Cestode larvae excite host neuronal circuits via glutamatergic signaling.

Hayley Tomes^{*1,2}, Anja de Lange^{*1,2}, Ulrich Fabien Prodjinotho³, Siddhartha Mahanty⁴, Katherine Smith^{2,5}, William Horsnell^{2,6,7}, Chummy Sikasunge⁸, Clarissa Prazeres da Costa³, Joseph Valentino Raimondo^{1,2}

*Authors contributed equally

¹Division of Cell Biology, Department of Human Biology, Neuroscience Institute and Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

²Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

³Institute for Medical Microbiology, Immunology and Hygiene, Center for Global Health, Technical University Munich (TUM), Munich, Germany

⁴Department of Medicine, The Peter Doherty Institute for Infection and Immunity and the Victorian Infectious Diseases Service, University of Melbourne, Melbourne, Australia

⁵Institute of Infection and Immunity, Cardiff University, Cardiff, UK

⁶Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom

⁶Laboratory of Experimental and Molecular Immunology and Neurogenetics (INEM), UMR 7355 CNRS-University of Orleans, Orleans, France

⁸School of Veterinary Medicine, Department of Paraclinicals, University of Zambia, Lusaka, Zambia

Correspondence:

Joseph V. Raimondo

email: joseph.raimondo@uct.ac.za

Keywords

Cestode, Taenia crassiceps, Taenia solium, neurocysticercosis, glutamate, seizures, epilepsy

Acknowledgements

Dr Philip Fortgens: Division of Chemical Pathology, Department of Pathology, University of Cape Town and National Health Laboratory Service (affiliation when the analyses were done). Current affiliation: Dietrich, Voigt, Mia & Partners (PathCare) and honorary senior lecturer. Research leading to these results. The research leading to these results has received funding from a Royal Society Newton Advanced Fellowship (NA140170) and a University of Cape Town Start-up Emerging Researcher Award to JVR and grant support from the Blue Brain Project, the National Research Foundation of South Africa, Wellcome Trust and the FLAIR Fellowship Programme (FLR\R1\190829): a partnership between the African Academy of Sciences and the Royal Society funded by the UK Government's Global Challenges Research Fund. CS, UFP and CPdC were supported by the Federal Ministry of Education and Research of Germany (BMBF), Project title: "CYSTINET-Africa" (01KA1610, Germany II). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Neurocysticercosis (NCC) is caused by the presence of *Taenia solium* larvae in the brain and is the leading cause of adult-acquired epilepsy worldwide. However, little is known about how seizures emerge in NCC. To address this knowledge gap we used whole-cell patch-clamp electrophysiology and calcium imaging in rodent hippocampal organotypic slice cultures to identify direct effects of cestode larval products on neuronal activity. We found both whole cyst homogenate and excretory/secretory (E/S) products of *Taenia* larvae have an acute excitatory effect on neurons, which trigger seizure-like events *in vitro*. Underlying this effect was *Taenia*-induced neuronal depolarization, which was mediated by glutamate receptor activation but not by nicotinic acetylcholine receptors, acid-sensing ion channels nor Substance P. Glutamate assays revealed the homogenate of both *Taenia crassiceps* and *Taenia solium* larvae contained high concentrations of glutamate and that larvae of both species consistently produce and release this excitatory neurotransmitter into their immediate environment. These findings contribute towards the understanding of seizure generation in NCC.

64 *Author summary*

65 Brain infection by larvae of the tapeworm *Taenia solium* (neurocysticercosis or NCC) is the leading
 66 cause of acquired epilepsy in adulthood. Little is understood about the mechanisms by which larvae
 67 cause seizures. To address this, we used electrophysiological and imaging techniques in rodent brain
 68 slices to investigate how tapeworm larvae directly impact neuronal function. We discovered that both
 69 the homogenate and secretory products of tapeworm larvae excite neurons and can trigger seizure-
 70 like events in brain slices. This effect was caused by the activation of glutamate receptors and not by
 71 activating other types of receptors in the brain. Finally, we observed that tapeworm larvae both
 72 contain and release the neurotransmitter glutamate into their immediate environment. These findings
 73 are relevant for understanding how tapeworm larvae cause seizures in NCC.

74

Introduction

Neurocysticercosis (NCC) is the most prevalent parasitic infection of the central nervous system (CNS) (1,2). It is caused by the presence of *Taenia solium* (*T. solium*) larvae in the brain (3). The most common symptom of NCC is recurrent seizures (4). As a result, NCC is the leading cause of adult-acquired epilepsy worldwide (5), resulting in significant morbidity and mortality. In endemic areas, approximately 29% of people with epilepsy also have NCC (6). Despite the impact of NCC, there are a paucity of studies investigating the seizure mechanisms involved (7). As a result, precisely how larvae are involved in seizure generation is still relatively poorly understood.

In NCC seizures may occur at any stage following initial infection (4). It is thought that inflammatory processes in the brain can play an important role in the development of recurrent seizures (8). Previous work exploring seizure development in NCC has therefore typically focused on how the host neuroinflammatory response to larvae might precipitate seizures (7,9). Robinson *et al.* found, for example, that production of the inflammatory molecule and neurotransmitter, Substance P, produced by peritoneal larval granulomas, can precipitate acute seizures (10). In addition to host-derived substances, cestodes themselves are known to excrete or secrete various products that interact with host cells in their vicinity. Cestode larvae derived factors are known to modulate the activation status of immunocytes such as microglia and dendritic cells (11,12). However, comparatively little is known about how factors contained in, or secreted by, cestode larvae might affect neurons and neuronal networks directly, including whether these may have pro-seizure effects.

To address this, we used whole-cell patch-clamp recordings and calcium imaging in rodent hippocampal organotypic slice cultures to demonstrate the direct effects of larval products on neuronal activity. We find that both the whole cyst homogenate and the excretory/secretory (E/S) products of *Taenia crassiceps* (*T. crassiceps*) larvae have a strong, acute excitatory effect on neurons. This was sufficient to trigger seizure-like events *in vitro*. Underlying seizure induction was *T. crassiceps*

101 larval induced neuronal depolarization, which was mediated by glutamate receptor activation and not
 102 nicotinic acetylcholine receptors, acid-sensing ion channels nor Substance P. Direct measurement of
 103 glutamate concentration revealed that the homogenate of both *T. crassiceps* and *T. solium* larvae
 104 contain high levels of glutamate. Lastly, we provide evidence that larvae of both species consistently
 105 produce and release this excitatory neurotransmitter into their immediate environment; suggesting
 106 that parasite released glutamate directly contributes to NCC seizures.

107

Materials and Methods

Ethics statement

All animal handling, care and procedures were carried out in accordance with South African national guidelines (South African National Standard: The care and use of animals for scientific purposes, 2008) and with approval from the University of Cape Town Animal Ethics Committee (Protocol No: AEC 015/015, AEC 014/035).

Taenia maintenance and preparation of whole cyst homogenates and E/S products

Larvae of *T. crassiceps* (ORF strain) were donated to us by Dr Siddhartha Mahanty (University of Melbourne, Melbourne, Australia) and propagated *in vivo* by serial intraperitoneal infection of 5-8 week old female C57BL/6 mice. Every 3 months parasites were harvested by peritoneal lavage and washed 6 times in phosphate buffered saline (PBS, 1X, pH 7.4; Sigma-Aldrich) before further processing.

For the preparation of *T. crassiceps* whole cyst homogenate, larvae were stored immediately after harvesting at -80 °C. Later these were thawed (thereby lysing the cells) and suspended in PBS (1X) containing a protease cocktail inhibitor (1% vol/vol, Sigma-Aldrich) at a larval:PBS ratio of 1:3. The larvae were then homogenised on ice using a Polytron homogenizer (Kinematica). The resulting mixture was centrifuged at 4000rpm for 20 minutes at 4 °C. The liquid supernatant (between the white floating layer and solid pellet) was collected and sterile filtered through a 0.22 µm size filter (Millex-GV syringe filter, Merck). This supernatant was then aliquoted and stored at -80 °C until use. To assess whether large or small molecules were responsible for the excitation of neurons, a portion of the whole cyst homogenate was dialysed using a Slide-A-Lyzer™ dialysis cassette (3kDa MWCO, Separations) in 2l of artificial cerebro-spinal fluid (aCSF) at 4 °C. The aCSF solution was changed twice over 24 hours. To determine the ionic composition of *T. crassiceps* whole cyst homogenate a Cobas

6000 analyser (Roche) was used, with ion specific electrodes for K⁺ and Na⁺. A Mettler Toledo SevenCompact™ pH meter S210 (Merck) was used to determine the pH of the homogenate.

For the preparation of *T. crassiceps* E/S products, after harvesting, washed larvae were maintained for 14-21 days at 37°C in 5% CO₂ in 50ml tissue culture flasks (approximately 7ml volume of larvae per flask) containing culture medium consisting of Earle's Balanced Salt Solution (EBSS) supplemented with glucose (5.3 g/liter), Glutamax (1X), penicillin (50 U/ml), streptomycin (50 ug/ml), gentamicin sulphate (100 ug/ml) and nystatin (11.4 U/ml) (Sigma-Aldrich). Culture medium was replaced after 24 hours and the original media discarded. Thereafter, culture media were replaced and collected every 48-72 hours, stored at -20 °C and at the end of the culture period, thawed and pooled. The pooled conditioned medium was termed total excretory/secretory products (total E/S). A portion of the total E/S was aliquoted and stored at -80 °C, while another portion was concentrated (about 100X) and buffer exchanged to PBS using an Amicon stirred cell with a 3 kDa MWCO cellulose membrane (Sigma-Aldrich). The concentrated product contained *T. crassiceps* E/S products larger in size than 3kDa (E/S > 3kDa), in PBS (1X). This was aliquoted and stored at -80 °C. The fraction of the total E/S that passed through the 3kDa membrane (E/S < 3 KDa, still in medium) was also retained, aliquoted and stored at -80 °C.

For the preparation of *T. solium* whole cyst homogenate, larvae of *T. solium* were harvested from the muscles of heavily infected, freshly slaughtered pig. After extensive washing with sterile 1X phosphate buffered saline (PBS; pH 7.4; Sigma-Aldrich), *T. solium* larvae were suspended in PBS containing phenylmethyl-sulphonyl fluoride (5mM) and leupeptin (2.5 µM) at a larval:PBS ratio of 1:3. Larvae were then homogenised using a sterile hand held homogenizer at 4 °C. The resulting homogenate was sonicated (4 x 60 s at 20 kHz, 1 mA, with 30 s intervals) and gently stirred with a magnetic stirrer for 2h at 4°C. Thereafter it was centrifuged at 15,000 g for 60 min at 4 °C and the liquid supernatant (between the white floating layer and solid pellet) was collected. The supernatant was filtered through 0.45 µm size filters (Millex-GV syringe filter, Merck), aliquoted and stored at -80 °C. All *T. crassiceps*

and *T. solium* larval products were assessed for protein concentration using a BCA protein or Bradford's assay kit respectively.

For the assessment of daily glutamate production both *T. crassiceps* and *T. solium* larvae were placed into 6 well plates (+/- 15 per well, of roughly 5mm length) with 2 ml of culture medium (*Taenia solium* medium: RPMI 1640 with 10 mM HEPES buffer, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml amphotericin B and 2 mM L-glutamine; *Taenia crassiceps* medium: EBSS with 100 U/ml penicillin, 100 µg/ml streptomycin, 11.4 U/ml nystatin and 2 mM glutamax). Every 24 hours 1 ml of culture media were collected from each well, stored at -80 °C, and replaced with fresh culture medium. The concentration of glutamate was measured using a Glutamate assay kit according to the supplier's instructions (Sigma-Aldrich).

Hippocampal brain slice preparation

Organotypic brain slices were prepared using 6-8 day old Wistar rats and C57BL/6 mice following the protocol originally described by Stoppini *et al.*, (1991). Brains were extracted and swiftly placed in cold (4°C) dissection media consisting of Geys Balanced Salt Solution (Sigma-Aldrich) supplemented with D-glucose (Sigma-Aldrich). The hemispheres were separated and individual hippocampi were removed and immediately cut into 350 µm slices using a McIlwain tissue chopper (Mickle). Cold dissection media was used to rinse the slices before placing them onto Millicell-CM membranes (Sigma-Aldrich). Slices were maintained in culture medium consisting of 25 % (vol/vol) Earles balanced salt solution (Sigma-Aldrich); 49 % (vol/vol) minimum essential medium (Sigma-Aldrich); 25 % (vol/vol) heat-inactivated horse serum (Sigma-Aldrich); 1 % (vol/vol) B27 (Invitrogen, Life Technologies) and 6.2 g/l D-glucose. Slices were incubated in a 5 % carbon dioxide (CO₂) humidified incubator at between 35 – 37 °C. Recordings were made after 6-14 days in culture.

Electrophysiology and calcium imaging

Brain slices were transferred to a submerged recording chamber on a patch clamp rig, which was maintained at a temperature between 28 and 30 °C, and were continuously superfused with standard aCSF bubbled with carbogen gas (95 % O₂: 5 % CO₂) using peristaltic pumps (Watson-Marlow). The standard aCSF was composed of: NaCl (120 mM); KCl (3 mM); MgCl₂ (2 mM); CaCl₂ (2 mM); NaH₂PO₄ (1.2 mM); NaHCO₃ (23 mM) and D-Glucose (11 mM) in deionised water with pH adjusted to between 7.35 - 7.40 using 0.1 mM NaOH (Sigma-Aldrich). Neurons in the CA3 region of the hippocampus were visualized using a Zeiss Axioskop or Olympus BX51WI upright microscope using 20x or 40x water-immersion objectives and targeted for recording. Micropipettes were prepared (tip resistance between 3 and 7 MΩ) from borosilicate glass capillaries (outer diameter 1.2 mm, inner diameter 0.69 mm, Harvard Apparatus Ltd) using a horizontal puller (Sutter). Recordings were made in current clamp mode using Axopatch 200B amplifiers (Axon Instruments) and data acquired using WinWCP (University of Strathclyde) or Igor (Markram Laboratory, Ecole polytechnique fédérale de Lausanne). Matlab (MathWorks) was utilised for trace analysis. Two internal solutions were used: a “standard” internal solution (K-gluconate (126 mM), KCl (4 mM), HEPES (10 mM) Na₂ATP (4 mM), NaGTP (0.3 mM) and Na₂-phosphocreatine (10 mM); Sigma-Aldrich) and a “caesium” internal solution (CsOH (120 mM), Gluconic acid (120 mM), HEPES (40 mM), Na₂ATP (2 mM), NaGTP (0.3 mM) and NaCl (10 mM); Sigma-Aldrich). Experimental substances were puffed onto neurons using an OpenSpritzer, a custom made pressure ejection system (14). Current was injected if required to ensure a neuronal resting membrane potential within 2 mV of -60 mV. In all puffing experiments each data point represents the mean peak puff-induced change in membrane potential from 10 sweeps. In some experiments tetrodotoxin (TTX) (2 μM) was added to the aCSF to block voltage-gated sodium channels.

For calcium imaging, organotypic hippocampal mouse brain slices were virally transfected with a genetically-encoded Ca²⁺ reporter (GCaMP6s under the synapsin promoter, AAV1.Syn.GCaMP6s.WPRE.SV40) one day post culture using the OpenSpritzer and imaged 5 days later using an Olympus BX51WI microscope, 20x water-immersion objective, CCD camera (CCE-B013-U,

Mightex) and 470 nm LED (Thorlabs). Images were collected using μ Manager (15) and analysed using Caltrac3 beta scripts in Matlab.

Pharmacological manipulations were performed by bath application of drugs using a perfusion system (Watson-Marlow). Mecamylamine, Amiloride, D-AP5 and CNQX were purchased from Tocris. Kynurenic acid and Substance P were acquired from Sigma-Aldrich.

Data analysis and statistics

Data was graphed and analysed using Matlab, Microsoft Excel and GraphPad Prism. All data was subjected to a Shapiro-Wilk test to determine whether it was normally distributed. Normally distributed populations were subjected to parametric statistical analyses, whilst skewed data was assessed using non-parametric statistical analyses. Parametric tests included: unpaired student's t-test; paired student's t-test; one-way analysis of variance (ANOVA) with Tukey's post hoc test and repeated measures ANOVA with Tukey's post-hoc test. Non-parametric tests included: Mann-Whitney test; Wilcoxin ranked pairs test; Kruskal Wallis one-way ANOVA with post hoc Dunn's Multiple Comparison test; and Friedman test with post-hoc Dunn's Multiple Comparison test. The confidence interval for all tests was set at 95%.

Results

Taenia crassiceps homogenate excites neurons and can elicit epileptiform activity.

To investigate the potential acute effects of *T. crassiceps* larvae on neurons, *T. crassiceps* larval somatic homogenate was prepared using larvae harvested from the peritonea of mice which were freeze-thawed and homogenized (see Materials and Methods and **Fig. 1A**). Whole-cell patch-clamp recordings were made from CA3 pyramidal neurons in rodent hippocampal organotypic brain slice cultures and pico-litre volumes of *T. crassiceps* homogenate were directly applied to the soma of neurons using a custom built pressure ejection system (**Fig. 1A**) (16). Application of the homogenate (20 ms puff) elicited immediate, transient depolarization of the membrane voltage in recordings from both rat and mouse (**Fig. 1B**) neurons. Increasing the amount of homogenate delivered by increasing the pressure applied to the ejection system resulted in increasingly large membrane depolarization, which could trigger single or multiple action potentials (**Fig. 1B, C**). Similar puffs of artificial cerebrospinal fluid (aCSF) did not affect the neuronal membrane potential, thereby excluding the possibility that mechanical effects might account for the homogenate-induced depolarization observed.

To further explore the acute excitatory effect of *T. crassiceps* on neurons, neuronal networks and the propagation of network activity, we performed fluorescence Ca^{2+} imaging in mouse hippocampal organotypic brain slice cultures. Neurons were virally transfected with the genetically-encoded Ca^{2+} reporter, GCAMP6s, under the synapsin promoter and imaged using widefield epifluorescence microscopy (**Fig. 1D** and Materials and Methods). To simulate a pro-ictal environment, a low Mg^{2+} aCSF was used (0.5 mM Mg^{2+}) and neurons in the dentate gyrus were imaged whilst small, spatially restricted puffs of *T. crassiceps* homogenate were delivered every 15 s using a glass pipette (**Fig. 1D**). The cells within the direct vicinity of the puffing pipette showed a sharp increase in fluorescence immediately following the delivery of *T. crassiceps* homogenate (**Fig. 1.1E t₁**) for all 3 puffs, indicating Ca^{2+} entry following membrane depolarization and action potential generation. Interestingly, cells in the periphery showed notable increases in fluorescence at a delayed interval following some (but not

all) puffs (**Fig. 1E t₂**). The excitation of these cells could only be as a result of being synaptically connected to the cells that were exposed to the puff itself, as they were not positioned close enough to the puffer pipette to be directly excited by the homogenate. Indeed, a current-clamp recording from a neuron in the same slice (**Fig. 1E, inset**) indicates that a single, spatially restricted puff of *T. crassiceps* homogenate can elicit the onset of a regenerative seizure-like event lasting far longer than the puff itself. Together, these results indicate that *T. crassiceps* larvae homogenate results not just in a transient depolarization of cells in the immediate vicinity of application, but can trigger a wave of excitation that propagates through the brain slice in both space and time. This demonstrates that *T. crassiceps* homogenate can initiate seizure-like activity under suitable conditions.

Potassium contributes modestly to *Taenia crassiceps* induced neuronal depolarization.

The extracellular concentration of K⁺ ([K⁺]_e, typically 3 - 4mM) in the brain is considerably lower than the intracellular concentration of K⁺ ([K⁺]_i, typically 100 mM) of neurons (17), which means that even small changes in [K⁺]_e result in relatively large changes to the transmembrane K⁺ gradient and consequently the reversal potential for K⁺ (E_{K⁺}). Due to the fact that constitutively activated K⁺ conductances, or K⁺ “leak” channels, are the major contributor to the resting membrane potential, addition of extracellular K⁺ and accompanying changes to E_{K⁺} result in membrane depolarization (18).

As most cell types tend to have a high [K⁺]_i, it is conceivable that the *T. crassiceps* homogenate could have a high K⁺ concentration, which could potentially account for its depolarizing and excitatory effects on neurons. To address this, we first directly measured the ionic composition of the *T. crassiceps* homogenate using a Roche Cobas 6000. The K⁺ concentration of the *T. crassiceps* homogenate was 11.4 mM as compared to 3.0 mM in our standard aCSF. The *T. crassiceps* homogenate Na⁺ concentration was 123.9 mM, and 144.2 mM for the aCSF. Although the K⁺ concentration is higher in the homogenate than the aCSF, the ionic composition more closely reflects that of the extracellular as opposed to intracellular compartment. Nonetheless, we set out to

determine what effect application of 11.4 mM K⁺ would have on neurons in our system. Whole-cell patch-clamp recordings in current clamp mode using a standard internal solution and aCSF (see Materials and Methods) were made from CA3 pyramidal neurons in rat hippocampal organotypic brain slice cultures. If necessary, current was injected to ensure that the baseline membrane potential was within 2 mV of -60 mV. To remove membrane potential fluctuations due to synaptic noise, and to prevent regenerative Na⁺ conductances due to voltage-gated sodium channels, 2 μM TTX was added to the aCSF. Puffs of aCSF containing 11.4 mM K⁺ were directed toward the soma of recorded neurons. Whilst this did cause a depolarization of the membrane potential, the effect was modest, a median positive shift of only 0.72 mV (IQR 0.51 - 1.04 mV, N = 8) in the membrane potential, which was significantly less than the depolarization caused by puffs of *T. crassiceps* homogenate (median 10.12 mV, IQR 9.93 - 12.49 mV, N = 5, p = 0.0016, Mann-Whitney U test, **Fig. 2A, B, D**). Next, to isolate neuronal depolarization induced by *T. crassiceps* homogenate, but not mediated by K⁺, we performed whole cell patch clamp recordings using a caesium based internal solution (see Materials and Methods), which blocks K⁺ channels. The addition of caesium did not greatly change the positive shift in membrane potential caused by *T. crassiceps* homogenate (median 13.76, IQR 10.87 - 17.24 mV, N = 49, p ≤ 0.0489, Mann-Whitney U test, **Fig. 2C, D**). Together, this indicates that although K⁺ in the *T. crassiceps* homogenate does contribute to membrane depolarization, the majority of the effect is mediated by a different component.

Taenia crassiceps homogenate induced neuronal depolarization is mediated by a small molecule.

Next, to determine the fraction of the *T. crassiceps* homogenate, which underlies the acute excitatory effect on neurons we observed, we used a dialysis membrane to separate the fraction of the homogenate bigger than 3 kDa from the total homogenate. To do so we used a 3 kDa dialysis cassette incubated overnight in standard aCSF. This effectively removed molecules smaller than 3 kDa from the homogenate. When this dialysed *T. crassiceps* was puffed onto the cells in the same recording conditions as in **Fig. 2B** and **C**, the depolarizing response was greatly reduced (p ≤ 0.0003,

Mann-Whitney U test, **Fig. 3 A-C**). The median positive shift in membrane potential for dialysed *T. crassiceps* homogenate was only 0.27 mV (IQR 0.20 - 0.85 mV, N = 5) as compared to a median of 13.76 mV (IQR 10.87 - 17.24 mV, N = 49) for the total homogenate (**Fig. 3**). This suggests that the excitatory component of the *T. crassiceps* homogenate is a molecule smaller than 3 kDa.

Taenia crassiceps excretory/secretory products also cause neuronal depolarization.

Helminths are well known to excrete or secrete products into their immediate environment. Therefore, having confirmed that a small molecule within the *T. crassiceps* homogenate causes acute neuronal depolarization we next sought to determine whether E/S products generated by *T. crassiceps* larvae could also result in neuronal depolarisation. *T. crassiceps* larvae were cultured in media. The media was collected over a period of 21 days and either used as is (*T. crassiceps* total E/S products) or separated into two fractions and buffer exchanged to PBS using an Amicon stirred cell with a 3 kDa cellulose membrane. The first fraction contained E/S products larger than 3 kDa (*T. crassiceps* E/S > 3 kDa) in PBS, and the second fraction contained E/S products smaller than 3 kDa (*T. crassiceps* E/S < 3 kDa) in media. To control for possible effects of molecules in the buffers, both media and PBS were independently puffed onto neurons.

Again, CA3 neurons in rat hippocampal organotypic slice cultures were whole-cell patched using a caesium based internal in the presence of 2 μ M TTX. Brief application of total *T. crassiceps* E/S products using a soma directed puff was sufficient to cause a neuronal depolarization (mean 12.29 mV, SEM 1.74 mV, N = 7) significantly larger than that of the media control (mean -0.48 mV, SEM 0.40 mV, N = 7, $p \leq 0.001$, One-way ANOVA, Tukey's post hoc test, **Fig. 4A, D, F**). Similarly, *T. crassiceps* E/S products containing components smaller than 3 kDa evoked a significantly larger shift in membrane potential than the media control (mean 16.09 mV, SEM 1.56 mV, N = 7, $p \leq 0.001$, One-way ANOVA, Tukey's post hoc test, **Fig. 4B, D, F**). The response evoked by the *T. crassiceps* E/S

products containing components smaller than 3 kDa did not differ significantly than that evoked by the total E/S ($p > 0.05$, One-way ANOVA, Tukey's post hoc test, **Fig. 4A, B, F**).

However, the *T. crassiceps* E/S product fraction larger than 3 kDa (mean 1.43 mV, SEM 0.43 mV, $N = 8$) did not generate significant neuronal depolarization when compared to the PBS control (mean 1.10 mV, SEM 0.22 mV, $N = 8$, $p \leq 0.001$, One-way ANOVA, Tukey's post hoc test, **Fig. 4C, E, F**). Both the total E/S and the E/S products < 3 kDa evoked a depolarization larger than that of the E/S products > 3 kDa ($p \leq 0.001$, One-way ANOVA, Tukey's post hoc test, **Fig. 4A, B, D, F**). Together this set of experiments demonstrated that *T. crassiceps* E/S products also contain an excitatory component, which is a small molecule.

Taenia crassiceps homogenate induced neuronal depolarization is not mediated by nicotinic acetylcholine receptors, ASIC receptors, or Substance P.

Our results demonstrate that the *T. crassiceps* derived small molecule induces neuronal depolarization very rapidly (within ms), as such, it is highly likely that this molecule is an agonist of a neuronal ionotropic receptor. Therefore, we utilized pharmacological methods to determine the ionotropic receptor system via which the *T. crassiceps* derived small molecule acts.

Nicotinic acetylcholine receptors (nAChRs) are well described ionotropic receptors in the nervous system, which mediate depolarization via a mixed Na^+ , K^+ conductance (19). Again, using a caesium based internal solution in the presence of TTX in the aCSF, CA3 neurons were whole-cell patched and the peak voltage during *T. crassiceps* homogenate induced neuronal depolarisation measured before, during and after bath application of the non-competitive nicotinic acetylcholine receptor antagonist mecamylamine hydrochloride ($10\mu\text{M}$) to block nAChRs (**Fig. 5A**). The blockade of nAChRs did not significantly alter the *T. crassiceps* homogenate induced depolarisation. The median *T. crassiceps* homogenate induced depolarization was 16.28 mV (IQR 13.54 - 23.63 mV) during baseline, 16.58 mV

(IQR 11.21 - 24.50 mV) in the presence of mecamlamine hydrochloride and 13.20 mV (IQR = 10.37 - 23.48 mV) following washout (N = 5, p = 0.6914, Friedman test, **Fig. 5A,B**).

Acid-sensing ion channels (ASICs) are proton-gated sodium channels known to be expressed by hippocampal neurons and result in neuronal depolarization when activated. We therefore considered that the pH of the *T. crassiceps* homogenate may be important as acidic conditions could activate ASICs and cause neuronal depolarization. We measured the pH of the *T. crassiceps* homogenate (mean = 7.99), which was too alkaline to activate ASICs, activated maximally at a pH of 5 (20). However, certain compounds can activate ASICs directly (21). Therefore, to determine whether activation of ASICs could underlie *T. crassiceps* homogenate induced depolarization we used the non-specific ASIC blocker amiloride (2 mM). The presence of amiloride did not significantly attenuate the effect of the homogenate (**Fig. 5C**), with the mean *T. crassiceps* homogenate induced depolarization being 14.55 mV (SEM 1.41 mV) during baseline and 12.12 mV (SEM 2.25 mV) in the presence of amiloride, whilst 17.06 mV (SEM 2.20 mV) following washout (N = 10, p = 0.1124, repeated measures ANOVA, **Fig. 5C, D**).

A further possible candidate for our excitatory small molecule was Substance P, an abundant neuropeptide and neurotransmitter (22,23). Robinson *et al* (2012) have found Substance P in close vicinity to human NCC granulomas. Garza *et al.* (2010) found that substance P signalling contributes to granuloma formation and Substance P enhanced NMDA channel function (25). We therefore investigated whether Substance P could elicit a similar neuronal depolarizing response to that of *T. crassiceps* homogenate. However, we found that 100 μ M Substance P had no acute effect on the membrane potential of CA3 hippocampal pyramidal neurons (median = 0.08 mV, IQR 0.01 – 0.12 mV, N = 5, Wilcoxon signed rank test with theoretical median = 0.00, p = 0.0625, **Fig. 5E, F**). Together these data indicate that *T. crassiceps* homogenate induced neuronal depolarization is not mediated by nicotinic acetylcholine receptors, ASIC receptors, nor by substance P.

The depolarizing effects of *Taenia crassiceps* are mediated by glutamate receptor activation.

The predominant excitatory neurotransmitter in the nervous system is glutamate (19). Glutamate activates a number of ionotropic glutamate receptors (GluRs) including AMPA, Kainate and NMDA receptors (19), therefore it is likely that *T. crassiceps* homogenate could cause neuronal depolarization by activation of GluRs. To test this possibility, whole cell current clamp recordings from CA3 hippocampal pyramidal cells were acquired using a caesium based internal in the presence of TTX to block voltage-gated sodium channels. *T. crassiceps* homogenate induced neuronal depolarization was measured before, during and after the application of 10 μ M CNQX, 50 μ M D-AP5 and 2mM kynurenic acid (in the aCSF) to block all three classes of GluRs. We found that GluR blockade significantly reduced the median *T. crassiceps* homogenate induced neuronal depolarization from 14.72 mV (IQR 13.39 - 15.68 mV) to 1.90 mV (IQR 1.09 - 2.21 mV, N = 9, $p \leq 0.01$, Friedman test with Dunn's multiple comparison test, **Fig. 6A, B**), which recovered to a median value of 14.37 mV (IQR 7.32 - 16.38 mV) following washout (N = 9, $p \leq 0.01$, Friedman test with Dunn's multiple comparison test, **Fig. 6A, B**). This indicated that activation of GluRs is the primary mediator of the depolarizing effect of *T. crassiceps* homogenate we observed.

Thereafter, we confirmed whether the depolarizing effect of *T. crassiceps* E/S products is also mediated by GluRs. Here, too, we found that application of GluR antagonists reduced the median total *T. crassiceps* E/S products induced depolarization from 16.35 mV (IQR 15.17 - 19.03 mV) to 2.19 mV (IQR 1.67 - 4.16 mV, N = 10, $p \leq 0.01$, Friedman test with Dunn's multiple comparison test, **Fig. 6C, D**), which recovered to a median value of 16.43 mV (IQR 14.41 - 23.14 mV) following washout (N = 10, $p \leq 0.01$, Friedman test with Dunn's multiple comparison test, **Fig. 6C, D**). These results indicate that the acute excitatory effect of both *T. crassiceps* homogenate and *T. crassiceps* E/S products is mediated through GluRs.

Taenia crassiceps and *Taenia solium* larvae contain and produce glutamate.

Glutamate is the major excitatory neurotransmitter in the brain and the prototypical agonist of GluRs (19). We therefore directly measured the concentration of glutamate in the *T. crassiceps* homogenate we had been puffing onto neurons using a glutamate assay (see Materials and Methods). We found that the mean concentration of glutamate in the *T. crassiceps* homogenate was 6.47 µg/mg (SEM 0.97 µg/mg, N = 5, **Fig. 7A**) – corresponding to approximately 112 µM for puffing experiments. Glutamate at this concentration robustly activates GluRs in hippocampal pyramidal neurons (Forman *et al.*, 2017). *T. crassiceps*, whilst a closely related species to *T. solium*, is not the causative pathogen in humans. We therefore also measured the glutamate concentration in homogenate prepared from larvae of *T. solium*, the predominant pathogen responsible for human NCC. We recorded a glutamate concentration of 4.56 µg/mg (N = 1), which indicates that the concentration of this excitatory neurotransmitter is also high within larvae of *T. solium* (**Fig. 7A**).

Whilst glutamate is a neurotransmitter in the CNS, it is also an ubiquitous metabolite present in the cytoplasm of the majority of cells (26). Therefore, it is possible that the *T. crassiceps* and *T. solium* homogenate only contain glutamate due to cell lysis during homogenate preparation. To determine whether both these larvae actively produce and excrete/secrete glutamate into their environments we measured the *de novo* daily production of glutamate by larvae following harvest of live larvae from the intermediate host (mice for *T. crassiceps* and pigs for *T. solium*, see Materials and Methods). Both species of larvae were observed to generate glutamate *de novo* and release this into the culture media (**Fig. 7B**). *T. crassiceps* larvae released a relatively constant daily amount of glutamate (mean 64.07 µg/15 larvae per day, SEM 4.31 µg/15 larvae per day, N = 3, **Fig. 7B**). *T. solium* larvae released a large amount of glutamate on the first day post-harvest, none on the 2nd and 3rd day *in vitro*, but again begin to produce large amounts of glutamate on days 4, 5 and 6 (mean 137.06 µg/15 larvae per day, SEM 58.49 µg/15 larvae per day, N = 1, **Fig. 7B**). Collectively these results demonstrate that larvae of both

431 *T. crassiceps* and *T. solium* contain and continually release glutamate into their immediate
432 surroundings.
433

Discussion

Here we used patch-clamp electrophysiology, and calcium imaging in hippocampal organotypic brain slice cultures to demonstrate that *Taenia* larval products cause neuronal depolarization and can initiate seizure-like events via glutamate receptor activation. Glutamate assays revealed that both *T. crassiceps* and *T. solium* larvae contain and release considerable quantities of glutamate.

Clinical evidence and animal models conclusively demonstrate that the presence of larvae in the brain can result in the generation of seizures (9,27). Previous work has focused on the involvement of the host inflammatory response in seizure generation (10,28). Whilst this is likely important, it does not preclude the involvement of additional or exacerbating pathogenic mechanisms for seizure generation and epileptogenesis in NCC. In this study we have identified that *Taenia* larvae have a direct excitatory effect on neurons via glutamatergic signaling. This is important, as the central role of glutamatergic signaling in epileptogenesis has conclusively been shown using multiple cell culture (29–31), slice (32–34), and *in vivo* models of epilepsy (35–37).

Clinically, in addition to NCC, the other major causes of adult acquired epilepsy are stroke, traumatic brain injury and CNS tumors (38,39). In these other causes of acquired epilepsy, glutamatergic signaling and glutamate excitotoxicity are thought to be central to the pathogenic process. Glutamate excitotoxicity occurs when depolarized, damaged or dying neurons release glutamate, which activate surrounding neurons via NMDA and AMPA receptors, resulting in sustained neuronal depolarization, Ca^{2+} influx and the subsequent activation of signaling cascades and enzymes. These in turn lead to cell death via necrosis and apoptosis (40), the further release of intracellular glutamate and the propagation of the excitotoxic process to neighboring cells. In the neurons that survive, the prolonged exposure to glutamate has been shown to cause hyperexcitability and seizures (29,31) via multiple mechanisms including enhanced intrinsic excitability and NMDA receptor dependent disruption of GABAergic inhibition (41–43). Given our finding that *Taenia* larvae contain and release significant

quantities of glutamate, it is possible that should homeostatic mechanisms for taking up and metabolizing glutamate fail to compensate for larval-derived glutamate in the extracellular space, similar glutamate-dependent excitotoxic and epileptogenic processes that occur in stroke, traumatic brain injury and CNS tumors are likely to also occur in NCC.

Glioma, a common adult primary brain tumor, which typically presents with seizures in over 80% of patients (44), is an intriguing condition for comparison to NCC. Here, the tumor cells themselves have been shown to release glutamate into the extracellular space via the system x_c^- -cystine-glutamate antiporter (43). Interestingly, there is compelling evidence that tumoral release of glutamate via this mechanism both causes seizures and favours glioma preservation, progression and invasion in cases of malignant glioma (45–47). Analogously it is possible that *Taenia* larvae in the brain utilize the release of glutamate and the induction of glutamate excitotoxicity to facilitate their growth and expansion, with the accompanying effect of seizure generation. In addition, the death of *Taenia* larvae, or larval-derived cells would also result in the release of metabolic glutamate, further contributing to glutamate release and excitotoxicity. In the case of glioma, where the mechanism of glutamate release by tumor cells is known, pharmacological agents (e.g. sulfasalazine), which block glutamate release have considerable potential as therapeutic agents for reducing seizure burden in this condition. Therefore, it is important that future work attempts to identify the molecular mechanisms underlying the *Taenia*-specific production of glutamate in order to inform the development of new therapeutic strategies to potentially reduce larval expansion and treat seizures in NCC.

An important consideration is how we might reconcile our findings of continuous glutamate release by *Taenia* larvae with the clinical picture of delayed symptom onset in NCC. In people with NCC, some experience acute seizures immediately following infection whilst others present with seizures months to years following initial infection (48,49). This suggests that multiple different, or possibly interacting

mechanisms might be involved in the epileptogenic process in NCC. Neuroinflammation has long been thought to be central to the development of seizures in NCC. In support of this, larval suppression of host inflammatory responses could explain why many patients may remain asymptomatic for years following infection. Our findings are not at odds with this line of thinking when considering the effect of neuroinflammation on extracellular glutamate uptake. In the healthy brain parenchymal astrocytes are optimized to maintain extracellular glutamate at low concentrations (50). However, the inflammatory transition of astrocytes to a reactive phenotype is known to impede their ability to buffer extracellular uptake (51). It is therefore conceivable that cyst-associated reactive astrocytosis may gradually erode the ability of homeostatic mechanisms to compensate for larvae derived extracellular glutamate resulting in delayed symptom onset in NCC.

It is clear that there is still considerable uncertainty regarding the precise sequences of events leading to seizure onset in NCC. Nonetheless our findings provide the first evidence that as is the case with the other common causes of adult acquired epilepsy (i.e. stroke, traumatic brain injury and glioma), increased extracellular, parasite-derived glutamate likely also plays an important role in the development of seizures in NCC.

Figure Legends

Figure 1: *Taenia crassiceps* homogenate excites neurons and can elicit epileptiform activity. A)

Schematic showing the experimental set up in which whole cell patch clamp recordings were made from CA3 hippocampal pyramidal neurons in rodent organotypic slice cultures while a puffer pipette delivered pico-litre volumes of homogenised *Taenia crassiceps* larvae (*T. crassiceps* hom.) targeted at the cell soma. B) Current clamp recording from a rat pyramidal neuron while increasing amounts of *T. crassiceps* homogenate was applied via the puff pipette (left to right, orange arrows). Small amounts of *T. crassiceps* homogenate resulted in depolarization (left), increasing amounts elicited single (middle left) or even bursts of action potentials (middle right) whilst a similar puff of aCSF had no effect on the neuronal membrane potential (right, blue arrow), showing that potential mechanical disruption of the cell by a puff is not sufficient to depolarise it. C) As in 'B', identical effects of *T. crassiceps* homogenate could be elicited in current clamp recordings from a CA3 hippocampal pyramidal neuron in a mouse organotypic brain slice culture. D) Top: widefield fluorescence image of neurons in the dentate gyrus of a mouse hippocampal organotypic brain slice culture expressing the genetically-encoded Ca^{2+} reporter GCAMP6s under the synapsin promoter in aCSF containing 0.5 mM Mg^{2+} . A subset of neurons used to generate the Ca^{2+} traces in 'E' are indicated by different colours. The orange dotted circle indicates where *T. crassiceps* homogenate was delivered using the puff pipette. Bottom, schematic of the experimental setup including puffer pipette and CCD camera for Ca^{2+} imaging using a 470 nm LED. E) Top, dF/F traces representing Ca^{2+} dynamics from the GCAMP6s expressing neurons labelled in 'D' concurrent with 3 puffs (30 ms duration) of *T. crassiceps* homogenate 15 s apart. Bottom: two images of raw Ca^{2+} fluorescence at two time points t_1 and t_2 . Note how at time point t_2 neurons distant to the site of *T. crassiceps* homogenate application are also activated, indicating spread of neuronal activity. Inset, top-right: current clamp recording from a neuron demonstrates that *T. crassiceps* homogenate application was able to evoke a seizure-like event.

Figure 2: Potassium contributes modestly to *Taenia crassiceps* induced neuronal depolarization. A)

Whole-cell patch clamp recording using a standard internal in current clamp mode from a CA3 pyramidal neuron in a rat organotypic hippocampal slice culture. A 20 ms puff of *T. crassiceps* homogenate (orange arrow) directed at the cell soma is delivered via a puffer pipette, causing depolarization. B) As in 'A' but puffing aCSF containing 11.39 mM K⁺ (equivalent to the K⁺ concentration of *T. crassiceps* homogenate) caused modest depolarization. 2 μM TTX was added to the aCSF to reduce synaptic noise in the voltage trace. C) As in 'A' but with a caesium based internal electrode solution to block K⁺ channels and 2 μM TTX in the aCSF. Puffs of *T. crassiceps* homogenate (orange arrow) resulted in sizeable depolarization. D) Population data showing that the response from the 11.39 mM K⁺ puff was significantly smaller than the *T. crassiceps* homogenate (with and without caesium internal). Values with means ± SEM; *** p ≤ 0.001.

Figure 3: *Taenia crassiceps* homogenate induced neuronal depolarization is mediated by a small molecule. Whole-cell patch recordings in current clamp mode were made from CA3 pyramidal

neurons in rat organotypic hippocampal slice cultures using a caesium based internal in the presence of 2 μM TTX. A) Delivery of a puff of *T. crassiceps* homogenate caused a depolarizing shift in membrane potential. B) The depolarizing response to *T. crassiceps* homogenate was largely abolished by dialysing out all molecules smaller than 3 kDa. C) Population data demonstrating that the *T. crassiceps* homogenate response is significantly reduced by dialysis, indicating that the excitatory component is a small molecule (< 3 kDa). Values with means ± SEM; *** p ≤ 0.001.

Figure 4: *Taenia crassiceps* excretory/secretory products cause neuronal depolarization. A) Current

clamp, whole-cell patch recording from a rat CA3 pyramidal neuron using a caesium based internal and 2 μM TTX in the aCSF. A 20 ms puff of *T. crassiceps* excretory/secreted (E/S) products caused a marked depolarizing shift in voltage. B) Similar application of *T. crassiceps* E/S products smaller than 3 kDa also resulted in a large depolarizing response. C) In contrast, a puff of *T. crassiceps* E/S products

larger than 3 kDa resulted in a negligible shift in membrane potential. D) No change in membrane voltage occurred in response to a puff of growth media, a control for the E/S products < 3 kDa. E) A negligible depolarization of the membrane voltage occurred in response to a puff of phosphate-buffered saline (PBS), a control for the E/S products > 3kDa. F) Population data demonstrating that *T. crassiceps* E/S products as a whole or when separated to E/S products < 3kDa cause significant depolarization when compared to either the larger constituent of E/S products or either of the control substances. Values with means $\pm$ SEM; *** $p \leq 0.001$; ns = not significant, $p > 0.05$.

Figure 5: *Taenia crassiceps* homogenate induced neuronal depolarization is not mediated by

nicotinic acetylcholine receptors, ASIC receptors, or Substance P. Whole-cell patch recordings were

made from CA3 pyramidal neurons in rat organotypic hippocampal slice cultures using a caesium based internal in the presence of 2 μ M TTX. If necessary, current was injected to maintain the membrane potential within 2 mV of -60 mV. A) The depolarizing response to a *T. crassiceps* homogenate puff (orange arrow) before (black trace), during (blue trace) and after (grey trace) the addition of a nicotinic acetylcholine receptor (nAChR) blocker (mecamylamine hydrochloride, 10 μ M). B) Population data demonstrating that nAChR blockade has no significant effect on the *T. crassiceps* homogenate induced depolarization. C) The depolarizing response to a *T. crassiceps* homogenate puff (orange arrow) before (black trace), during (blue trace) and after (grey trace) the addition of an ASIC receptor blocker (amiloride, 2 mM) D) Population data demonstrating that ASIC receptor blockade has no significant effect on the *T. crassiceps* homogenate induced depolarization. E) Current clamp trace showing that a puff of 100 μ M Substance P (blue arrow) does not affect the membrane potential of CA3 pyramidal neurons. F) Population data for Substance P application. ns = not significant, $p > 0.05$.

Figure 6: The depolarizing effects of *Taenia crassiceps* are mediated by glutamate receptor

activation. A) Whole-cell current clamp recording from a rat CA3 pyramidal neuron in a hippocampal

organotypic slice culture. Neuronal membrane potential in response to a 20 ms puff of *T. crassiceps*

homogenate (orange arrow) before (black trace), during (blue trace) and following wash out (grey trace) of a pharmacological cocktail to block glutamate receptors (10 μ M CNQX, 50 μ M D-AP5 and 2mM kynurenic acid). B) Population data shows that the depolarization response to *T. crassiceps* homogenate is significantly reduced in the presence of glutamate receptor blockers and returns upon wash out. C) The depolarization response to a puff of *T. crassiceps* E/S products is also markedly reduced during glutamate receptor blockade. D) Population data for glutamate receptor blockade during *T. crassiceps* E/S products application. Values with means $\pm$ SEM; *** $p \leq 0.001$; ** $p \leq 0.01$.

Figure 7: *Taenia crassiceps* and *Taenia solium* larvae contain and produce glutamate. A) Glutamate levels in whole cyst hom. of *T. crassiceps* and *T. solium* larvae. B) Glutamate production per day per 15 larvae for 6 days *in vitro* for *T. crassiceps* and *T. solium* larvae. Note that both species of larvae produce glutamate *de novo*, particularly on days 4 – 6.

References

1. Diop AG, Boer HM De, Mandlhate C, Prilipko L, Meinardi H. The global campaign against epilepsy in Africa. *Acta Trop.* 2003;87:149–59.
2. Preux P-M, Druet-Cabanac M. Epidemiology and aetiology of epilepsy in sub-Saharan Africa. *Lancet Neurol.* 2005 Jan;4(1):21–31.
3. Trevisan C, Mkupasi EM, Ngowi HA, Forkman B, Johansen M V. Severe seizures in pigs naturally infected with *Taenia solium* in Tanzania. *Vet Parasitol.* 2016;220:67–71.
4. Garcia HH, Del OH. Antiparasitic treatment of neurocysticercosis - The effect of cyst destruction in seizure evolution. *Epilepsy Behav.* 2017;76:158–62.
5. Nash TE, Mahanty S, Garcia HH. Neurocysticercosis-More Than a Neglected Disease. *PLoS Negl Trop Dis.* 2013;7(4):7–9.
6. Ndimubanzi PC, Carabin H, Budke CM, Nguyen H, Qian YJ, Rainwater E, et al. A systematic review of the frequency of neurocysticercosis with a focus on people with epilepsy. *PLoS Negl Trop Dis.* 2010;4(11).
7. de Lange A, Mahanty S, Raimondo J V. Model systems for investigating disease processes in neurocysticercosis. *Parasitology.* 2018 Nov 15;1–10.
8. Vezzani A, Fujinami RS, White HS, Preux P, Blümcke I, Sander JW, et al. Infections, inflammation and epilepsy. *Acta Neuropathol.* 2016;131:211–34.
9. Nash TE, Mahanty S, Loeb J a., Theodore WH, Friedman A, Sander JW, et al. Neurocysticercosis: A natural human model of epileptogenesis. *Epilepsia.* 2015 Feb;56(2):177–83.
10. Robinson P, Garza A, Weinstock J, Serpa J a, Goodman JC, Eckols KT, et al. Substance P causes seizures in neurocysticercosis. *PLoS Pathog.* 2012 Feb;8(2):e1002489.
11. Sun Y, Chauhan A, Sukumaran P, Sharma J, Singh BB, Mishra BB. Inhibition of store-operated calcium entry in microglia by helminth factors: implications for immune suppression in neurocysticercosis. *J Neuroinflammation.* 2014 Dec 24;11(1):210.
12. Vendelova E, Camargo de Lima J, Lorenzatto KR, Monteiro KM, Mueller T, Veepaschit J, et al. Proteomic Analysis of Excretory-Secretory Products of *Mesocostoides corti* Metacestodes Reveals Potential Suppressors of Dendritic Cell Functions. *PLoS Negl Trop Dis.* 2016;10(10):1–27.
13. Stoppini L, Buchs P-A, Muller D. A simple method for organotypic cultures of nervous tissue. *J Neurosci Meth.* 1991 Apr;37(2):173–182.
14. Forman CJ, Tomes H, Mbobo B, Baden T, Raimondo J V. Openspritzer: an open hardware pressure ejection system for reliably delivering picolitre volumes. *bioRxiv.* 2016;093633.
15. Edelstein A, Amodaj N, Hoover K, Vale R, Stuurman N. Computer Control of Microscopes Using µManager. In: *Current Protocols in Molecular Biology.* Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2010. p. 14.20.1-14.20.17.
16. Forman CJ, Tomes H, Mbobo B, Burman RJ, Jacobs M, Baden T, et al. Openspritzer: an open hardware pressure ejection system for reliably delivering picolitre volumes. *Sci Rep.* 2017 May;7(1):2188.
17. Jiang C, Haddad GG. Effect of anoxia on intracellular and extracellular potassium activity in hypoglossal neurons in vitro. *J Neurophysiol.* 1991;66(1):103–11.
18. Lesage F. Pharmacology of neuronal background potassium channels. *Neuropharmacology.* 2003;44:1–7.

- 636 19. Bear MF, Connors BW, Paradiso MA. Neuroscience: Exploring the brain. Third. Lupash E,
637 Connolly E, Dilerenia B, Williams P, editors. Philadelphia, Baltimore, New York, London, Buenos
638 Aires, Hong Kong, Sydney, Tokyo: Lippincott Williams & Wilkins; 2007.
- 639 20. Weng X, Zheng J, Gai X, Li J, Xiao W. Two types of acid-sensing ion channel currents in rat
640 hippocampal neurons. *Neurosci Res.* 2004;50:493–9.
- 641 21. Osmakov DI, Koshelev SG, Andreev YA, Kozlov SA. Endogenous isoquinoline alkaloids agonists
642 of acid-sensing ion channel type 3. *Front Mol Neurosci.* 2017;10(September):1–7.
- 643 22. Liu H, Mazarati AM, Katsumori H, Sankar R, Wasterlain CG. Substance P is expressed in
644 hippocampal principal neurons during status epilepticus and plays a critical role in the
645 maintenance of status epilepticus. *Proc Natl Acad Sci.* 2002;96(9):5286–91.
- 646 23. Harrison S, Geppetti P. Substance P. *Int J Biochem Cell Biol.* 2001;33(6):555–76.
- 647 24. Garza A, Tweardy DJ, Weinstock J, Viswanathan B, Robinson P. Substance P signaling
648 contributes to granuloma formation in taenia crassiceps infection, a murine model of
649 cysticercosis. *J Biomed Biotechnol.* 2010;2010.
- 650 25. Lieberman DN, Mody I. Substance P enhances NMDA channel function in hippocampal dentate
651 gyms granule cells. *J Neurophysiol.* 1998;80(1):113–9.
- 652 26. Newsholme P, Procopio J, Ramos Lima MM, Pithon-Curi TC, Curi R. Glutamine and glutamate—
653 their central role in cell metabolism and function. *Cell Biochem Funct.* 2002;21:1–9.
- 654 27. Verastegui MR, Mejia A, Clark T, Gavidia CM, Mamani J, Ccopa F, et al. Novel Rat Model for
655 Neurocysticercosis Using Taenia solium. *Am J Pathol.* 2015;185(8):2259–68.
- 656 28. Stringer JL, Marks LM, White AC, Robinson P. Epileptogenic activity of granulomas associated
657 with murine cysticercosis. *Exp Neurol.* 2003 Oct;183(2):532–6.
- 658 29. Sun DA, Sombati S, DeLorenzo RJ. Glutamate Injury–Induced Epileptogenesis in Hippocampal
659 Neurons. *Stroke.* 2001 Oct 1;32(10):2344–50.
- 660 30. Sombati S, Delorenzo RJ. Recurrent spontaneous seizure activity in hippocampal neuronal
661 networks in culture. *J Neurophysiol.* 1995 Apr;73(4):1706–11.
- 662 31. DeLorenzo R, ... SP-P of the, 1998 undefined. Prolonged activation of the N-methyl-d-aspartate
663 receptor–Ca²⁺ transduction pathway causes spontaneous recurrent epileptiform discharges in
664 hippocampal neurons. *Natl Acad Sci.*
- 665 32. Anderson WW, Anderson WW, Lewis D V., Scott Swartzwelder H, Wilson WA. Magnesium-free
666 medium activates seizure-like events in the rat hippocampal slice. *Brain Res.* 1986;398(1):215–
667 9.
- 668 33. Stasheff S, Anderson W, Clark S, Science WW-, 1989 undefined. NMDA antagonists
669 differentiate epileptogenesis from seizure expression in an in vitro model.
670 science.sciencemag.org.
- 671 34. Ziobro JM, Deshpande LS, DeLorenzo RJ. An organotypic hippocampal slice culture model of
672 excitotoxic injury induced spontaneous recurrent epileptiform discharges. *Brain Res.* 2011 Jan
673 31;1371:110–20.
- 674 35. Croucher MJ, Bradford HF. NMDA receptor blockade inhibits glutamate-induced kindling of the
675 rat amygdala. *Brain Res.* 1990;506(2):349–52.
- 676 36. Croucher MJ, Bradford HF, Sunter DC, Watkins JC. Inhibition of the development of electrical
677 kindling of the prepyriform cortex by daily focal injections of excitatory amino acid antagonists.
678 *Eur J Pharmacol.* 1988;152(1–2):29–38.

37. Rice AC, Delorenzo RJ. NMDA receptor activation during status epilepticus is required for the development of epilepsy. *Brain Res.* 1998;782(1–2):240–7.
38. Hauser WA, Annegers JF, Kurland LT. Prevalence of Epilepsy in Rochester, Minnesota: 1940–1980. *Epilepsia.* 1991 Aug;32(4):429–45.
39. Forsgren L, Beghi E, Oun A, Sillanpaa M. The epidemiology of epilepsy in Europe - a systematic review. *Eur J Neurol.* 2005 Apr 1;12(4):245–53.
40. Ankarcrona M, Dypbukt JM, Bonfoco E, Zhivotovsky B, Orrenius S, Lipton SA, et al. Glutamate-induced neuronal death: A succession of necrosis or apoptosis depending on mitochondrial function. *Neuron.* 1995 Oct 1;15(4):961–73.
41. Lee HHC, Deeb TZ, Walker JA, Davies PA, Moss SJ. NMDA receptor activity downregulates KCC2 resulting in depolarizing GABAA receptor-mediated currents. *Nat Neurosci.* 2011 Jun 1;14(6):736–43.
42. Terunuma M, Vargas KJ, Wilkins ME, Ramírez OA, Jaureguiberry-Bravo M, Pangalos MN, et al. Prolonged activation of NMDA receptors promotes dephosphorylation and alters postendocytic sorting of GABAB receptors. *Proc Natl Acad Sci.* 2010 Aug 3;107(31):13918–23.
43. Buckingham SC, Campbell SL, Haas BR, Montana V, Robel S, Ogunrinu T, et al. Glutamate release by primary brain tumors induces epileptic activity. *Nat Med.* 2011 Oct 11;17(10):1269–74.
44. Santosh V, Sravya P. Glioma, glutamate (SLC7A11) and seizures-a commentary. *Ann Transl Med.* 2017 May;5(10):214.
45. Sontheimer H. A role for glutamate in growth and invasion of primary brain tumors. *J Neurochem.* 2008 Apr 1;105(2):287–95.
46. Takano T, Lin JH-C, Arcuino G, Gao Q, Yang J, Nedergaard M. Glutamate release promotes growth of malignant gliomas. *Nat Med.* 2001 Sep;7(9):1010–5.
47. Huang W, Choi W, Chen Y, Zhang Q, Deng H, He W, et al. A proposed role for glutamine in cancer cell growth through acid resistance. *Cell Res.* 2013 May 29;23(5):724–7.
48. Adalid-Peralta L, Arce-Sillas A, Fragoso G, Cárdenas G, Rosetti M, Casanova-Hernández D, et al. Cysticerci drive dendritic cells to promote in vitro and in vivo tregs differentiation. *Clin Dev Immunol.* 2013;2013:1–9.
49. Verma A, Prasad KN, Cheekatla SS, Nyati KK, Paliwal VK, Gupta RK. Immune response in symptomatic and asymptomatic neurocysticercosis. *Med Microbiol Immunol.* 2011 Nov 1;200(4):255–61.
50. Perea G, Araque A. GLIA modulates synaptic transmission. *Brain Res Revs.* 2010 May;63(1–2):93–102.
51. Seifert G, Carmignoto G. Astrocyte dysfunction in epilepsy. *Brain Res Rev.* 2010 May 1;63(1–2):212–21.

Figure 1: *Taenia crassiceps* homogenate excites neurons and can generate epileptiform activity

bioRxiv preprint doi: <https://doi.org/10.1101/859009>; this version posted November 28, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Figure 2: K⁺ contributes modestly to *Taenia crassiceps* induced neuronal depolarization

bioRxiv preprint doi: <https://doi.org/10.1101/859009>; this version posted November 28, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Figure 3: *Taenia crassiceps* homogenate induced neuronal depolarization is mediated by a small molecule

Figure 4: *Taenia crassiceps* excreted / secreted products cause neuronal depolarization

Figure 5: *Taenia crassiceps* homogenate induced neuronal depolarization is not mediated by acetylcholine receptors, ASIC receptors nor Substance P

Figure 6: The excitatory effects of *Taenia crassiceps* are mediated by glutamate receptor activation

Figure 7: *Taenia crassiceps* and *Taenia solium* larvae contain and produce glutamate.