Quantifying the Effects of Local and Global Glutamate Expression on Electrical and Calcium Signaling Dynamics

Glutamate Increasing Neuronal Activity

Glutamate receptors have been known to function as channels to allow the entry of certain ions into nerve cells. This occurs because when glutamate binds to these ionotropic receptors, positively charged ions such as sodium and sometimes calcium ions, flow into the cell. When this instance occurs multiple times, a coordinated flow of ions across nerve cell membranes is created, which leads to the generating of nerve signals (National Cancer Institute, 2014). This process, which was originally initiated by glutamate and controlled by glutamate leads to an increase in neuronal activity in the neuromuscular junction (NMJ). The neuromuscular junction is the place in the human body where a motor neuron connects with a muscle, allowing the neuron to signal the muscle to contract. However, an excess amount of activation of ionotropic glutamate receptors such as NMDA by glutamate can lead to a dangerous condition called excitotoxicity. Excitotoxicity is the process in which neurons are damaged or killed by excessive activation of glutamate receptors. This may increase the localized vulnerability of neurons which is a consequence of a changed regional distribution of NMDA receptors (Hynd, 2004).

Glutamate in the Cockroach NMJ

Cockroaches have been established as an excellent model for neuroscience experiments as they have neurons that are very similar to humans. Though their nervous systems are simpler, they still exhibit basic functions like sensing, moving, and learning, which can be easily manipulated using technology. Scientists have identified glutamate to be a primary neurotransmitter in certain neuromuscular junctions of cockroaches, specifically Periplaneta *americana*. They found that L-glutamate, one of the most abundant neurotransmitters in the brain, was able to cause a depolarization of the muscle fiber in cockroaches, showing how

glutamate plays a significant role in the cockroach NMJ. Additionally, the effects that glutamate and GABA (an inhibitory receptor) had on the cockroach were opposite to each other, as the threshold concentration that allowed L-glutamate to increase the amplitude and frequency of the cockroach muscle contractures and Mepps was the same threshold concentration that allowed GABA to decrease the Mepps and contractures of the cockroach muscle (Kerkut & Walker, 1966). These findings show how glutamate has a significant role as a neurotransmitter in the neuromuscular junctions of cockroaches.

Glutamate as a Modulator and Mediator in the NMJ

It is still unknown where glutamate receptors have precisely localized at the presynaptic terminals of neuromuscular junctions, as well as in motoneurons. However, glutamate at the neuromuscular junction has been shown to act on the modulation of cholinergic synaptic transmission (neurotransmission by acetylcholine at synapses) by interacting with presynaptic receptors. In the tail muscles of frogs, glutamate has been found to activate presynaptic ionotropic (ion channels) receptors, such as AMPA and NMDA. This activation leads to an increase in the spontaneous release of acetylcholine. This effect of glutamate on the increased spontaneous release of acetylcholine is controlled by influx of calcium ions that occurs through the NMDA receptors at the opening of voltage activated calcium ion channels. This same effect regarding glutamate and an increase in spontaneous acetylcholine release also occurred in zebrafish larvae (Colombo

& Francolini, 2019). Glutamate can increase neuronal activity through the modulation of the cholinergic synapse, as increased amounts of glutamate leads to larger releases of the neurotransmitter acetylcholine, leading to increased neuron signals and a larger neural output. This is evidence of how glutamate can affect neuronal activity in humans.

Glutamate and Calcium Dynamics

Muscle contraction can be demonstrated by the sliding filament model, which consists of actin and myosin heads that bind to each other to generate a force, and this process is mediated by calcium ions. A calcium transient is the trigger for actin-myosin crossbridge binding, which is where the actin (thin filament) is grabbed by myosin (filament “heads”), and the crossbridge is where the myosin grabs the actin (Figure 1). These cross bridges pull, causing the muscle to contract, which generates a force. More spikes means that there is more calcium, which means there is more actin available for the myosin to grab, and therefore more cross bridges are formed, leading to more/stronger contractions and forces. Recently, studies conducted on ALS have demonstrated that changing glutamate receptor signaling influences calcium dynamics. When too much glutamate was activated, this led to calcium being overloaded and caused neurons to die off. ALS mutations caused more neurons to die off and increased the severity of calcium overloading. They found that calcium resting levels were elevated, there were larger infrequent spikes in muscle force, and the cells could not restore calcium fast enough (Bursch et al., 2019). This study indicates that local and global changes in glutamate influence the baseline, amplitude, and frequency of calcium dynamics. Scientists have previously identified the effect of electrical stimulation on the baseline, amplitude, and frequency of calcium dynamics; however, the effects of neuromodulation on calcium dynamics under electrical stimulation have yet to be studied.

ALS and Calcium Transients

In ALS, or Amyotrophic Lateral Sclerosis, the motor neurons die off, leading patients to lose functions such as breathing, walking, talking, etc. This occurs due to ALS causing a deficiency in nerve cells’ ability to clear up excess glutamate, leading to glutamate build up. This buildup of glutamate causes the intercellular levels of calcium to change inside the nerve cells, which eventually kills them off. The proteins that clear up glutamate are located on the spinal cord, and since the neurons on the spinal cord die off, the proteins also die off and are unable to complete

their function. Scientists have tried multiple medications to reduce the onset of ALS, such as reducing the speed of glutamate being excreted from the cell. However, these medications have lots of toxic side effects and have not been extremely effective in treating ALS (Aggarwal & Cudkowicz, 2008). Scientists have also tried to treat ALS using electrical stimulation, however they have yet to identify how different frequencies of electrical stimulation can affect the calcium dynamics and the specific changes to the calcium amplitude, baseline, and frequency in the motor nerve cells. Though scientists know that lower frequencies of electrical stimulation do decrease calcium transients in a non-neuromodulated environment, they have not yet studied what frequencies can reduce calcium transients in a neuromodulated environment, such as with glutamate added.