A POST-SUBMISSION MATERIALS

The COVID-19 pandemic has severely impacted the ability to conduct bench research at academic institutions around the world. At the College of Staten Island, reseach activity was stopped in March 2020 and the return to research was prioritized for faculty with external funding beginning in August 2020. My laboratory, which was not externally funded, reopened in October 2020, graduate students were permitted to resume limited activity in my lab in March 2021, and undergraduates will resume activity in June 2021. The pause in research activity has resulted in the limited ability to contribute preliminary data to this R15 proposal, and has thus neccesitated this one page post-submission document. The preliminary data shown here, collected after the February 2021 proposal submission, provide direct support for the central hypothesis that social and environmental factors in the colony nest, specifically oxytocin and carbon dioxide, enhance KCC2 membrane stability and GABAergic inhibition in the adult NM-R.

While it has been known for some time that carbon dioxide (CO_2) can supress seizure activity [1], and we have demonstrated that nest levels of carbon dioxide can suppress seizures in NM-R [2], the neuronal mechanisms driving this effect are not well understood. In the current R15 proposal, we will test whether enhanced CO_2 comensates for the NM-R KCC2 impairment by increasing membrane stabilization of KCC2, specifically through phosphorylation at the ser940 locus. The R15 proposal includes preliminary data to demonstrate antibody specificity for phosphorylated ser940 KCC2 in NM-R.

The hypothesis that oxytocin (OXT) can also supress seizure activity has less support in the extant literature (see [**? ? ? ?**]. However, unlike CO_2, OXT has already been demonstrated to increase KCC2 membrane stabilization through phosphorylation at the ser940 locus [3]. This likely plays an important role in the developmental regulation of GABA efficacy, particularly at birth [4], but the potential role for this mechanism of oxytocin to inhibit seizure activity has not yet been tested.

We have observed previously that NM-R hippocampal slices, prepared under typical *in vitro* conditions, exhibit spontaneous epileptiform burst discharges in the pyramidal cell layer of area CA3 [2], similar to what we have observed previously in chronic epileptic adult rats [5]. These epileptiform bursts are significantly exacerbated when CO_2 is removed from the NM-R slice by replacing the carbogen gas with 100% O_2 oand bicarbonate in the buffer solution is replaced with HEPES (data not shown). They are also significantly exacerbated when $GABA_A$ receptor agonist isoguvacine is added to the slice, demonstrating an excitatory response to GABA (see [2]).

To test whether OXT receptor activation suppresses epileptiform activity in the NM-R slice, we applied the selective OXT receptor agonist Thr^4,Gly^7-oxytocin (TGOT) to NM-R slices with epiletiform activity. Figure 3 shows the robust suppression of epileptiform events we observed in response to 200 nM TGOT, using the exact dose and method of application outlined in Experiment 1.1 of the proposal. The TGOT suppression of epileptiform activity did not readily wash upon returning the slices to normal ASCF for 30 minutes, indicating potential downstream transcription; potentially KCC2 membrane stabilization as hypothesized in the proposal. To test whether GABA enhancement has occurred following TGOT treatment, we again administered isoguvacine following TGOT and a 30 minute wash period. In this condition isoguvacine further reduced activity in the slice, consistent with a typical GABAergic effect, indicating the OXT receptor activation may ”rescue” GABAergic function in the NM-R brain.

Although these data are still preliminary, and proper controls must be performed, they provide support for our central hypothesis that social and environmental factors in the colony nest, specifically oxytocin and carbon dioxide, enhance KCC2 membrane stability and GABAergic inhibition in the adult NM-R. With the requested funding, we hope to properly test this hypothesis and pave the way to new approaches in the prevention and treatment of epilepsy and other chloride dysfunction disorders.

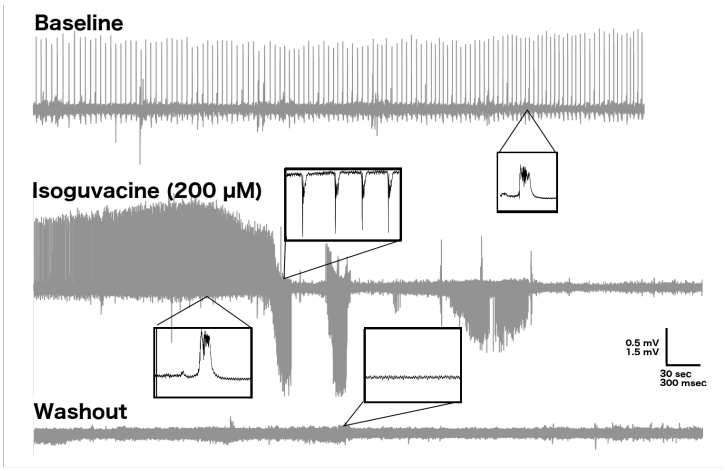

Figure 1: Baseline recording from hippocampal area CA3 under standard conditions showing epileptiform bursts. When the $GABA_A$ agonist isoguvacine (200 µM) was added to the perfusate, slices increased their bursting activity and developed spreading depression in all slices tested (n=3 slices from 3 animals), indicating an excitatory response to $GABA_A$ receptor activation. From [2]

LITERATURE CITED

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