Examples of the type of data available from each of our experimental approaches are given below. The data in this section was obtained from *Ascaris* somatic muscle.

Illustrative results using two electrode current-clamp

Figure 3 is a current-clamp recording from *Ascaris* somatic muscle. Figure 3a is a low time resolution display covering approximately 30 min. The blue arrow (dark gray) indicates the resting membrane potential of the cell (-37 mV in this experiment). The red arrow (light gray) shows the voltage response to the -40 nA injected current pulses. The size of the voltage response is inversely related to the input conductance of the cell. The size of the response increases as the conductance decreases (when ion channels close) and vice versa. Figure 3a clearly demonstrates that levamisole application induces a rapid depolarization. When the trace is examined in more detail (Fig. 3b, c) the effect on the cell's conductance also becomes apparent. In Fig. 3b, c, the red arrow (light gray) again represents the response to injected current and the blue arrow (dark gray) this time represents the depolarization induced by levamisole. In Fig. 3b the depolarization induced by levamisole (blue arrow, dark gray) is clearly seen. Levamisole is an agonist of the nicotinic acetylcholine receptor (nAChR) ion channel, application of the drug causes these channels to open and cations to enter the cell thus causing the depolarization. The opening of the ion channels causes an increase in input conductance during the depolarization. The red arrow highlights the voltage response to injected current and at the peak of the depolarization this response is reduced, reflecting the conductance increase due to nAChR opening. Figure 3b is the levamisole response after a 2 min application of the neuropeptide AF2 (1 μ M). It is apparent that both the levamisole induced depolarization (blue arrow) and conductance change are substantially increased by treatment with this peptide. Figure 3a also demonstrates that AF2 treatment prolongs the recovery time after levamisole treatment.

Illustrative results using two electrode voltage-clamp

A sample experiment using two electrode voltage-clamp recording on *Ascaris* muscle is shown in Fig. 4. In this experiment, we have isolated the voltage gated potassium currents and examined the effects of the potassium channel blocker 4-amino pyridine (4-AP). To study the potassium currents in isolation we have replaced calcium (a permeant ion) in our recording solutions with the same concentration of magnesium (an impermeant ion) to remove the voltage activated inward currents carried by calcium. Figure 4a are the outward currents carried by potassium in response to 40 ms step voltage changes in the holding potential of the cell. In this instance, the cell was held at -35 mV and stepped to -25 , -20 , -15 , -10 , -5 , 0 , 5 , 10 , 15 , and 20 mV. The same voltage step protocol was applied in the presence of 5 mM 4-AP (Fig. 4b) which substantially reduced the amplitude of the outward potassium currents. After a 30-min wash period the currents had partially recovered (Fig. 4c). The maximum current at each

voltage step was plotted (Fig. 4d) and clearly shows the inhibitory effect of 4-AP and this effect was partially reversible.

Illustrative results using single-channel patch-clamp

A sample of a recording from an *Ascaris* muscle derived vesicle is shown in Fig. 5a. The isolated inside-out patch was held at +75 mV and the patch pipette contained 30 μ M levamisole. Rectangular channel openings are clearly visible ranging from ~2 to 4 pA in size and ~0.3 to 10 ms in duration. In this experiment, there are openings to more than one level indicating the presence of multiple subtypes of nAChR present in this isolated patch of membrane. In Fig. 5b, we plotted an amplitude histogram of all openings in the recording and fitted with gaussian distributions to calculate the mean amplitude for each of the three peaks. By using multiple agonists, concentrations and antagonists we have been able to characterize three subtypes of nAChR on *Ascaris* muscle cells that have different single-channel and pharmacological properties. Figure 6 is a summary diagram of these findings where N-type refers to a nicotine preferring subtype of nAChR, L-type refers to a levamisole preferring subtype of nAChR and B-type refers to a buprenorphine preferring subtype of nAChR.

Discussion

The development of electrophysiological methods has taken >50 years to mature. Early studies concentrated on large easily observable cells that were easy to impale, e.g. the squid giant axon. Interestingly, *Ascaris suum* muscle cells were investigated as early as the 1950s (Jarman 1959). As the techniques were refined the need for large cells decreased. Additionally, Brading and Caldwell (1971) found that *Ascaris* had different properties to other more typical cell types. These developments possibly led to the conclusion that *Ascaris* was not necessarily a good model for general electrophysiology studies of cells and have thus restricted the amount of research carried out on this and other parasitic nematodes using electrophysiological techniques.

We have described some of the electrophysiological methods that can be used to study ion-channels in *Ascaris* and other nematodes. Included in the methods section are additional details that we have found important for successful studies. Details that are seldom discussed at length in other publications due to space constraints. The aim of this report is to provide detailed information to facilitate the study of ion channels in parasitic nematodes by any interested researchers.

The importance of studying these parasite ion-channels is readily apparent. There are a number of groups of anthelmintic compound that act on channels in parasites. These include: the cholinomimetics (pyrantel, etc.) that act as agonists of nAChRs on muscle (Harrow and Gratton 1985); the avermectins are allosteric activators of glutamate-gated chloride channels in the pharynx (Wolstenholme and Rogers 2005) and/or GABA-gated chloride channels on muscle; piperazine an agonist of GABA-gated chloride channels on muscle (Martin 1982); emodepside is proposed to have an effect on potassium currents (Guest et al. 2007); and recently the amino acetonitrile derivatives (AADs) are proposed to be nAChR antagonists (Kaminsky et al. 2008).

We have detailed our approaches on nematode muscle. Several other groups have successfully used electrophysiological techniques in a variety of preparations including the musculature (Holden-Dye and Walker 1990) to examine ion-channel properties, drug action and more basic biological questions. The pharynx of *Ascaris* has been investigated using whole cell current-clamp (Martin 1996) and voltage-clamp (Byerly and Masuda 1979), while Adelsberger et al. (1997) successfully developed vesicle production from the pharynx to make patch recordings of glutamate-gated chloride channels. The electrophysiological properties of parasite nerve

cells have also been investigated in detail (Davis and Stretton 1996). While more recent work on *C. elegans* has developed techniques for recording whole cell currents from body wall muscle (Richmond and Jorgensen 1999), single-channel recording of nAChRs from body wall muscle (Qian et al. 2008) and even electrical recording of pharyngeal activity (Cook et al. 2006).

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References

- Adelsberger H, Scheuer T, Dudel J. A patch clamp study of a glutamatergic chloride channel on pharyngeal muscle of the nematode *Ascaris suum*. *Neurosci Lett* 1997;230:183–186. [PubMed: 9272691]doi: 10.1016/S0304-3940(97)00512-0
- Brading AF, Caldwell PC. The resting membrane potential of the somatic muscle cells of *Ascaris lumbricoides*. *J Physiol* 1971;217(3):605–624. [PubMed: 5098084]
- Byerly L, Masuda MO. Voltage-clamp analysis of the potassium current that produces a negative-going action potential in *Ascaris* muscle. *J Physiol* 1979;288:263–284. [PubMed: 469718]
- Cook A, Franks CJ, Holden-Dye L. Electrophysiological recordings from the pharynx. *WormBook* 2006;17:1–7. [PubMed: 18050442]
- Davis RE, Stretton AO. The motoneuronal system of *Ascaris* electrophysiology and anatomy of the neurons and their control by neuromodulators. *Parasitology* 1996;113:S97–S117. [PubMed: 9051930]
- Guest M, Bull K, Walker RJ, Amliwala K, O'Connor V, Harder A, Holden-Dye L, Hopper NA. The calcium-activated potassium channel, SLO-1, is required for the action of the novel cyclo-octadepsipeptide anthelmintic, emodepside, in *Caenorhabditis elegans*. *Int J Parasitol* 2007;37(14):1577–1588. [PubMed: 17583712]doi: 10.1016/j.ijpara.2007.05.006
- Harrow ID, Gratton KAF. Mode of action of the anthelmintics morantel, pyrantel and levamisole in the muscle cell membrane of the nematode *Ascaris suum*. *Pestic Sci* 1985;16:662–672.doi: 10.1002/ps.2780160612
- Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, Sachs JD, Savioli L. Control of neglected tropical diseases. *N Engl J Med* 2007;357(10):1018–1027. [PubMed: 17804846]doi: 10.1056/NEJMra064142
- Holden-Dye L, Walker RJ. Avermectin and avermectin derivatives are antagonists at the 4-aminobutyric acid (GABA) receptor on the somatic muscle cells *Ascaris*—is this the site of anthelmintic action? *Parasitology* 1990;101:265–271. [PubMed: 2175874]
- Jarman M. Electrical activity in the muscle cells of *Ascaris lumbricoides*. *Nature* 1959;184:1244. [PubMed: 14406817]doi:10.1038/1841244a0
- Kaminsky R, Ducray P, Jung M, Clover R, Rufener L, Bouvier J, Weber SS, Wenger A, Wieland-Berghausen S, Goebel T, Gauvry N, Pautrat F, Skripsky T, Froelich O, Komoin-Oka C, Westlund B, Sluder A, Mäser P. A new class of anthelmintics effective against drug-resistant nematodes. *Nature* 2008;452(7184):176–180. [PubMed: 18337814]
- Kaplan RM. Drug resistance in nematodes of veterinary importance: a status report. *Trends Parasitol* 2004;20(10):477–481. [PubMed: 15363441]doi:10.1016/j.pt.2004.08.001
- Martin RJ. Electrophysiological effects of piperazine and diethylcarbamazine on *Ascaris suum* somatic muscle. *Br J Pharmacol* 1982;77:255–265. [PubMed: 7139188]
- Martin RJ. An electrophysiological preparation of *Ascaris suum* pharyngeal muscle reveals a glutamate-gated chloride channel sensitive to the avermectin analogue, milbemycin D. *Parasitology* 1996;112(Pt 2):247–252. [PubMed: 8851865]
- Martin RJ, Kusel JR, Pennington AJ. Surface properties of membrane vesicles prepared from muscle cells of *Ascaris suum*. *J Parasitol* 1990;76(3):340–348. [PubMed: 1693673]doi:10.2307/3282663
- Martin RJ, Valkanov MA, Dale MVE, Robertson AP, Murray I. Electrophysiology of *Ascaris* muscle and anti-nematodal drug action. *Parasitology* 1996;113:S137–S156. [PubMed: 9051932]

- McLeod RS. Costs of major parasites to the Australian livestock industries. *Int J Parasitol* 1994;25(11): 1363–1367. [PubMed: 8635886]doi: 10.1016/0020-7519(95)00071-9
- Qian H, Robertson AP, Powell-Coffman JA, Martin RJ. Levamisole resistance resolved at the single-channel level in *Caenorhabditis elegans*. *FASEB J* 2008;22(9):3247–3254. [PubMed: 18519804] doi: 10.1096/fj.08-110502
- Richmond JE, Jorgensen EM. One GABA and two acetylcholine receptors function at the *C. elegans* neuromuscular junction. *Nat Neurosci* 1999;2:791–797. [PubMed: 10461217]doi:10.1038/12160
- Robertson AP, Martin RJ. Ion-channels on parasite muscle: pharmacology and physiology. *Invert Neurosci* 2007;7(4):209–217. [PubMed: 17999098]doi: 10.1007/s10158-007-0059-x
- Robertson AP, Bjorn HE, Martin RJ. Resistance to levamisole resolved at the single-channel level. *FASEB J* 1999;13:749–760. [PubMed: 10094935]
- Wolstenholme AJ, Rogers AT. Glutamate-gated chloride channels and the mode of action of the avermectin/mylbemycin anthelmintics. *Parasitology* 2005;131:S85–S95. [PubMed: 16569295]doi: 10.1017/S00 31182005008218

Fig. 1.
a Photograph of adult *Ascaris suum*; **b** photograph of muscle flap preparation showing muscle cell bags (~200 μm diameter) suitable for two-electrode recording techniques. The *faint horizontal line* is the ventral nerve cord in this preparation

Fig. 2.

a Photograph; **b** diagram of two electrode current-clamp “rig”; **c** photograph and **d** diagram of the recording chamber for current-clamp experiments. The muscle flap is clearly seen with both microelectrodes visible. The perfusate is applied via a 20-gauge needle (*gray arrow* in diagram) and excess removed by gravity through the outflow on the bottom right of the photograph and diagram (*gray circle*)

Fig. 3.

a Low time resolution current-clamp trace illustrating the effects of 20 s applications (asterisk) of 1 μ M levamisole (4 ml/min flow rate) before and after treatment of the muscle flap with 1 μ M AF2 (a nematode FMRF-related neuropeptide). Blue arrow illustrates the resting membrane potential while the red arrow illustrates the size of the voltage response to -40 nA injected current. Levamisole induces an obvious depolarization of the cell; **b**, **c** higher time resolution view of sections of the recording in **a**. Red arrow (light gray) illustrates the voltage response to injected current and blue arrow (dark gray) illustrates the amplitude of levamisole induced depolarization. It can be clearly seen that both the depolarization and conductance change in response to levamisole are larger after AF2 treatment (**c**) than before (**b**)

Fig. 4.

Voltage activated potassium currents from *Ascaris* muscle bags recorded under two electrode voltage-clamp; **a** under control conditions (Ca free APF solution); **b** during application of 5 mM 4-amino pyridine (4-AP); and **c** after 30 min wash in calcium free APF solution; **d** current-voltage relationship for the recordings in **a-c** clearly showing the inhibitory effect of 4-AP and that it is partially reversed on washing

Fig. 5.

a Sample of a single-channel recording from a membrane patch of *Ascaris* muscle vesicle held at +75 mV. Discrete single-channel openings are visible as rectangular current pulses of ~2–4 pA. *Blue asterisk* highlight the presence of three separable open levels and therefore three different ion channel molecules in this membrane patch; **b** histogram of all channel openings from the recording illustrated in **a**. Three separable peaks are obvious and have been fitted using Gaussian distributions to determine the amplitude of channel opening at +75 mV for each channel type

Fig. 6.
Summary diagram representing a membrane patch containing the three nAChR subtypes present on *Ascaris* muscle with some of their single-channel and pharmacological properties illustrated