The role of gap junctions on mammalian spinal locomotor activity
Emelia Krakora, 2024

Project

This summer I explored the role that gap junctions play on mammalian spinal locomotor activity. Gap junctions physically connect cells by allowing small molecules and ions to pass between cells connecting them electrically (Takeuchi et al. 2011). In previous research conducted on gap junctions, it was found that they are present in different levels of motor systems and they help in coordinating motor activity patterns (Kiehn and Tresch 2002). It was also found that gap junctions are indeed present in the mouse spinal cord (Blivis et al., 2019). However, there is limited research on the function and location of these gap junctions, especially at the level of the central pattern generator (CPG) neural network located within the lumbar spinal cord. The CPG is a network of neurons that control rhythmic outputs such as walking, swimming, heartbeat, and circadian rhythms. In our project we used a gap junction blocker, carbenoxolone (CBX), to assess what effects inhibiting electrical coupling would have on different parameters pertaining to motor neuron bursts produced in the lumbar spinal cord. We measured peak amplitude (height of the burst), burst duration (time from onset to offset of burst), and cycle period (duration from onset of one burst to the onset of the next burst). It was hypothesized that when gap junctions were blocked this would reduce the excitability of the lumbar CPG neural network by decoupling electrical activity between motor neurons affecting rhythmic output. Specifically, it was predicted that peak amplitude would decrease while burst duration and cycle period would increase.

Methods

To test our hypothesis, we used similar methods to the research performed by Acevedo et al., 2016. The spinal cord from 0-5 day old mice was extracted using a ventral laminectomy and then the ventral roots that were different combinations of lumbar 2 (L2) and lumbar 5 (L5) were sucked up using small electrodes to record electrical activity. To elicit locomotor-like activity, 9μM of serotonin (5-HT) and 6μM of NMDA were perfused into the spinal cord in a bath of normal mouse ringer (NMR) which served as the control solution. Then 100 μM CBX was perfused into the spinal cord along with 9μM 5-HT, 6M NMDA, and NMR. Then a wash was attempted by removing CBX with the control solution.

Results

The results matched the predictions that there was an overall trend of a decrease in burst amplitude and increase of burst duration and cycle period. Although when a one-way analysis of variance was performed (ANOVA) there was no significant difference between the control and experimental data, there was an overall trend of what was previously predicted. It is believed that burst amplitude decreased because this is a measure of neuronal recruitment and when CBX inhibits this recruitment by blocking gap junctions, the connections between networks of neurons are lost. This means that less neurons are able to fire at once so there is less summation which decreases the amplitude. The cycle period increased because CBX inhibits the synchronization of neurons so less neurons are able to fire at the same time. This decreases the chances of the membrane potential reaching threshold which leads to more time between bursts. Lastly, burst duration is believed to increase because CBX inhibits electrical coupling between neurons which causes less overall CPG network excitability.

Conclusions

Gap junctions play an important role in coupling neurons in the lumbar CPG network of the neonatal mouse spinal cord and CBX can block these gap junctions to disrupt locomotor activity. In a study performed by Takeuchi et al., 2011 it was theorized that blocking gap junctions could be used as a potential therapeutic treatment for neurodegenerative diseases. Excess glutamate release in microglial cells is believed to cause neurodegenerative diseases so when CBX blocks these gap junctions it is able to suppress this excess glutamate release. This summer project has helped provide some insight on the function and location of gap junctions in the lumbar spinal cord which can be useful in future research pertaining to the treatment of neurodegenerative diseases.

Faculty Mentor: Manolo Diaz- Rios
Funded by the James Stacy Coles Summer Research Fellowship

References

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