Journal of International Research in Medical and Pharmaceutical Sciences

Volume 19, Issue 3, Page 36-51, 2024; Article no.JIRMEPS.12437
ISSN: 2395-4477 (P), ISSN: 2395-4485 (O)

Evaluation of the Effects of a Neurotransmitter on the Action Potential Parameters in Lumbricus Terrestris

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: https://doi.org/10.56557/jirmeps/2024/v19i38888

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.ikprress.org/review-history/12437

Original Research Article

Received: 02/08/2024
Accepted: 05/10/2024
Published: 09/10/2024

ABSTRACT

Background of the Study: Action potentials (APs) are crucial for transmitting electrical signals in neurons. They are influenced by the balance of ions across the membrane and the permeability of sodium and potassium channels. Acetylcholine (ACh) affects neural activity, but its specific impact on AP parameters needs to be understood, mainly when administered near the ventral nerve area.
Aim: This study investigated the effects of acetylcholine on action potential parameters in L. terrestris, focusing on changes in response time, peak point, valley point, and wave width of AP evoked by electrical stimulation.
Methodology: The L. terrestris were placed on electrode arrays of the data acquisition system to record APs in their ventral nerve under 90 mV electrical stimulation. Baseline recordings were taken, followed by the administration of ACh near the ventral nerve cord. The resulting changes in

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Cite as: *Park, Rebecca. 2024. "Evaluation of the Effects of a Neurotransmitter on the Action Potential Parameters in Lumbricus Terrestris". Journal of International Research in Medical and Pharmaceutical Sciences 19 (3):36-51. https://doi.org/10.56557/jirmeps/2024/v19i38888.*

AP parameters were analyzed to assess the impact of these substances on neural conduction.
Conclusions: Acetylcholine increased the response time and peak amplitude of action potentials, while wave width and valley point were not dependent on the amount of Ach volume injected. Further clinical or in-vitro studies might be needed to improve our system understanding.

Keywords: Acetylcholine effects; action potential; electrical stimulation; neural conduction; ventral nerve cord.

1. INTRODUCTION

An action potential is a rapid sequence of changes in the voltage across a membrane [1, 2]. The membrane voltage, or potential, is determined at any time by the relative ratio of ions, extracellular to intracellular, and the permeability of each ion. In neurons, the rapid rise in potential, depolarization, is an all-or-nothing event initiated by the opening of sodium ion channels within the plasma membrane [3, 4]. The subsequent return to resting potential, repolarization, is mediated by the opening of potassium ion channels. To re-establish the appropriate balance of ions, an ATP-driven pump (Na/K-ATPase) induces the movement of sodium ions out of the cell and potassium ions into the cell [5].

Conduction velocity tests, specifically within the peripheral nerves, can determine if there are deficits in transmitting action potentials. Yet, further testing is needed to identify the conduction block's specific mechanism(s) or decreases in conduction velocity [6]. For example, a decrease in conduction velocity may be due to injured axons followed by remyelination with short internode length, nerve constriction as observed in carpal tunnel syndrome, or axonal tapering in the distal limbs [7]. Additionally, injury to the nerve, diabetic neuropathy, or demyelination caused by autoimmune disorders such as multiple sclerosis or Guillain–Barré syndrome could decrease the speed or even block the conduction of electrical signals within the nerves [8]. These pathological disturbances on nerve codes must change the AP parameters that were monitored in the study.

In addition to the pathologies that could modify the action potential in the peripheral nervous system, genetic disorders affecting ion channels, so-called channelopathies, might result in many different pathologies, depending on which tissues the channels are typically expressed [9]. Channelopathies may cause neuromyotonia, epileptic seizures, migraines, ataxia, or a host of heart, muscular, or GI conditions [10]. Local anesthetics act by blocking voltage-gated sodium channels, thus preventing the transmission of signals in pain and sensory fibers. Specifically, local anesthetics must pass through the plasma membrane, then bind to and block the channel pore while it is open [11].

Acetylcholine is the neurotransmitter at neuromuscular junctions, synapses in the visceral motor system's ganglia, and various sites within the central nervous system [12, 13]. Much is known about the function of cholinergic transmission at the neuromuscular junction and ganglionic synapses, but the actions of ACh in the central nervous system are not understood either [14]. The importance of this study might be seen as the fact that the effect of ACh on AP was investigated on the intact nerve code in vivo from invertebrate animals.

Acetylcholine is synthesized in nerve terminals from acetyl coenzyme A (acetyl CoA, which is synthesized from glucose) and choline in a reaction catalyzed by choline acetyltransferase (CAT) [15]. The presence of CAT in a neuron is thus a strong indication that ACh is used as one of its transmitters. Choline is present in plasma at a concentration of about 10 m*M* and is taken up into cholinergic neurons by a high-affinity Na+/choline transporter. About 10,000 molecules of ACh are packaged into each vesicle by a vesicular ACh transporter [16, 17].

The organophosphates are among the many exciting drugs that interact with cholinergic enzymes [18]. Compounds such as diphenyl trichloroethane (DTT) and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) were initially developed as insecticides [19]. This group also includes some potent chemical warfare agents. One such compound is the nerve gas “Sarin,” which was made notorious a few years ago after a group of terrorists released this gas in Tokyo's underground rail system [20].

Organophosphates can be lethal to humans and insects because they inhibit AChE, causing ACh to accumulate at cholinergic synapses. This

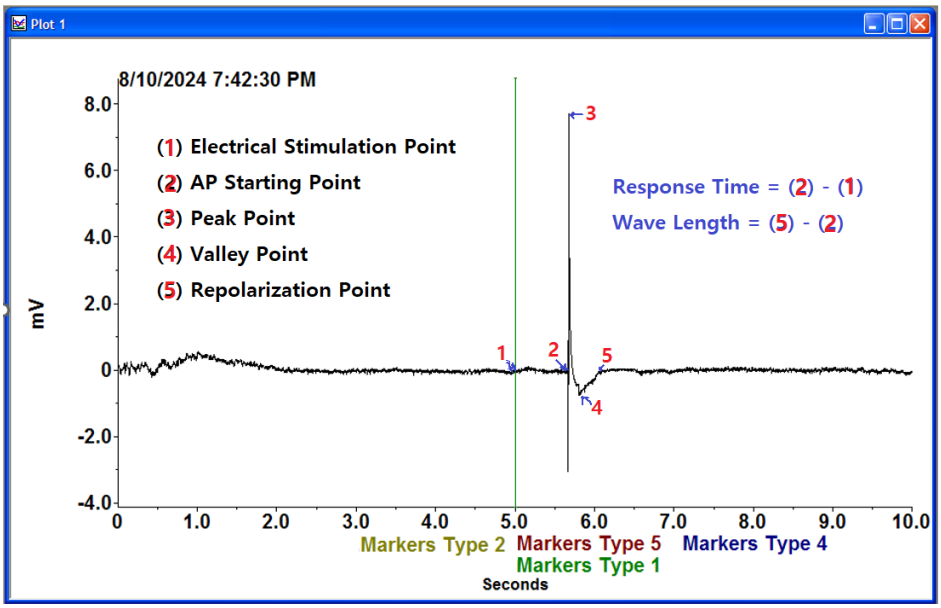

Fig. 1. One of the AP waveforms 90 mV that were electrically invoked for a baseline study

build-up of ACh depolarizes the postsynaptic cell and renders it refractory to subsequent ACh release, causing, among other effects, neuromuscular paralysis [21].

Earthworms possess three giant fibers within their ventral nerve cord, one median, and two lateral fibers [22]. These fibers are responsible for flight reflexes and twitches of the worm. Skin sensory cells of the worm's front end are connected to the median giant fiber (MGF), which has a diameter of up to 0.07 mm. The two lateral giant fibers (LGF) have a diameter of up to 0.05 mm and receive their input mainly from skin sensory cells of the hind end [23]. These two fibers have segmental cross-connections and can be regarded as a single functional unit. In general, earthworms and similar Oligochaetes have the advantage that recordings from these fibers can be performed without dissection. Because the skin and body muscle walls are thin, it is sufficient to place worms upon arrays of electrodes that make contact with the outer ventral part of the body. This has obvious advantages, including using either awake or anesthetized worms and the animals surviving the experiment intact.

This experiment aimed to examine the changes in AP parameters by injecting ACh near the nerve cord region. Our study defined the AP parameters as seen in Fig.1, which was retrieved from an electrically invoked AP of a baseline study. Other scientists have used the same definitions [24, 25]. However, the analysis method may be different from others. In addition, study purposes and injection materials were different as well. Fig.1 was a single event retrieved from AP waveforms evoked with 90 mV electrical stimulation. The AP parameters were defined as shown. Response time was calculated by subtracting the electrical stimulation point (1) from the AP starting point (2). The peak point was read from the highest point as (3), while the valley point from the lowest point as (4) in Fig.1. The wavelength was evaluated by subtracting AP starting point (2) from the repolarization point (5). The AP parameters might change by various factors, such as the conditions of sodium channels, potassium channels, and other pharmacological effects. In this study, we would like to investigate which parameters would have the most impact by administering ACh.

2. EXPERIMENTAL METHODS

2.1 Materials and Reagents

The 10% ethyl alcohol for anesthesia was prepared with 70% rubbing alcohol, diluting using distilled water. Our data acquisition system was purchased from Data Science International and consisted of a transmitter, AD converter, electrodes, receivers, data conditioning matrix, ambient pressure sensor module, and PC computer. The L. terrestris, as generally called Canadian nightcrawlers, was purchased from local Dick's Sports Center in Paramus, NJ.

Dissection pins and surgical boards were prepared before every experiment. An electrical balance was needed to monitor the size and weight of the earthworm. At the same time, a magnet and a radio were used to confirm the functionality of the transmitter, which had an internal magnetic switch that worked by magnetism.

2.2 Study Procedures

Our study took eight steps, from data acquisition system preparation to data analysis, as illustrated in Diagram 1 below. Each subsection is described as subsection as below.

2.2.1 Setup data acquisition system for software

Our system consisted of a transmitter, receiver, AD converter, ambient pressure sensor, data distributor multiplexer, and a PC computer. All the systems were purchased from Data Science International (St. Paul, MN). The transmitter had a positive and a negative electrode for measuring the electrical potentials passing through the ventral nerve cord. The flexibility of the coiled wire electrode facilitated the AP measurement despite minor movement during the animal study. The potentials were analyzed as an action potential induced by electrical stimulation.

The diagnostic software was first opened to do the acquisition, and everything on the computer system was tested to ensure everything was running smoothly. After testing was done, the Acquisition system was opened. From there, "configuration" was opened, followed by settings. In the study folder section, at the end of the document description, the experimenter's name, along with the year, month, and day, was typed. For the alternate folder, the same thing was typed in. Next, "Rat 1" was chosen to collect data because this telemetric system was assembled initially to study small animals like rats. Control was opened, the sampling started, and " continuous " was selected. The "save and trace" box was then marked off. Once all this was done, the ECG graph showed.

2.2.2 Stock solution preparation

Acetylcholine chloride (C7H16ClNO2) was purchased from Sigma-Aldrich (Burlington, MA, USA). Its molecular weight was 146.2 g/mole, and its solubility in water was 100 mg/ml.

For creating 20 mM ACH, 300 mg Ach was weighed out and transferred to a 100 mL beaker, to which 100 mL distilled water was added. The solution was stirred for 5 minutes on a magnetic stirrer. For the study, a serial dilution of the stock solution with a lower Ach concentration was performed.

2.2.3 Animal preparation and electrode placement

To start this experiment, an earthworm was randomly picked from its container and washed in water to remove the dirt off the earthworm's body. Then, the earthworm was weighed on the scale, and the weight was recorded. After weighing, the earthworm was immersed in 10% alcohol for 7-10 minutes to get the worm's subconscious. After several minutes, with a Q-tip, touching the worm's skin was to make sure the worm was subconscious.

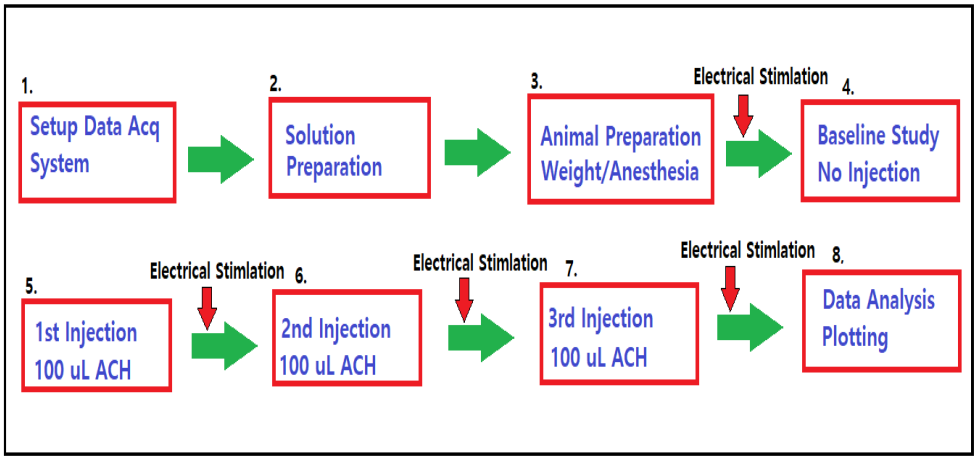

Diagram 1. Presents a stepwise illustration of our study

Fig. 2. Our telemetric data acquisition system, which consists of a transmitter, receiver, AD converter, and multiplexer

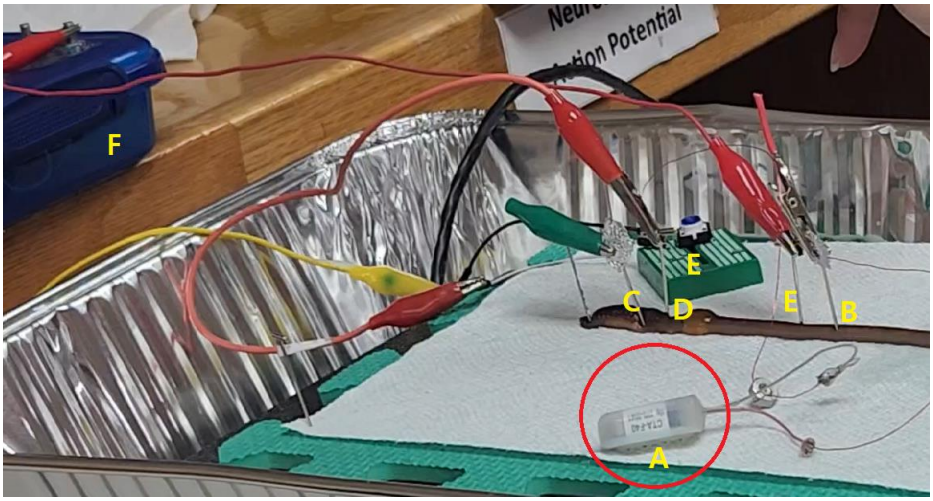

Fig. 3. The placement of electrodes from the transmitter and electrical stimulator

Then, the worm faced upward, which was the darker side of the body. Then, the earthworm was placed onto the surgical board while ensuring the worm's darker side was face up, spread out evenly, and pinned on the head and back end of the worm, as in Fig. 1. Two more pins were then pinned, one 2 cm in front of the clitellum (C) and 5 cm behind the clitellum (B). The transmitter (A) was turned on to clip the red line, the positive (B), onto the back pin and the clear line, the negative (C), onto the front pin. Two more pins were pinned opposite those two clips and 1 cm away from each pin. The stimulator was turned on, and with a wire, the P1 positive charge connected with the back clip in front of the first clip that was put 5 cm away from the clitellum. Another wire connected a handmade button on the front pin and the P2 negative charge with the front clip. To connect the button, the white wire of the button was connected with a wire onto the front clip, and the black wire was attached to the P2 stimulation point on the stimulator that was made in-house using a series of resistors. Finally, the earthworm was prepared for the experiment.

2.2.4 Experimental procedures

With the data acquisition system and animal preparation, baseline data was recorded for approximately 5 minutes while observing the waveforms of the resting state. After confirming the high quality of baseline data streaming, the first electrical stimulation was given, and an event marker was marked before any injection. Generally, three consecutive stimulations were

given for data averaging, all the time with event marking. Then, three minutes were waited to stabilize any physiological disturbance. 100 ul of ACH solution was filled into a 1 ml tuberculin syringe, and 2 cm posterior to the clitellum was injected into the earthworm's skin. The needle tip was placed close to the abdominal nerve cord area. After injection, the punctured site was pressed with the index finger for approximately 10 seconds to prevent any solution leaking. After the injection, another 3 minutes was spent to diffuse the solution to the active nervous site. Then, three electrical stimulation sessions were given with event marking activities. Two more consecutive ACH administrations were performed using procedures identical to the first injection. Therefore, 300 ul of ACH administrations were completed accumulatively in the same area of the animal.

2.3 Data analysis

Data was acquired as a bipotential waveform with event markers placed at every critical moment, such as injection and electric stimulation times. After a preliminary study with multiple magnitudes of electric potentials, 90 mV stimulation was chosen for the following experiment. After retrieving the survey, each injection and stimulation point was identified using the event markers. We prepared a data summary template in Google worksheet, in which data averages and plots were created simultaneously by reading the original retrieved data and typing it in. Final plots were made in Microsoft Excel since its functionality was more versatile and easy to use. The trendline function was used with the polynomial equation with regression coefficients.

3. RESULTS AND DISCUSSION

3.1 Injection Volume Relation to Response Time

In this group of studies, we used a single concentration, while stimulation potentials and injection volumes were changed to find an optimal magnitude of electrical potential. The relationship of response time (RT) with an injection volume of 1ug/ul Ach was deemed necessary, and it was examined first, as seen in Fig. 4 below, for injection volumes up to 600 ul. It was found that RT had a high correlation with injection volume with a regression coefficient of 0.9866, while the stimulation potentials seemed to be in no uniform patterns. It could be inferred from the data that the high stimulation potential might hold the electrons slower in the system. More clarification studies might be needed. We can see that AI nerve conduction velocity (NCV) slows as electrical potential increases [26].

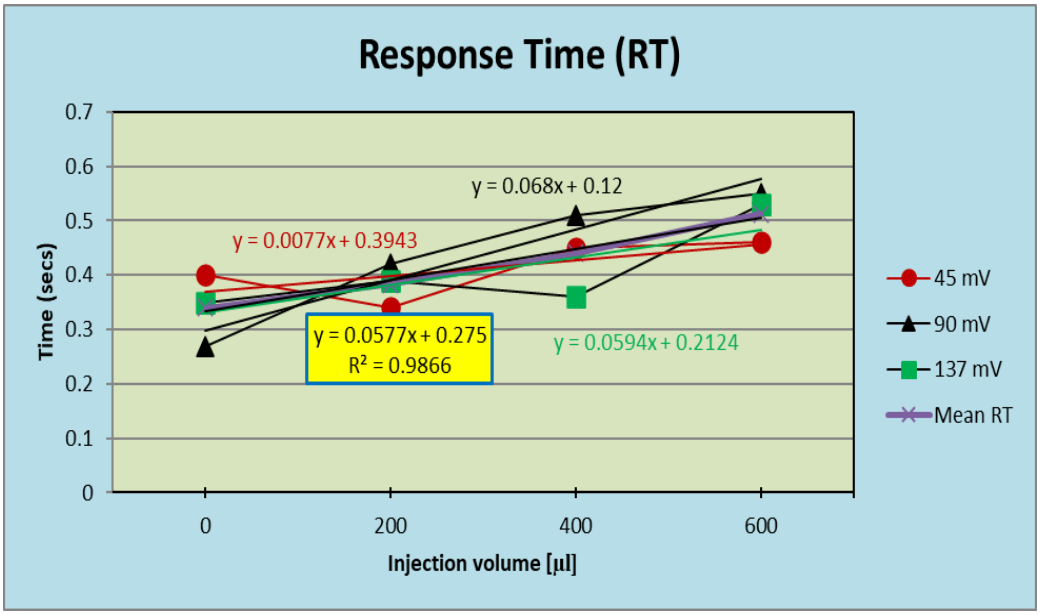

Fig. 4. The relationship of response time for injection volume (n=3)

3.2 Injection Volume Relation to Peak Point (PP)

The positive feedback of the rising phase slows and comes to a halt as the sodium ion channels become maximally open. At the peak of the action potential, the sodium permeability is maximized, and the membrane voltage Vm is nearly equal to the sodium equilibrium voltage [27]. The relationship of the peak point with an injection volume of 1ug/ul Ach might be necessary, as measured in Fig. 5 below. The data suggested that PP had a relatively weak correlation with injection volume with a regression coefficient of 0.2080, while the stimulation potentials seemed to be in no uniform patterns. It was understood that the peak point might not change much by the magnitude of any stimulation in principle.

3.3 Injection Volume Relation to Valley Point (VP)

The lowest point of an action potential is defined here as a valley point, which is usually around -50 to -55 mV if measured at cellular levels [28]. However, our measured point might be different from the actual potential points. Still, this is the minimum potential difference a neuron needs to reach to fire an action potential. For most neurons in humans, the resting membrane potential is -70 mV, so a signal to a resting cell must raise the membrane potential to -55 mV to trigger an action potential. The relationship of the valley point with an injection volume of 1ug/ul ACH was considered valuable and measured as presented in Fig. 6 below. It was found that PP was decreased with injection volume with a polynomial regression coefficient of 0.7542, while PP was not much different by the stimulation potentials.

3.4 Injection Volume Relation to Wave Width (WW)

The width of an action potential in cortical neurons typically ranges from 1 to 6 milliseconds (ms). The width of an action potential is linearly correlated with its height, which usually varies from 0 to 30 millivolts (mV) [29]. In our system, WW was far more significant compared to the actual number at the cellular level. This could happen since all the in vivo study systems are complicated, with innumerable interconnections and extra signals from other cells. Therefore, our data should be accepted as an integrated expression from groups of nerve cells away from their original points. Still, our valley point with an injection volume of 1ug/ul ACH was deemed essential and measured, as seen in Fig. 7 below. PP increased with injection volume with a regression coefficient of 0.7238, while the stimulation potentials seemed independent.

3.5 Action Potential Parameter Changes at 20 mM Ach Injection

From this group of studies, we measured the AP parameter changes for injection with various Ach concentrations from 20 mM. Fig. 8 presents the action potential parameter changes when injected with a 20 mM Ach solution.

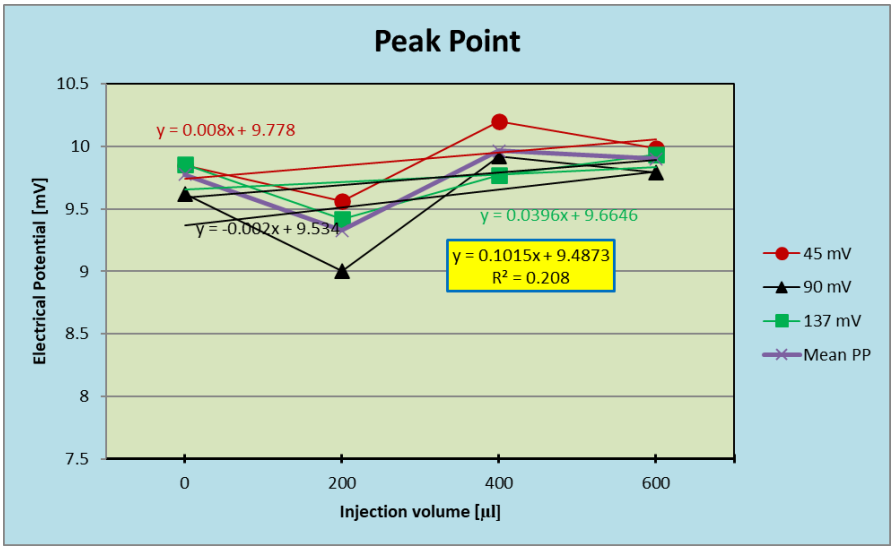

Fig. 5. The relationship of peak point for injection volume (n=3)

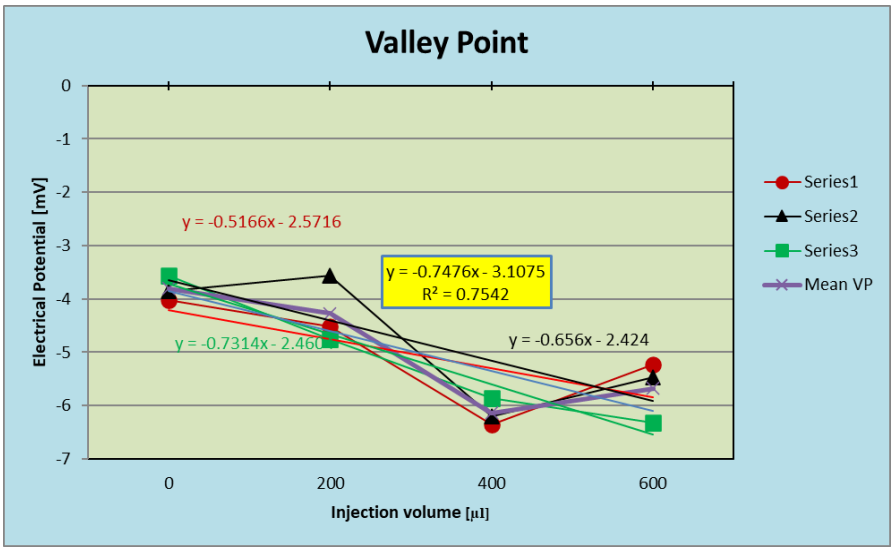

Fig. 6. The relationship of valley point time for injection volume (n=3)

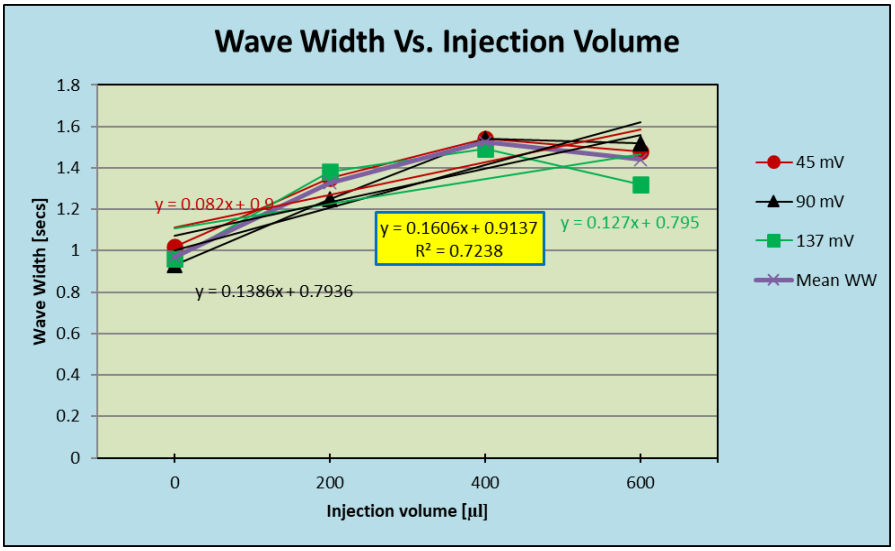

Fig. 7. The relationship of wave width for injection volume

The points on the graph are the average of ECG action potential marks at each injection volume. As seen below, Fig. 8a shows the response time change for the accumulated volume. The response time was not dependent on the injected volume. At the same time, wave width, as in Fig.8b, looked consistent with a polynomial relationship for the accumulated volume. Its R2 was equal to 0.999, which was an almost perfect fit. Fig.8c presents the peak point change for the ACH accrued volume.

Interestingly, the first three points were minimally changed, and then the peak point lowered to 9.50. There might be some critical point between 200 and 300 ml of accumulated ACH. The valley points in Fig. 8d didn't change significantly for the accumulated volume of ACH.

3.6 Action Potential Parameter Changes at 10 mM ACH Injection

Fig. 9 presents collections of graphs from the AP potentials with an ACH injection of 10 mA. The data was compared with those of the previous set with 20 mA ACH, as shown in Fig.9. At this concentration, the trend of parameter value changes looked similar, except for peak paint, which increased straight up for accumulated volume. We didn't know at this point why it could happen. Further study would give a better clue for the increase.

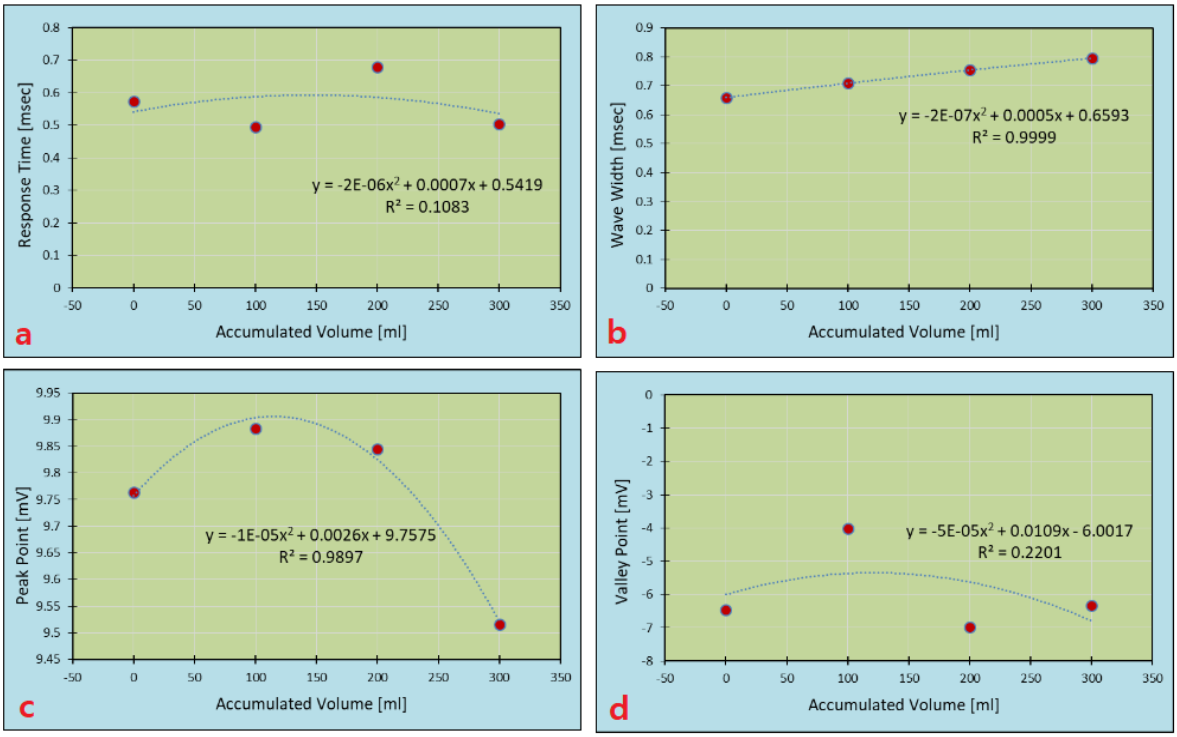

Fig. 8. The change of AP parameters with respect to the accumulated volume of 20 mM ACH (n=3)

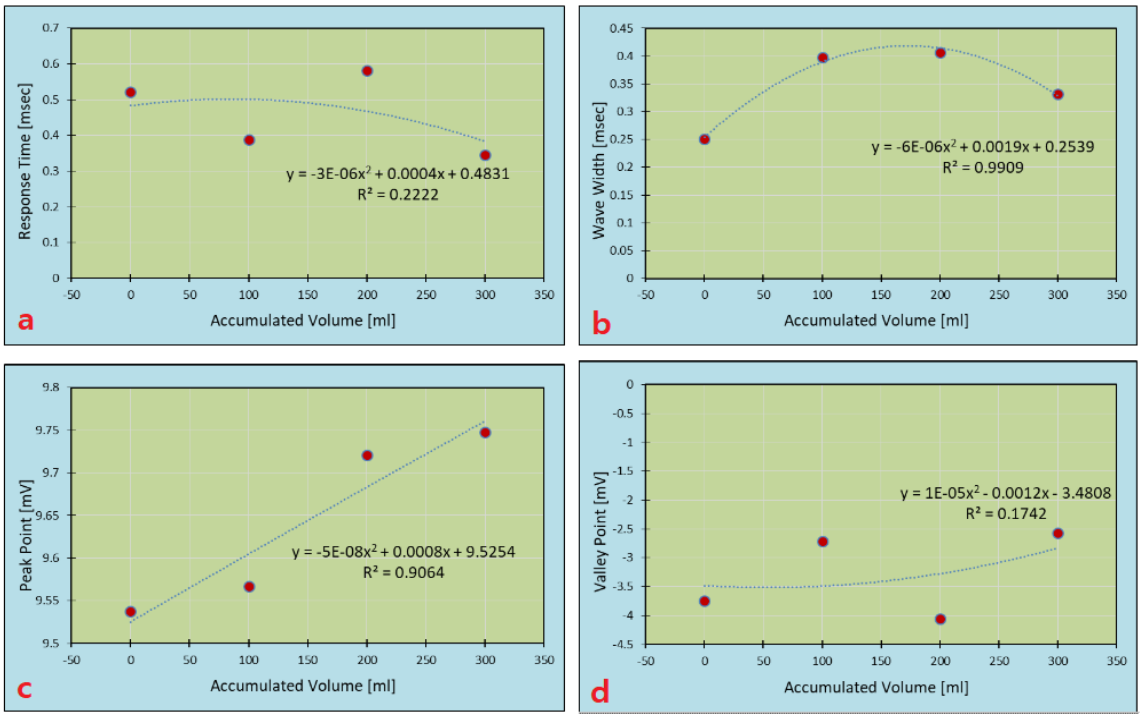

Fig. 9. the change of AP parameters with respect to the accumulated volume of 10 mM ACH (n=3)

3.7 Action Potential Parameter Changes at 5 mM ACH Injection

Fig. 10 is a collection of graphs from action potentials with ACh injection of 5mM. At this concentration of ACh, the parameter values seem to be fluctuating in the response time throughout each accumulated volume, as shown in Fig. 10a. As more acetylcholine (ACH) was injected into an earthworm, the increased wave width distance on the ECG, as shown in Fig. 10b, indicated a slowing of electrical activity due to prolonged depolarization and repolarization of the cells. This suggests that ACh is reducing the conduction velocity of action potentials, likely through its hyperpolarization effects on the cell membrane. The line of best fit (trendline) for the peak points shows an over-drastic decrease as more ACh was injected. However, the data points regarding the y-coordinates are relatively close and don't have much difference. The valley points' line of best fit represents the gradual decrease, suggesting that excessive acetylcholine leads to receptor desensitization or overstimulation. Overall, the more acetylcholine wasinjected, the more the physiological response was reduced.

3.8 Action Potential Parameter Changes at 3 mM ACH Injection

Fig. 11 presents the graphs from action potentials with ACh injection of 3 mM. When the accumulated volume in mL of acetylcholine increased. The Response Time graph shows a decrease in the first half, then increases back up to around the same as at baseline with 0 mL injected. The Wave Width graph shows a consistent increase with the injection of acetylcholine. The least tendency of changes when injected with acetylcholine is in the Peak Point graph. The Valley Point graph shows a decrease mainly due to the injection of acetylcholine.

3.9 Action Potential Parameter Changes at 0 mM ACH Injection

Fig. 12 is a collection of graphs from action potentials with ACh injection of 0 mM. The graphs below show how the effects on action potential on a Lumbricus terrestris change when only water is injected. It can also compare the acetylcholine-injected graphs and the water-injected graphs. Both the Response

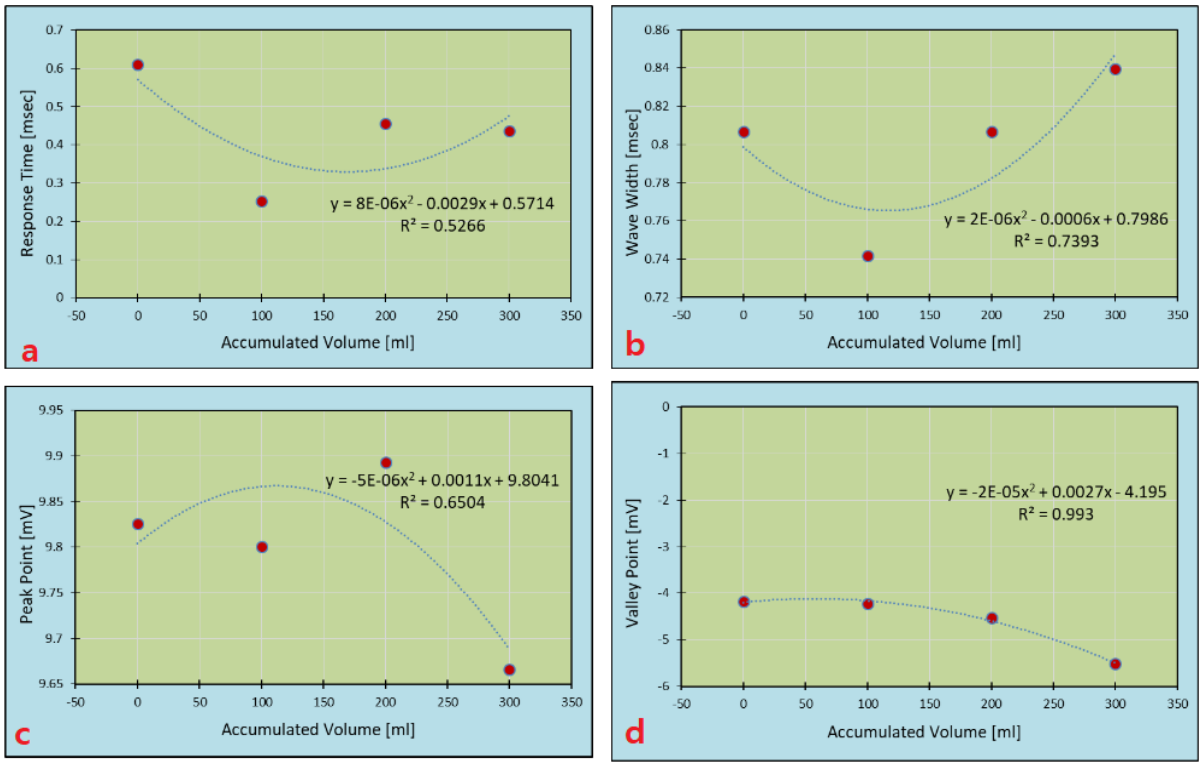

Fig. 10. The change of AP parameters with respect to the accumulated volume of 10 mM ACH (n=3)

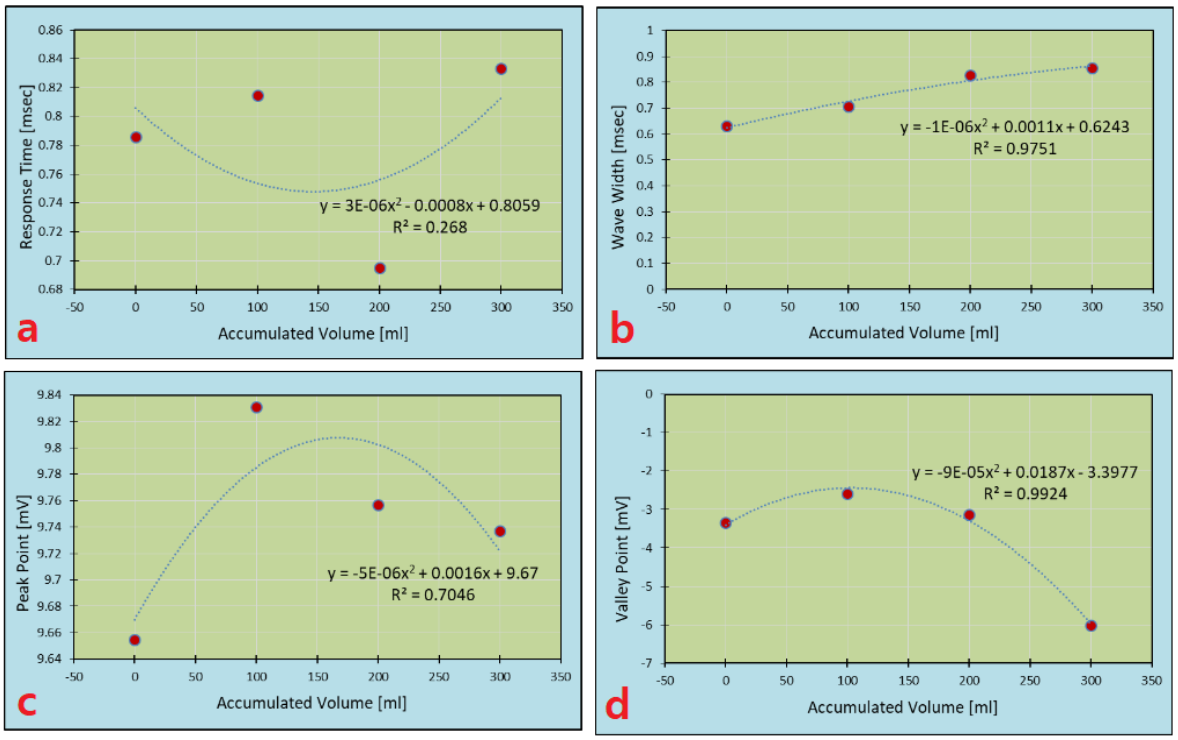

Fig. 11. The change of AP parameters with respect to the accumulated volume of 3 mM ACH (n=3)

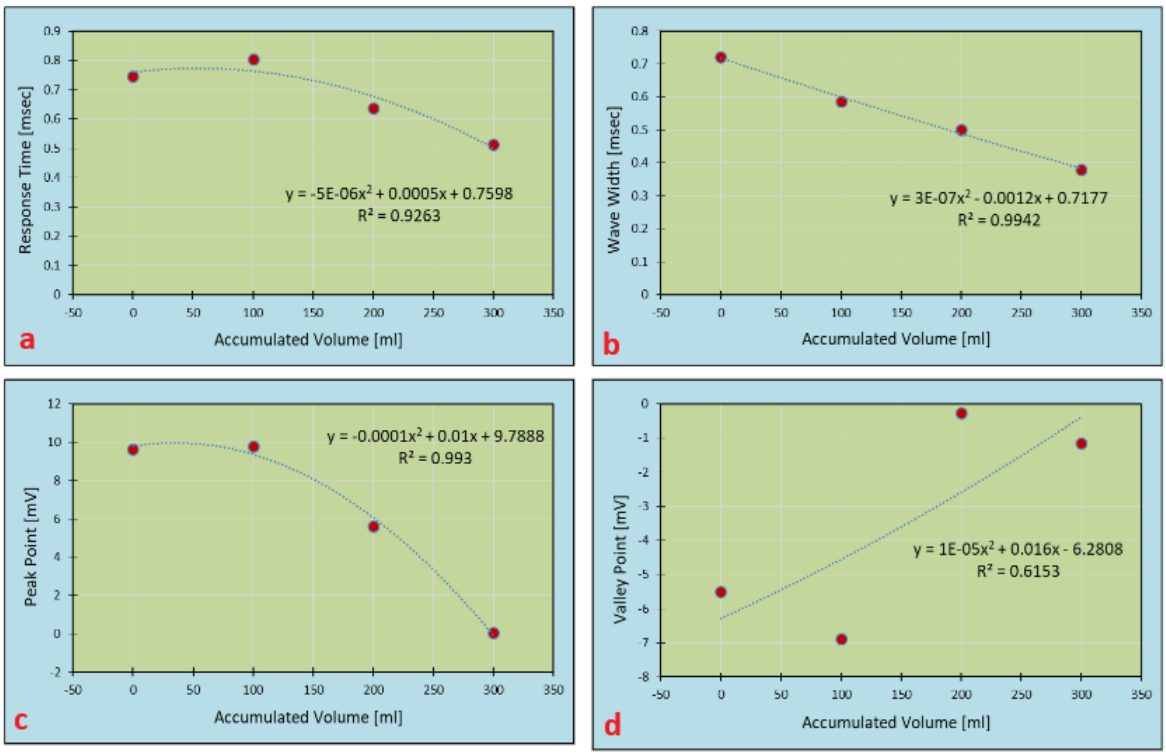

Fig. 12. The change of AP parameters with respect to the accumulated volume of 0 mM ACH (n=3)

Time and Wave Width graphs show a decrease trend when more volume is accumulated. While the Peak Point graph also shows a decrease, there is a more substantial decrease on the y-axis of the graph.

3.10 Comparison of Response Time Change

The response time was defined as starting action potential after the electrical stimulation. Our data showed that the response time was relatively consistent, within a 10% change for each point. However, the magnitude of response time seemed more dependent on the concentration of injected ACH volume with more significant change, though still having high variability.

3.11 Comparison of Wave Width Change

Wave width is estimated by subtracting the time point of AP start from the time point of AP end at which its AP was returned to the baseline levels. The data showed increased wave width due to increased accumulated volumes. However, the slope was not very steep, except for an Ach injection of 0 mM, at which time the magnitude of wave width decreased while the accumulated volume increased.

3.12 Comparison of Peak Point Change

The peak point was defined as the highest point of the action potential. Our data showed that the peak points were almost consistent with the increase in accumulated volume injected into the animals, except for the injection of 0 mM Ach solution, in which the peak points decreased with respect to the accumulated volume. The consistency of the peak point should be more predictable, while the decreased change of peak point at 0 mM should be re-confirmed for doing more repeated studies.

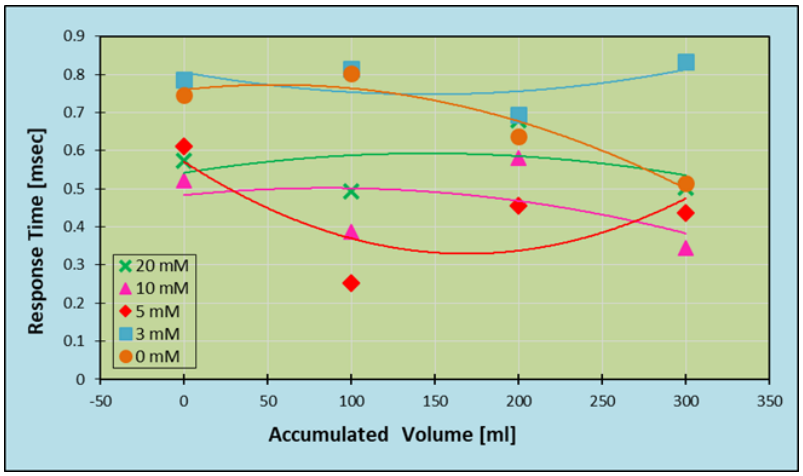

Fig. 13. the comparison of response time change for accumulated volume

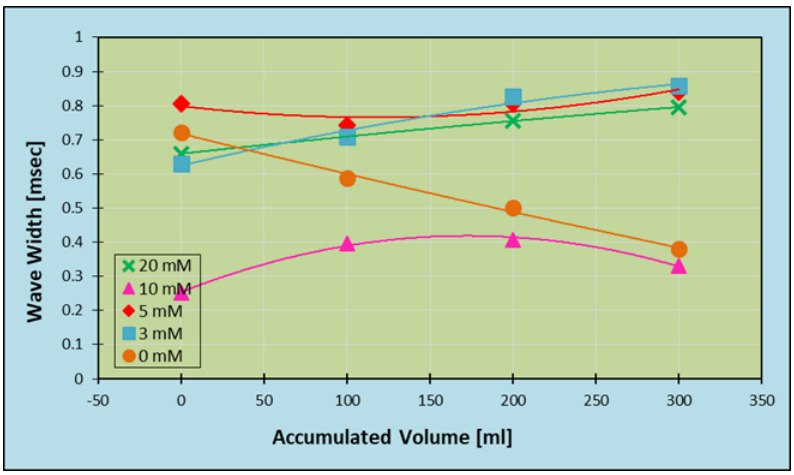

Fig. 14. the comparison of wave width change for accumulated volume

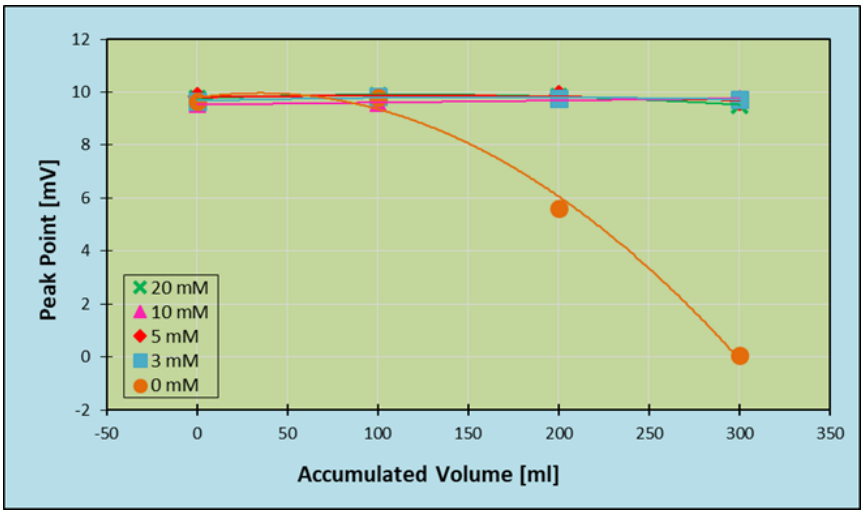

Fig. 15 the comparison of peak point change for accumulated volume

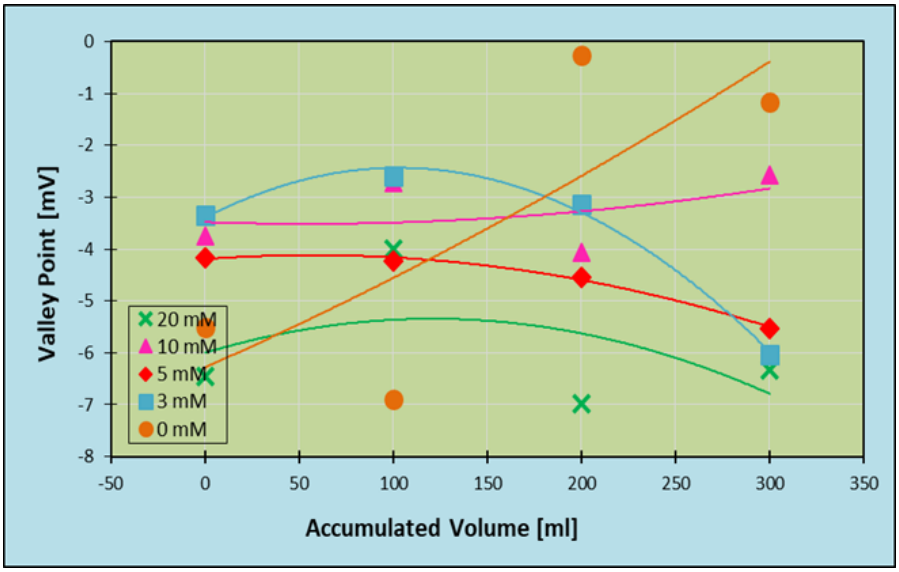

Fig. 16 the comparison of valley point change for accumulated volume

3.13 Comparison of Valley Point Change

The valley point was measured as the lowest point of the action potential. Our data showed that the valley points were almost consistent with the increase in the accumulated volume injected into the animals, except for the case of injection of 0 mM ACH solution, in which the peak points were increased with respect to the accumulated volume.

4. CONCLUSIONS

An action potential is a rapid change in voltage across a cell membrane with a characteristic pattern. A nerve impulse propagates along axon surfaces without reduction, affecting all parts of an excitable membrane once initiated. Acetylcholine is an ester of choline and acetic acid that serves as a transmitter substance of nerve impulses within the central and peripheral nervous systems. This study investigated the effects of acetylcholine on action potential parameters in L. terrestris, focusing on changes in response time, peak amplitude, valley point, and wave width of induced AP by electrical stimulation.

The L. terrestris were placed on electrode arrays of the data acquisition system to record APs in their ventral nerve under 90 mV electrical stimulation. Baseline recordings were taken,

followed by the administration of ACh. The resulting changes in AP parameters were analyzed to assess the impact of these substances on neural conduction. Acetylcholine increased the response time and peak amplitude of action potentials, while wave width and valley point were not dependent on the amount of Ach volume injected. Further, detailed cellular studies might help improve our understanding of the relationship between Ach injection and AP parameters.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.ikprress.org/review-history/12437