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The effect of potassium chloride on *Aplysia Californica* abdominal ganglion activity

Fanrui Fu, Munish Chauhan, Rosalind Sadleir

School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ 85287, USA.

Abstract

Objective: Spontaneous activity in the abdominal ganglion of *Aplysia* can be used as a convenient bioelectricity source in tests of novel MRI-based functional imaging methods, such as functional Magnetic Resonance Electrical Impedance Tomography (fMREIT). In these tests, it is necessary to find a consistent treatment that modulates neural activity, so that these results can be compared with control data. Effects of MREIT imaging currents combined with treatment were also of interest.

Approach: Potassium chloride (KCl) was employed as a rhythm modulator. In a series of experiments, effects of adding different volumes of KCl solution were tested and compared with experiments on control groups that had artificial sea water administered. In all cases, neuronal activity was measured with micro electrode arrays.

Main results: It was possible to reversibly stop spontaneous activity in ganglia by increasing the extracellular potassium chloride concentration to 89 mM. There was no effect on experimental outcomes when current was administered to the sample chamber between recordings.

Significance: KCl can be used as a reversible neural modulator for testing neural detection methods.

Keywords

Aplysia; action potential; extracellular potassium; microelectrode array

1. Introduction

Magnetic Resonance Electrical Impedance Tomography (MREIT) is a recently developed conductivity imaging method that may have applications in functional neuroimaging (Seo and Woo, 2011, Sadleir et al., 2010). In MREIT, small external currents are applied to the body during MR scans. This current flow produces a magnetic field that affects MRI phase images. These phase images may then be used to reconstruct current density or conductivity tensor images (Kwon et al., 2016, Jeong et al., 2016, Kasinadhuni et al., 2017, Chauhan et al., 2017).

[†]**Corresponding Author:** Rosalind Sadleir, PhD, School of Biological and Health Systems Engineering, Arizona State University, Box 879709, Tempe, Arizona 85287-9709, USA, **Phone** +1 480 727-9790, **Fax:** +1 480 727-7264, Rosalind.Sadleir@asu.edu.

During neural spiking, conductivities of active neural tissue membranes change, affecting the passage of imaging current paths near this tissue, principally affecting MR phase images. Neural activity may therefore be detected directly in functional MREIT (fMREIT) by evaluating regions of increased phase variability (Sadleir et al., 2017). Several other direct and indirect MR based functional neuroimaging techniques exist or have been proposed, including BOLD-fMRI (Ogawa et al., 1990), ncMRI (Bandettini et al., 2005), Lorentz force imaging (Truong et al., 2008) and Ultra low field MRI (Zotev et al., 2008). The principal advantage of fMREIT is that it is direct and sensitive to a scalar quantity (conductivity contrast), and is therefore not susceptible to cancellation effects (Sadleir et al., 2010), as is ncMRI (Cassarà et al., 2008).

Early testing in fMREIT has involved in vitro monitoring of changes in isolated neural preparations (Sadleir et al., 2017). Such tests are more convenient to perform using tissue from invertebrate animals. A method of controlling neural activity is critical in order to verify that test how phase images depend upon activity levels. In many systems activity levels can be controlled using evoked responses, for example light or mechanical responses, but these are difficult to achieve in isolated neural structures or in invertebrates. However, it is generally possible to use chemical means to modulate activity in tissue.

We chose to use the abdominal ganglion of *Aplysia Californica* (AAG) as a source of spontaneous neural activity in our study. The AAG is a valuable tissue for neural study not only for its spontaneous bioelectric activity but also for its large cell size. The largest neuron in the ganglion can reach 1mm in diameter and the majority of small cells in the ganglion are about the size of the biggest neuron in vertebrate brain (Frazier et al., 1967). The large size of the AAG makes visual observation and neural activity localization easier when also performing recordings using microelectrode arrays (Novak and Wheeler, 1986). Tests of ncMRI were previously performed using tetrodotoxin (TTX) as a chemical modulator (Petridou et al., 2006). However, TTX is dangerous to humans, and its effects on tissue preparations are generally irreversible.

In this study, different volumes of potassium chloride (KCl) solution were administered to tissue preparations to determine their effects on this tissue, and to assess if KCl could be used to consistently decrease neural activity. Previous studies have shown that moderate increases in extracellular potassium concentration can change neural activity patterns and increase spiking rates (Dominguez and Fozzard, 1970). This effect was also used in a previous fMREIT study (Sadleir et al., 2017). However, the relation between KCl concentration and AAG activity has not been thoroughly investigated. Here, we used high concentrations of KCl, and combined KCl administration with an MREIT-like current injection to see if current combined with this treatment produced changes in spontaneous AAG activity levels. The effect of KCl on AAG samples was measured using microelectrode arrays. Because the purpose of the study was to verify the feasibility of using KCl as a neural activity modulator in the context of fMREIT experiments, our experiments tested effects of increasing extracellular potassium concentrations by adding of two different quantities of KCl-doped solution into the artificial sea water (ASW) containing the preparation. We did not consider the precise patterns of neural activity in this study, since we wished only to assess changes in relative activity levels when different amounts of KCl were

added, and when current injection was combined with this treatment. Matched controls were employed in all cases. Finally, we were also curious to discover if any treatment effects from KCl were reversible, therefore we tested the response of ganglia treated with KCl to the removal of the solution and reinfusion with ASW.

2. Methods

2.1. Animal handling

Thirty-six late-juvenile (around 50g) *Aplysia Californica* were obtained from NIH/University of Miami National Resource for *Aplysia* Facility. After delivery, animals were maintained in an aquarium tank filled with artificial sea water (ASW). The ASW was composed of deionized water and sea salt (Instant Ocean, Spectrum Brands, Blacksburg, VA, USA). The overall salinity was adjusted to 30 ppt, and the environmental temperature was set to 16 C°. The initial molarity of potassium in the ASW was 8.24 mM (Atkinson and Bingman, 1998). Prior to experiments, *Aplysia* were fed with dried seaweed sheets (OMEGA ONE, Omega Sea LLC, Painesville OH, USA) every three days.

2.2. Dissection

The *Aplysia* were anesthetized with MgCl₂ solution (77 g/L of MgCl₂ and 3.6 g/L of HEPES buffer). After muscles relaxed fully, the foot was cut open and the ganglion was removed. Following removal, the ganglion was stored in ASW.

2.3. Equipment setup

A 3-D-printed sample chamber was constructed to house the AAG (figure 1(b)). The cylindrical chamber had a diameter of approximately 12.5 mm. Four equally spaced holes (diameter 2 mm) were printed into one plane of the sample chamber for MREIT electrode placement. Copper sheets were used as MREIT electrodes. A Multichannel Systems (Reutlingen, Germany) Flex MEA36 micro electrode array (MEA) was inserted into the chamber base via a rectangular slot at the bottom of the chamber. The array was fixed to the chamber base with double sided tape, and the slot was sealed with Teflon tape.

A good connection between AAG and the MEA is key to achieving successful recordings. We applied a cellulose nitrate coating to the MEA (Egert and Meyer, 2005) to ensure the ganglion did not move during the experiment. Nitrocellulose stock solution was made by dissolving 25 mg (5cm²) nitrocellulose transfer membranes (protran® BA83/85) in 2 ml of 100% methanol. A working solution was made by diluting the stock solution with methanol in a ratio of 1:10. Five microliters of working solution were then introduced on the surface of the MEA and allowed to dry for approximately 3 mins. The AAG was then gently pushed down onto the MEA with tweezers. The ganglion orientation used was the same for each experiment (figure 1(d)).

During experiments, the chamber was placed in a small grounded metal enclosure to minimize environmental electromagnetic interference. The enclosure was located on a vibration isolation table (Newport Corporation, Irvine, CA, USA) to avoid movement noise (figure 1(c)). Microscope (ZEISS) images were collected after each ganglion was positioned

on the MEA. A current source system from Tucker-Davis Technologies (RZ5D BioAmp Processor, IZ2 Stimulator) was used for current injection.

2.4. Experimental design

Three different groups of experiments were designed to investigate the behavior of the ganglia when different volumes of KCl were added, with or without current injection. The KCl solution was made by adding 0.6g KCl to a 20-ml volume of ASW, thus the concentration of potassium in the KCl solution was 411 mM. For each experiment, each treatment (KCl) group was matched to a control (ASW) group. There were 6 specimens in each group. We sought to quantify the effect of these factors by counting the number of spikes generated by ganglia in the treatment groups, compared to those in control groups. All experiments commenced with the ganglion in 200 μ l of ASW solution.

In the first group of experiments, the treatment consisted of 20 μ l of KCl solution. Addition of the treatment increased the extracellular potassium concentration to 39 mM. The experimental procedure used was the same as used in fMREIT experiments, as shown in figure 2. There were 35-minute gaps between recordings. In fMREIT experiments, these periods were used for imaging. The 20 μ l KCl solution was added into the sample chamber after the third recording with a micropipette. The second group was the same as the first, but the volume of KCl solution added was 50 μ l (final potassium concentration 89 mM). The third group used 50 μ l KCl treatments, as well as interleaving MEA recordings with current injections between recordings 2 and 3, 5 and 6, and 7 and 8. Where current was used, the current produced by the current source was a bipolar pulse. Each pulse had a width of 8 ms, an amplitude of 250 μ A and a frequency of 1 Hz (figure 3). Combination of current administration with KCl or ASW administration served to confirm that persistent changes were not caused by current administration, allowing us to confirm that changes in activity levels were likely due to ionic concentration changes alone. In actual fMREIT experiments, where variability in magnetic flux density distributions are measured, changes in bulk solution conductivity should not result in changes in current density distributions. However, increases in media volume caused by control or treatment administration may cause dilution of current density and therefore a slight decrease in current densities in the neighborhood of ganglia. Conductivities of ASW and KCl-doped solutions were measured to be approximately 5.7 and 6.3 S/m respectively. Increasing the media volume from 200 μ l to 250 μ l resulted in a decrease in magnetic flux densities of approximately 0.15%.

2.5. Recovery Observation

In the treatment group of Experiment 2, we monitored for AAG recovery activity after KCl was removed and replaced by ASW. Therefore, after recording 8, the solution in the chamber was removed and replaced with 200 μ l ASW. A one-hour recording of AAG activity was obtained immediately afterwards to monitor the recovery response.

2.6. Data processing

MC_Rack software (MultiChannel Systems) was used to visualize and record the neural activity and to detect and count spikes. Each recording lasted 9 minutes. Neural spikes were detected using the spike sorting function built into the MC_Rack. A sample of 500 ms of

background noise at the beginning of each data trace was used to calculate noise standard deviations. A spike was defined as having an amplitude 5 times the noise standard deviation (5σ).

Recorded ganglion activity levels vary widely between samples due to factors such as differences in connective tissue thickness, positions of ganglia relative to MEA electrodes, and electrode contact quality as well as individual anatomical and baseline differences between animals, thus a normalized statistical analysis was used to reveal typical behavior changes. After spike detection, all spike counts for all ganglia were normalized to an initial value of 100. The normalized spike count data from all 32 flexMEA channels was computed over each 10-minute recording. Normalized recordings from all 6 specimens in each control or treatment group were used to calculate the mean and standard deviation in spike rates at each time point. Treatment and control group data were compared using Welch's t-tests. Significance was set at $\alpha=0.05$. We used Cohen's d value to quantify effect sizes (Sullivan and Feinn, 2012), that is

$$d = \frac{M_1 - M_2}{SD_{pooled}}, \text{ such that}$$

$$SD_{pooled} = \sqrt{\frac{SD_1^2 + SD_2^2}{2}}$$

where M_1 and SD_1 were the mean and standard deviation of the initial measurement in each experiment respectively (recording 1, $M_1=100$, $SD_1=0$), and M_2 and SD_2 were the mean and standard deviation of the subsequent recording data (recordings 2–8) in each experiment.

3. Results

3.1. Effects of K^+ concentration and current administration

Figure 4 shows the results of the three initial experiments. We found that normalized spike rates decreased continuously in the 200 minutes after administration of 20 μ l of KCl solution (figure 4(a)). By comparison, normalized spike rates in the control (ASW) group stayed relatively constant over the same period, with the smallest mean normalized spiking rate in this group post ASW administration being 73% of original rates. Five minutes after administration of 50 μ l KCl to the treatment group of Experiment 2, ganglion activity ceased in all cases (no spikes detected, figure 4(b)). This was also found in Experiment 3 both where 50 μ l administration was combined with current injection (figure 4(c)).

3.2. Effect sizes and statistical comparisons

Welch's t-tests assume unequal variance and are similar to one-tailed unpaired t-tests. Table 1 shows Cohen's d effect sizes (compared to initial spike rates) and Welch's t-test results comparing control and treatment groups for each experiment. Tests were performed comparing groups at each experimental time point, except Recording 1, which was used to normalize data. Significant differences were found between treatment and controls for 50 μ l

groups in all instances of recordings 5–8 (post media addition). Results for the 20 μ l treatment group were less definite, with p-values close to 0.05 for all post-administration measurement points.

Effect sizes in Experiments 2 and 3 were very large in all post-administration recordings, with typical values in recordings 5–8 being over 100.

3.3. Observation of recovery

In Experiments 2 and 3, we observed that ganglion activity ceased consistently after added 50 μ l of KCl treatment solution. This behavior was observed in the initial data obtained for the 6 animals used in the recovery experiment. These animals were the treatment group for Experiment 2. Normalized data for each of the six animals monitored in this test is shown in figure 5. It is clearly from figure 5 that all ganglia recommenced spiking after the solution was changed to ASW, with all animals producing spikes at 40 mins after treatment solution was replaced with 200 μ l of control media.

Figure 6 shows raw neural activity recordings from one ganglion in the recovery group (AAG 5). Five sets of recordings are shown, at two different timescales. The left side of figure 6(a) shows short-term recordings on a scale of 1 second, whereas the right side (b) shows the recording over a 10-minute time period. Each sub-image shows signals from each electrode of the 32-channel array shown in figure 1(a). For this AAG, the spiking rate was on average 1 spike/second at the beginning at the experiment. The AAG became more active (a maximum of 3 spikes/second) in the first minute after KCl treatment, but activity was not detected after 3 minutes. After the original solution was replaced by ASW, activity did not recover immediately. However, spiking recommenced 40 mins post-ASW replacement, as shown in the last recording in figure 6(b). At this point the maximum spiking rate in some channels was 3 spikes/s, but overall spiking was depressed, leading to a lower average spike rate. Similar behavior time courses were observed in other samples.

4. Discussion

In our experiments, we found out that increasing the potassium ion concentration from 8.24 mM to 88.72 mM caused activity in the AAG to cease. Interleaving MEA monitoring with 250 μ A current injection did not change this result, which indicates that these external currents did not persistently affect activity levels.

Increases in extracellular potassium concentration should raise resting membrane potentials. This is because intracellular potassium concentrations are typically much higher than extracellular ones, and increases in extracellular potassium concentrations should reduce potassium Nernst potentials, depolarizing cell membranes, and possibly making them more excitable. Neural membrane potential was found to be linearly related to the log of extracellular potassium concentration in a range that depends on the tissue species (Huxley and Stämpfli, 1951, Nicoll, 1979), at higher concentrations. However, membrane depolarization does not always lead to greater excitability (Malenka et al., 1981). Extended depolarizations have been found to result in reduced sodium inactivation, ultimately leading to conduction failure (Hodgkin and Huxley, 1952). For example, Hablitz and Lundervold

(Hablitz and Lundervold, 1981) found that when the concentration of extracellular K^+ was higher than 15.25 mM, evoked activity in guinea pig hippocampal slices terminated. This behavior was reversible in most cases, with spiking resuming when the tissue was returned to a 3.25 mM potassium environment. Hablitz and Lundervold's study was not able to follow the time course of the activity cessation, but it is possible that activity in their tissue followed a similar time course to that found in our study, that is, that activity increased for a short period of time following administration of the high concentration KCl solution, after which time it stopped.

Studies of osmotic stress and ion stress also point out a potential mechanism for neuron behavior during these manipulations. Addition of high-concentration potassium to extracellular space may have resulted in cell shrinkage, which is likely to be reversible, whereas use of smaller concentrations of potassium may have resulted in cell swelling. In Pichon and Treherne's study on *M. squinado* (Pichon and Treherne, 1976) it was reported that a hyperpolarization of the axon and decline in the amplitude of action potentials occurred after salinity in the external medium was reduced. It was supposed that osmotic swelling increased potassium permeability of the axonal membrane and led to a net potassium efflux. It is also possible that a reverse mechanism may manifest, that is, that osmotic shrinking decreases potassium permeability and overall activity.

We note three limitations in this experiment. First, we set a 5σ threshold for spike detection. Thus, for Experiments 2 and 3, we cannot say that that AAG activity stopped completely after KCl addition. It is possible that spike amplitudes decreased and were too low to be detected, while spiking rates stayed the same. Second, in our analysis we summed all channel data to obtain an average number representing each recording, which means that there was no spatial discrimination in our experiment. A more detailed experiment would investigate effects on individual channels or structures within the ganglion. However, on average, it was clear that KCl solution inhibited all neural activity after the 50 μ l of the 411 mM KCl solution was added. Finally, MEA recordings were not synchronized with current injection, since spike detection was complicated by the presence of external current injection waveforms. Therefore, differential spike rates during current injection could not be directly measured. However, the result of the third experiment showed that the external current injection did not persistently modulate the neuronal activity. This is important because if baseline activity was changed by current administration alone this may confound treatment effects relative to controls.

Our results indicate that this treatment should be useful for MREIT experimental models, as it affected the ganglia in a rapid, consistent and reversible way. This reversibility may be an advantage compared to other treatments such as TTX. Further experiments should be performed to investigate the relationship between KCl concentration and activity level changes in more detail.

5. Conclusion

It was found that increasing extracellular potassium concentration to approximately 90 mM can be used to rapidly inhibit AAG activity. The current injection protocol used in our

experiment did not appear to affect activity levels. The effect of KCl administration could be reversed by rinsing the preparation in ASW, which may be useful in experimental settings where repeated images are gathered on the same animal. We conclude from these observations that hypertonic KCl can be used to reliably decrease neural activity levels in functional neuroimaging experiments.

6. References

- ATKINSON MJ & BINGMAN C 1998 Elemental composition of commercial sea salts. *Journal of Aquaculture and Aquatic Sciences*, VIII, 39–43.
- BANDETTINI PA, PETRIDOU N & BODURKA J 2005 Direct detection of neuronal activity with MRI: Fantasy, possibility, or reality? *Applied Magnetic Resonance*, 29, 65–88.
- CASSARÀ AM, HAGBERG GE, BIANCIARDI M, MIGLIORE M & MARAVIGLIA B 2008 Realistic simulations of neuronal activity: A contribution to the debate on direct detection of neuronal currents by MRI. *NeuroImage*, 39, 87–106. [PubMed: 17936018]
- CHAUHAN M, INDAHLASTARI A, KASINADHUNI AK, SCHÄR M, MARECI TH & SADLEIR RJ 2017 Low-Frequency Conductivity Tensor Imaging of the Human Head in vivo using DT-MREIT: First Study. *IEEE Transactions on Medical Imaging*, in press.
- DOMINGUEZ G & FOZZARD HA 1970 Influence of extracellular K⁺ concentration on cable properties and excitability of sheep cardiac Purkinje fibers. *Circulation Research*, 26, 565–574. [PubMed: 5443134]
- EGERT U & MEYER T 2005 Heart on a chip - extracellular multielectrode recordings from cardiac myocytes in vitro. *Practical Methods in Cardiovascular Research*.
- FRAZIER WT, KANDEL ER, KUPFERMANN I, WAZIRI R & COGGESHALL RE 1967 Morphological and functional properties of identified neurons in the abdominal ganglion of *Aplysia californica*. *Journal of Neurophysiology*, 30, 1288–1351.
- HABLITZ JJ & LUNDERVOLD A 1981 Hippocampal excitability and changes in extracellular potassium. *Experimental Neurology*, 71, 410–420. [PubMed: 7449908]
- HODGKIN AL & HUXLEY AF 1952 The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*. *The journal of Physiology*, 116, 497. [PubMed: 14946715]
- HUXLEY AF & STÄMPFLI R 1951 Effect of potassium and sodium on resting and action potentials of single myelinated nerve fibres. *The journal of Physiology*, 112, 496–508. [PubMed: 14825229]
- JEONG WC, SAJIB SZK, KATOCH N, KIM HJ, KWON O & WOO EJ 2016 Anisotropic conductivity tensor imaging of canine brain using DT-MREIT. *IEEE Transactions on Medical Imaging*, 1–8. [PubMed: 26151933]
- KASINADHUNI AK, INDAHLASTARI A, CHAUHAN M, SCHÄR M, MARECI TH & SADLEIR RJ 2017 Imaging of Current Flow in the Human Head During Transcranial Electrical therapy. *Brain Stimulation*, 10, 764–772. [PubMed: 28457836]
- KWON OI, SAJIB SZ, SERSA I, OH TI, JEONG WC, KIM HJ & WOO EJ 2016 Current Density Imaging During Transcranial Direct Current Stimulation Using DT-MRI and MREIT: Algorithm Development and Numerical Simulations. *IEEE Trans Biomed Eng*, 63, 168–75. [PubMed: 26111387]
- MALENKA RC, KOCSIS JD, RANSOM BR & WAXMAN SG 1981 Modulation of parallel fiber excitability by postsynaptically mediated changes in extracellular potassium. *Science*, 214, 339–341. [PubMed: 7280695]
- NICOLL RA 1979 Dorsal root potentials and changes in extracellular potassium in the spinal cord of the frog. *The journal of Physiology*, 290, 113–127. [PubMed: 224169]
- NOVAK JL & WHEELER BC 1986 Recording from the *Aplysia* abdominal ganglion with a planar microelectrode array. *IEEE Transactions on Biomedical Engineering*, BME-33, 196–202.
- OGAWA S, LEE TM, KAY AR & TANK DW 1990 Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proceedings of the National Academy of Science USA*, 87, 9868–9872.

- PETRIDOU N, PLENZ D, SILVA AC, LOEW M, BODURKA J & BANDETTINI PA 2006 Direct magnetic resonance detection of neuronal electrical activity. *Proceedings of the National Academy of Science USA*, 103, 16015–16020.
- PICHON Y & TREHERNE J 1976 The effects of osmotic stress on the electrical properties of the axons of a marine osmoconformer (*Mala squinado*, Brachyura: crustacea). *Journal of Experimental Biology*, 65, 553–563. [PubMed: 1018162]
- SADLEIR RJ, FU F, FALGAS C, HOLLAND SK, BOGGESS M, GRANT SC & WOO EJ 2017 Direct Detection Of Neural Activity In Vitro Using Magnetic Resonance Electrical Impedance Tomography (MREIT). *NeuroImage*, 161, 104–119. [PubMed: 28818695]
- SADLEIR RJ, GRANT SC & WOO EJ 2010 Can high-field MREIT be used to directly detect neural activity? Theoretical Considerations. *NeuroImage*, 52, 205–216. [PubMed: 20382240]
- SEO JK & WOO EJ 2011 Magnetic resonance electrical Impedance tomography (MREIT). *SIAM Review*, 53, 40–68.
- SULLIVAN GM & FEINN R 2012 Using effect size - or Why the P value is not enough. *Journal of Graduate Medical Education*, 4, 279–282. [PubMed: 23997866]
- TRUONG T-K, AVRAM A & SONG AW 2008 Lorentz effect imaging of ionic currents in solution. *Journal of Magnetic Resonance*, 191, 93–99. [PubMed: 18180187]
- ZOTEV VS, MATLASHOV A, VOLEGOV P, SAVUKOV IM, ESPY M, MOSHER JC, GOMEZ JJ & KRAUS RH 2008 Microtesla MRI of the human brain combined with MEG. *Journal of Magnetic Resonance*, 194, 115–120. [PubMed: 18619876]

Figure 1.

(a) 32-channel flexible multi electrode array (flexMEA) (Multichannel Systems, Reutlingen, Germany). (b) Sample chamber schematic, showing dimensions. There were four ports in the perimeter for current introduction. A slot at the bottom was used to introduce the flexMEA (c) Close up of the experiment setup. The metal enclosure was used to shield environmental electromagnetic fields. (d) Ganglion placed on MEA.

Figure 2. Experimental schedule. Part (a) shows the procedure for the first and second experimental groups. Part (b) shows the procedure used for groups in the third experiment involving current injection.

Figure 3.
Current patterns used in Experiment 3 protocol.

Figure 4.

Averaged normalized ganglion activity in each experiment. Comparison of pooled control and treatment groups for (a) 20 μ l KCl, (b) 50 μ l KCl and (c) 50 μ l KCl combined with current administration experiments. Error bars indicate standard errors. Dotted lines in each plot indicate media administration time points. Black rectangles in (c) represent current injection periods between monitoring periods.

Figure 5.

Normalized data from animals in the 50 μ l KCl treatment recovery experiment. AAG 1–6 were the treatment group from Experiment 2. Dotted lines indicate times at which the KCl solution was added, and when ASW solution was restored to the ganglion.

Figure 6. Recordings from AAG 5 in treatment recovery group (treatment AAGs from Experiment 2), showing typical responses before and after each manipulation stage. Part (a) plots show 1 s of recording from all 32 channels, and (b) show 10 minute recordings for all channels. Detected spikes are indicated with green lines.

Table 1.

Effect sizes and p-values in experimental comparisons for Experiments 1, 2 and 3. Effect sizes compare initial control (C) and treatment (T) measurements (Record 1) with subsequent measurements. Welch's t-test p-values are also shown comparing normalized control and treatment spike data at each time point. Effect sizes (C or T) or p-values are indicated by the measure (M) column. Recording 1 (R1) results are not shown because they were used to normalize data. Significance was set at $\alpha = 0.05$. Significant differences are denoted in red text, effect sizes >10 are highlighted with green text.

Exp	M	R1	R2	R3		R4	R5	R6	R7	R8
(1) 20 μ l	C	-	1.1	-0.15		1.58	1.00	-0.07	0.036	0.145
	T	-	-0.27	-0.253		1.64	3.64	7.80	14.22	28.03
	P	-	0.7989	0.6114		0.0722	0.0121	0.0506	0.035	0.0168
(2) 50 μ l	C	-	-0.33	-0.44		1.08	1.09	0.91	1.12	0.67
	T	-	-0.11	-0.03	Solution Added	3.53	756	513	857	641
	P	-	0.5294	0.3677		0.0127	0.0019	0.0053	0.0043	0.0059
50 μ l (3) + Current	C	-	-1.2	-1.4		-0.38	-0.47	0.19	0.04	0.52
	T	-	-0.01	-0.37		7.9	100	219	199	176
	P	-	0.1232	0.4528		0.0096	0.0018	0.0163	0.0207	0.0299