

Full Length Research Paper

Neurotoxicity of arsenite (As III): *In vitro* electrophysiological approach to studying neural activity in rat brain slices

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Arsenite (Arsenic III) is ubiquitous and a widely distributed environmental toxin implicated in the aetiology of several neurological disorders. It is found in sea food, chemicals and pesticides. As(III) is also a mitochondria poison that induces reactive oxygen species (ROS) formation and membrane distortion. This study is aimed at proposing an *in vitro* method to detecting neural activity in rat's brain treated with As(III). Neurotoxic effects of As(III) on brain slices in rat model and description of its varying cytotoxic pathways using electrophysiological approach shall constitute another focus of the study. Brain slices were sub-cultured in Artificial Cerebro Spinal Fluid (ACSF) and treated with 50 µl of 0.1 M arsenite for 30 min. A superficial pin electrode was used to record the electrical activity following each stimulation. The spike train was analyzed to obtain the frequencies in db and this was recorded for 0.00 to 0.16 ms. As(III) induced changes in the electrical activities of the brain to a varying extent and such changes vary with the cytotoxic pathways for such regions. In the parietal cortex and upper brainstem, As(III) reduced the firing rate when compared with increased neuronal firing rates in the lower brainstem as depicted by spike peaks.

Key words: Arsenic, neuron, peaks, frequency, toxicity, action potential.

INTRODUCTION

Arsenic (As) is a widespread environmental metalloid toxin released from several anthropogenic sources such as agrochemicals, herbicides, rodenticides, wood preservatives, minerals and industrial waste. It occurs naturally in earth's crust and widely distributed in soil, water and foods (Zhang et al., 2013). The presence of this environmental toxin in human foods (Calatayud et al., 2011), though in small amounts, is traceable to its ubiquitous nature and processes inherent to human activities.

It exhibits several oxidation states such as elemental (0), trivalent (-3, +3) and pentavalent (+5) (Dopp et al., 2004). The trivalent species (for example, arsenite) are known to exhibit greater acute toxicity than the pentavalent species (for example, arsenate) which are most frequently found naturally (Zhang et al., 2013).

It has been reported that human arsenic exposure causes several health problems such as cancer, liver damage, dermatosis and nervous system disturbances

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such as polyneuropathy, electroencephalography (EEG) abnormalities and in extreme cases, hallucinations, disorientation and agitation (Rodríguez et al., 2002). Although, previous studies showed that liver is one of the most important targets for As(III) toxicity (Chen et al., 2013a, b). It is also evident that arsenic exposure has a toxic effect on the nervous system (Rodríguez et al., 2003). It has been previously reported that the content of catecholamines in striatum, hippocampus and other cerebral regions changes in mice and rats exposed to arsenic (Rodríguez et al., 2001). Studies by Kalimuthu et al. (2013) revealed that As(III) induces both apoptosis and necrosis when administered *in vitro* in different cellular systems through failure of mitochondrial electron transport system, induction of mitochondrial permeability transition and subsequent release of reactive oxygen species (ROS).

Oxidation of As(III) to As(V) is evident in liver metabolism as well as the release of iron from ferritin by trivalent arsenic species (Wu et al., 2012). In biologic cells, both As (V) and As (III) are methylated to yield monomethylarsonic (MMA) and dimethylarsinic acids (DMA), forms which are more rapidly excreted in urine. But a recent proposal by Kitchin and Wallace (2005) submitted a new metabolic pathway for As(III) that it does not involve oxidative methylation but rather is mediated by As-glutathione complexes, S-adenosylmethionine (SAM) and human arsenic methyltransferase Cyt19 to monomethyl-(MADG) and dimethyl-(DMAG) conjugates which are hydrolyzed to MMA and DMA, respectively. Although, pentavalent methylated metabolites are also known to result from oxidation of their trivalent forms rather than the reverse (Lau et al., 2004).

This study examined the effect of As(III) on patterns of neural activity generated by freshly isolated rat brainstem and cortical tissues to propose an *in vitro* electrophysiological approach to studying neural functions. We hope to describe cytotoxic pathways (if possible) associated with arsenite induced neural dysfunction.

MATERIALS AND METHODS

Adult male Wistar rat was used for this study. The animal was procured and kept in the animal holding facility of Afe Babalola University and allowed to acclimatize. All procedures in the animal handling were in accordance with the guidelines of Animal use Ethical Committee of the Afe Babalola University. The animal was sacrificed by cervical dislocation and the brain removed using bone forceps. The brain tissue was perfused with cold dextrose saline before transfer into cold ACSF. The ACSF composition was as follows: 18 mM glucose, 119 mM NaCl, 2.5 mM KCl, 1.3 mM MgSO₄, 2.5 mM CaCl₂, 26.2 mM NaHCO₃ and 1 mM NaH₂PO₄. The solution was bubbled with 95% oxygen and 5% carbon dioxide. The tissue perfusion set up was maintained for 30 min both for the parietal cortex, upper and lower brainstems. For each type of set up, we had 2 different groups: the test group treated with 50 µl of

0.1 M arsenic III and the other is the control without any treatment.

Electrophysiology

The study utilizes the Spiker Box from Backyard Brains (www.backyardbrains.com). The device was connected to a computer interface (Audacity). Stimulation was at 1.5 mV and the action potential generated was recorded as spikes on the Audacity software. This was done for all the four setups.

- A: Upper Brain stem in ACSF
- B: Upper Brain Stem in ACSF + As(III)
- C: Parietal Lobe in ACSF
- D: Parietal lobe in ACSF + AS(III)

Spectrum analysis

The recorded spikes were processed using the menu on the audacity software (pc version). The project rate was set at 44100 Hz and the spike regions were trimmed out and zoomed to an interval of 0.010 s. The peak patterns were selected at regular intervals and analyzed by spectrum plotting to generate an algorithm in enhanced autocorrelation through which the frequency in db at 0.00 to 0.16 ms at an interval of 0.02 ms were obtained. The spectrum plot was later depicted in the Hanning window to show peak patterns at the recorded intervals.

Measure of the root mean square (RMS)

This is also known as the quadratic mean. It is the statistical measure of average magnitudes at a rate that account for both heights and peaks in a positive and negative direction. For this purpose we have employed the use of software SigView Version 2.6. By working on the signal tools, noise was eliminated below 350 Hz using the filter function and following the methods of Dagda et al. (2013). Other statistically determined parameters include the mean, max, standard deviation and summation.

RESULTS

In this study, the trend of action potential (AP) generated is described in different phases. It is also imperative that we distinguish the peaks (positive and negative deflections) within the spike trains. At 0.02 ms, the frequency of the AP (peaks) is highest with a value of 3158 db for the control with a decline observed in the As(III) treated group where the peak occurred at 1862 db. This decline for the treatment was observed for the 3 consecutive peaks. Although at $P < 0.05$, the difference between the frequency of the control and treatment was insignificant for the peaks observed at 0.14 ms (Figure 4).

In general, a reduction in neuronal firing was observed in the treated groups when compared with the control (Figures 4 and 5). But duration of AP and spike train potentiation was prolonged in the treated upper brainstem (Figure 5). The spike train for the upper brainstem shows that at 0.02 and 0.06 ms, the control recorded higher peaks

Table 1. Statistical analysis.

Parameter	Max	Mean	Standard deviation	Sum	RMS
Cortex control	32019	-193.086	11906.2	1.87E007	11906.2
Cortex-arsenic	32767	-209.601	12951.9	2.07E007	12951.9
Brain stem control	30697	-49.6714	9177.53	1.24E007	9177.53
Brain stem arsenic	31426	90.006	12085.8	1.942E007	12085.8

with value of 4553 and 2459 db which is higher than the treatment when compared at less than $P < 0.05$ (95% confidence interval) (Figure 5). The result shows that at 0.12 and 0.14 ms, the frequencies of the treatment were higher than the control. As depicted by the tracings (Figures 2A to 2D), premature potentials staggered with full term spiking were also observed in the treated groups. Inconsistency (also disorganized) in spiking was more in the treated upper brainstem than the treated parietal cortex.

Table 1 show the summary of data extrapolated from analyzing the spike trains and peaks-cortex (Figure 1A to D) and brain stem (Figure 2A to D). The RMS shows that for the arsenic treated cortex *in vitro*, an increase (12,951.9) was observed when compared to the control (11,906.2). In the brain stem, a larger increase was recorded; from 9177.3 in the control to 12085.8 in the treatment. Examining the spike train for the cortex also showed a negative mean value for the peaks (-193.086 for the control and -209.601 for the arsenic treatment) which further confirms that arsenic affects the activity and firing on these neural tissues. However in the brain stem, the control recorded a negative value [(-49.67) versus a positive value in the test (90.066)].

DISCUSSION

This study investigated the effect of As(III) treatment and its associated changes on the electrical activity of the parietal cortex and upper brainstem to describe an electrophysiological approach to studying neural functions. The characteristic nature of neuronal integration and spike generation is a function of imminent impulse transmission and temporal coding of information in excitable tissues. Most experiments in sensory neurophysiology have widely recorded arrival time of AP generated in nerve cells, temporal coding of information in the patterns of spikes and most importantly spike timing which plays a major role in encoding various aspects of the stimulus. Mostly neglected is the neurological analysis of spike peaks as well as the contributory role of neuronal membrane in impulse transmission induced by changes in conductance and ion fluxes.

The results of this study showed that there was a general

decline in neuronal firing rates in the As(III) treated groups when compared with the control. This is an indication of altered nerve function within the selected regions of the brain which may possibly result from changes in membrane permeability due to induced oxidative damage on membrane lipids and proteins, as previously reported by Jin et al. (2010). As(III) like any other metal toxins, such as Lead (Pb), Cadmium and Mercury, can affect mitochondrial oxidative enzymes. It is possible that this toxin interferes with energy coupling process by altering the redox states of cytochrome C enzyme. The resultant ROS formed will in turn induce peroxidation of membranes and loss of its ion channels.

Prolonged AP observed in the treated upper brainstem may likely be due to a sustained electrolyte leakage resulting from gradual loss of membrane integrity. Such damage could result from various mechanisms including oxidation and inhibition of key membrane proteins such as H^+ -ATPase or changes in the composition and fluidity of membrane lipids (Meharg, 1993; Demidchik et al., 1997). It is also thought to be due to suppression of efflux mechanism similar to inhibiting K^+ pump or down regulation or inhibition of Na^+ pump. Although the mechanism of As(III) toxicity is still not clearly understood but affinity of As(III) for Na ion and how sodium arsenic oxide (a potent toxin) induced processes such as oxidative stress, genotoxicity, cytotoxicity and cell cycle arrest have been previously described by Jiang et al. (2013). In normal physiologic conditions, the permeability of the membrane to potassium (PK) is much greater than the permeability to sodium (PNa) because there are many more leakage (non-gated) channels in the membrane for K^+ than in the membrane for Na^+ . Hence, K^+ leakage is considered a natural phenomenon which is responsible for repolarization and after hyperpolarization.

Since heavy metals have been widely reported to interfere with efflux of K^+ from plant cells (Meharg, 1993), we suggest that the observed arsenite-induced alteration in firing rate (Prolonged AP) of nerve cells in the treated upper brainstem employs recruitment of efflux mechanisms which is also similar to the findings by Namgung et al. (2003). Other studies also show that the sodium and potassium channels are either depressed or down regulated in this toxicity process (Shaya et al., 2013). This function can also be taken down to the organelle level where

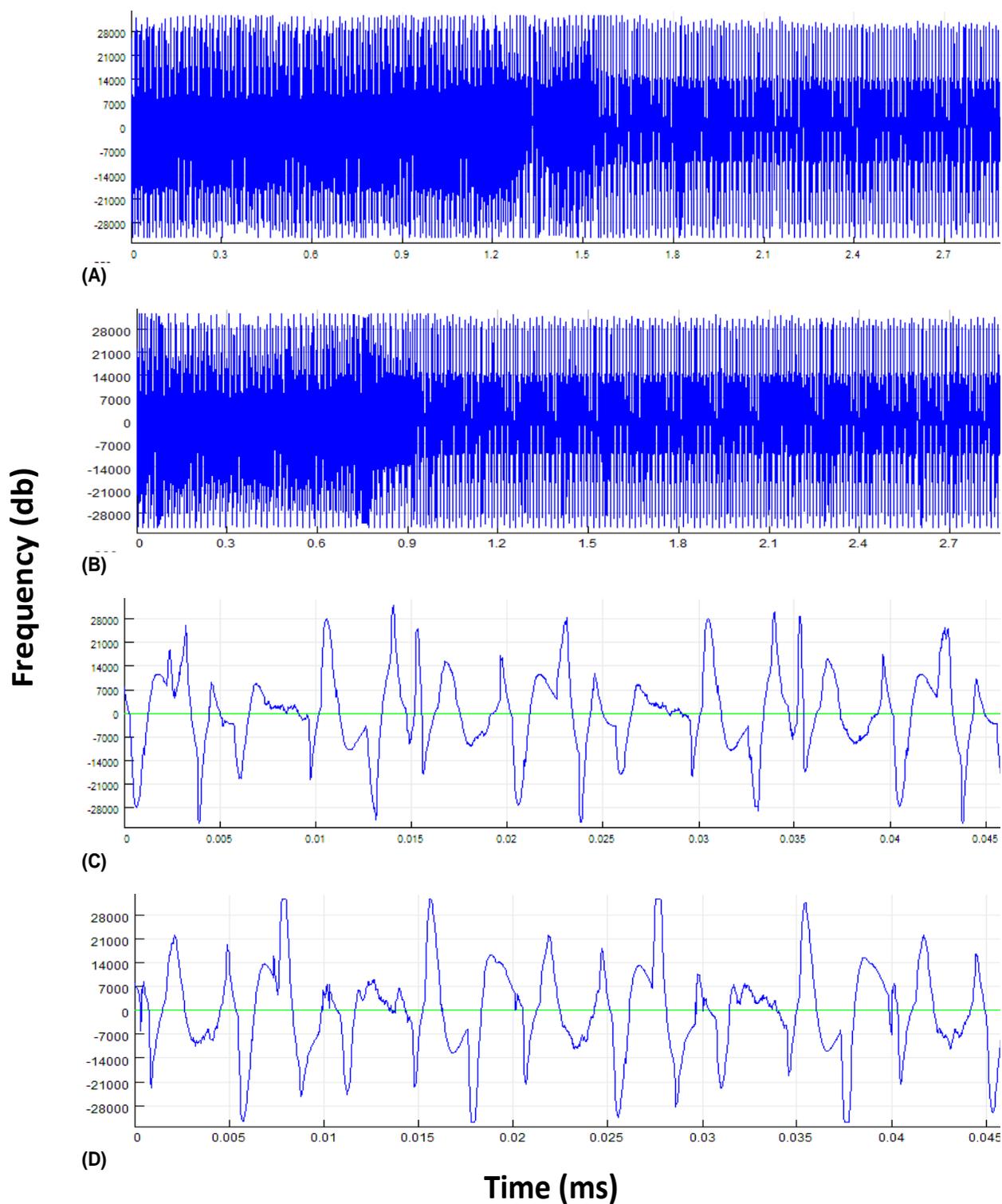


Figure 1. Tracing of spike train and peak patterns representing electrode placements and recordings for the control (1A and C) and the Arsenic III treated parietal cortex (1B and D). The control cortex; RMS 11906.2 and treated cortex RMS is 12951.9 at 0.02 s. The maximum peak recorded for control is 32019, while for the treated group an increase was observed up to 32767.

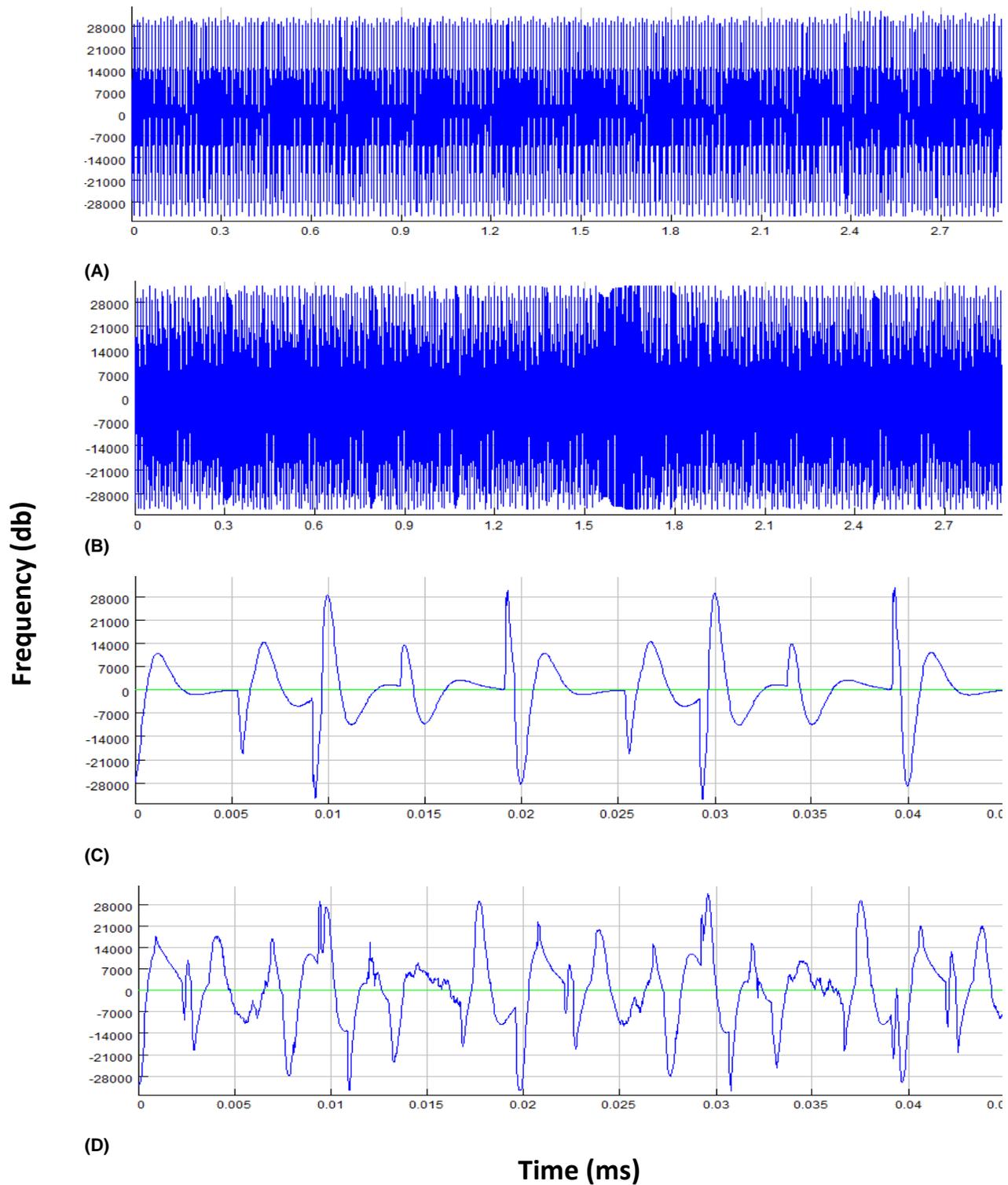


Figure 2. Tracing for spike train and peak patterns representing electrode placements and recordings for the control (2A and C) and the Arsenic III treated brain stem (at the level of upper pons) (2B and D). The control brain stem; RMS 9177.53 and Treated brain stem RMS is 12085.8 at 0.02 s. The maximum peak recorded for control is 32019 while for the treated group an increase was observed up to 32767.

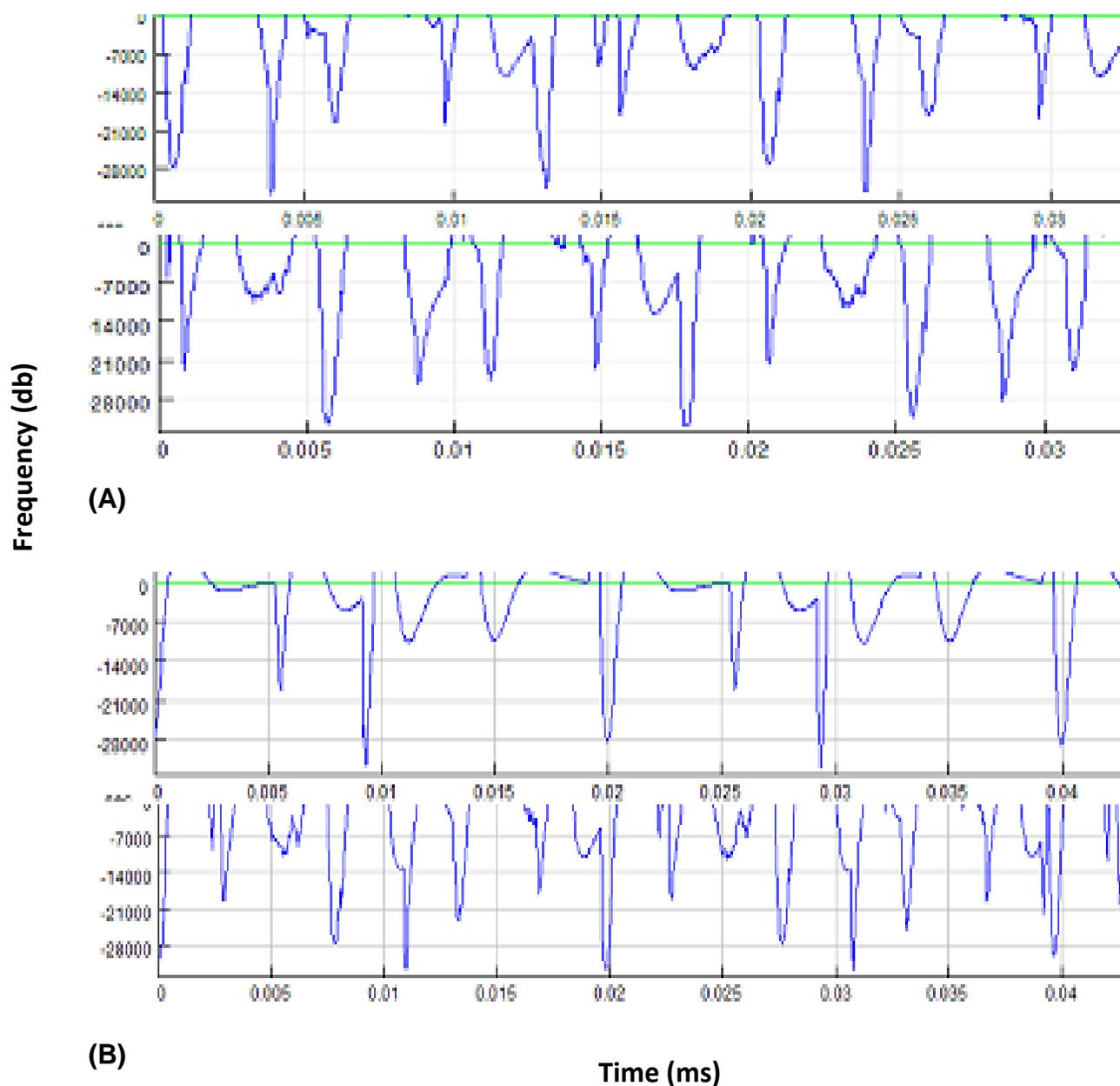


Figure 3. Tracing for lower peak comparative analysis; (3A) for the control and arsenic III treated parietal cortex. This shows an increase in the time required for the lower peak to be reached. (3B) In the brainstem a similar effect was achieved although at 0.04 and 0.02 second. A balance was achieved between the spike trains for the treatment and control.

the mitochondria have been found to be rich in H^+ pumps that will in essence affect the summation of electro-positive ions. Calcium imbalance has also been observed following arsenic exposure (Pachauri et al., 2013). Thus As(III) induced changes in the electrical activities of the brain to a varying extent and such changes vary with the

cytotoxic pathways for such regions.

Conclusion

Neuronal membrane may also play an important role in

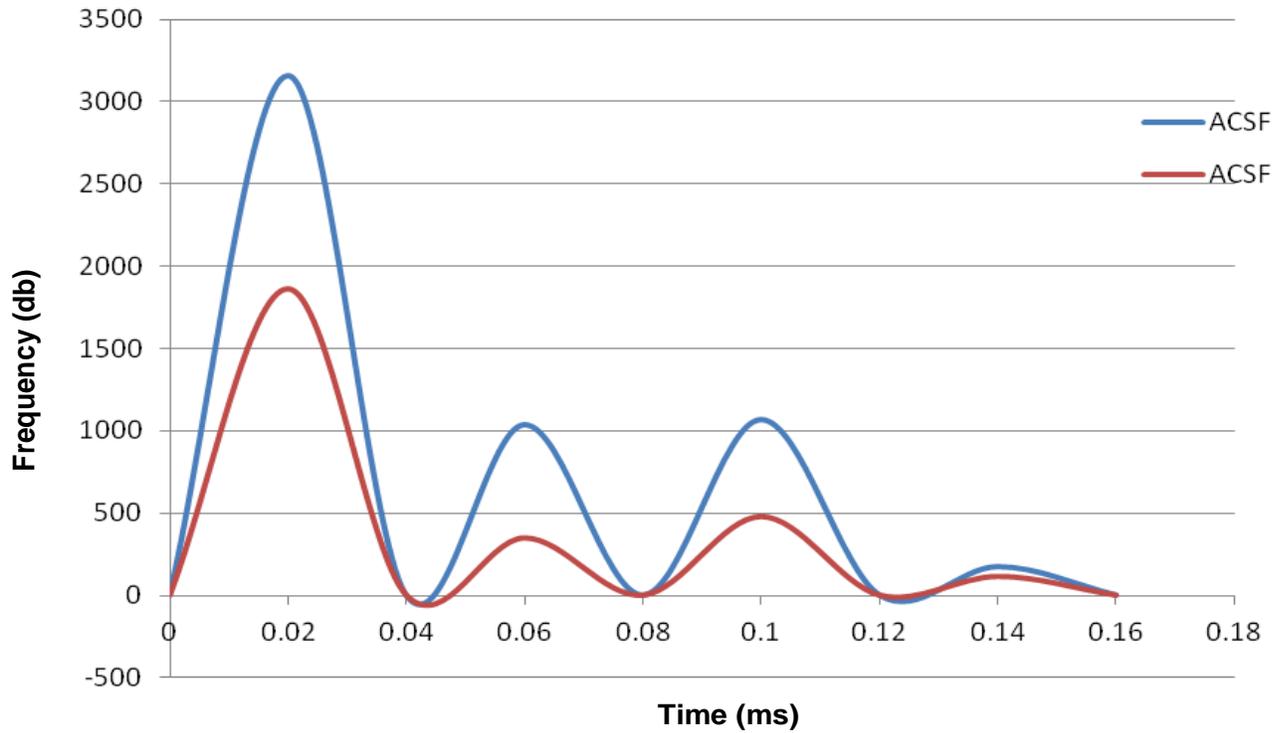


Figure 4. A curve showing the action potential (at an interval of 0.02 ms) for the control parietal cortex (in ACSF) and the parietal cortex treated with As (III) in ACSF. The result shows reduced firing rate at these intervals.

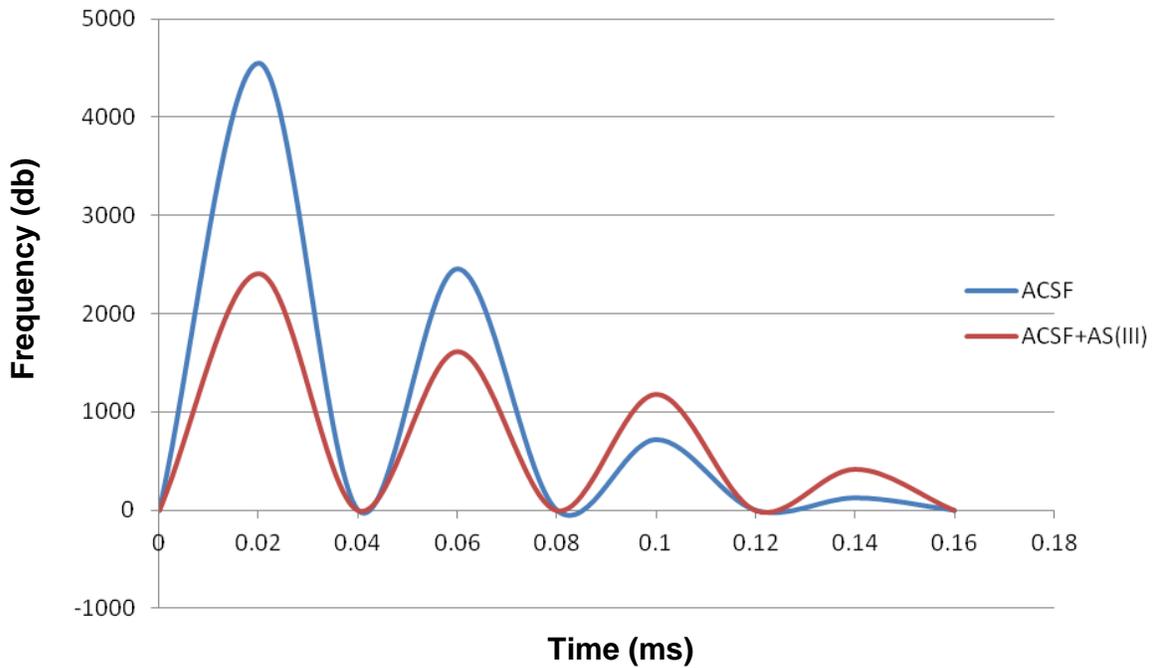


Figure 5. A curve showing the action potential (at an interval of 0.02 ms) for the control upper brain stem (in ACSF) and the upper brain stem treated with As (III) in ACSF. The result also shows reduced firing rates in the treated upper brainstem at these intervals, but prolonged AP when compared with the control.

neurotoxicity through alteration of its electrical potentials either through inhibition or limiting entry of ions into cells or through efflux mechanisms. We present this methodological template as a revised approach to studying neural functions.

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