

Bumetanide as a Candidate Therapy for Alzheimer’s Disease: Insights From Retrospective Clinical Databases and Preclinical Models

[1]Oran McNamara, [1] Aoife O’Connell, [1] Ying Zhai, [1] Tom Delany, [1] Ciara Ní Dhochartaigh, [1] Leo Quinlan, [1] Andrea Kwakowsky. [1] School of Pharmacy and Medical Sciences, Discipline of Pharmacology and Therapeutics, Galway Neuroscience Centre, University of Galway



Background & Experimental Overview

E/I Balance Dysfunction in Neurological Disorders

E/I balance dysfunction represents a core, convergent mechanism across both neurodevelopmental and neurodegenerative disorders. Alterations in the ratio of cortical excitation to inhibition are implicated in the pathophysiology of neurological disorders such as Autism Spectrum Disorder, Huntington’s Disease, Schizophrenia, and Alzheimer’s Disease [1].

Mechanisms contributing towards the loss of E/I balance include dysfunction of interneurons, glutamatergic hyperexcitability, and altered GABAergic signalling [2]. These alterations lead to network desynchronisation, excitotoxicity, and impaired synaptic plasticity [3].

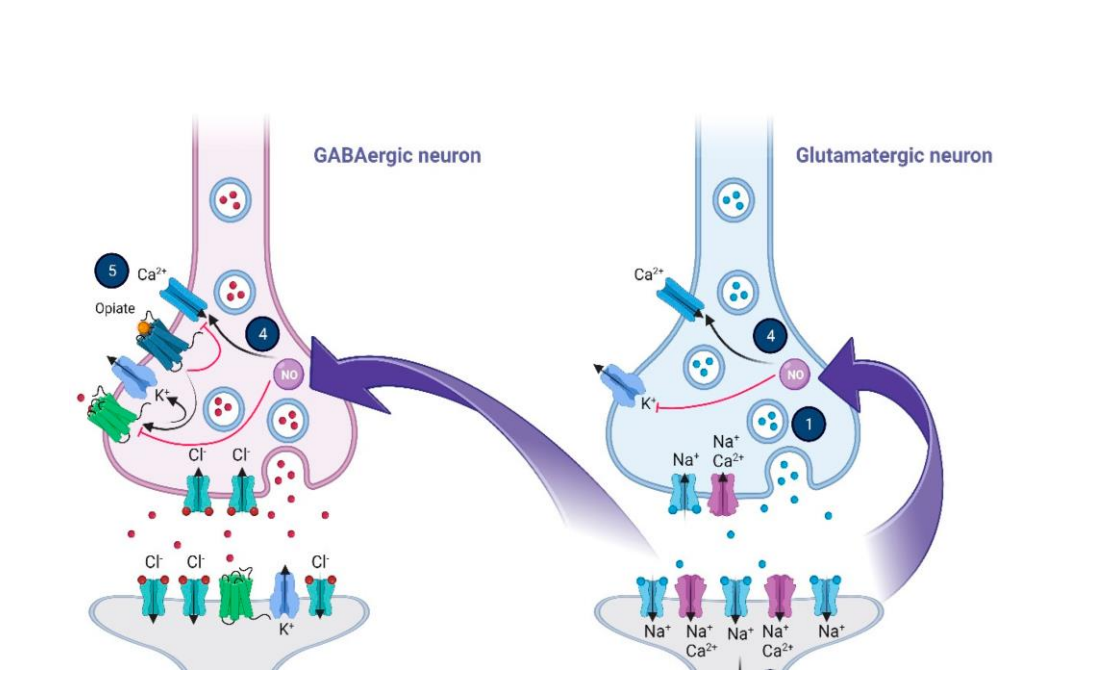


Fig. 1: Schematic representation of glutamatergic and GABAergic signalling within the CNS. Adapted from Sahafi et al., 2023.

NKCC1 in Disease and Development

During neurodevelopment, elevated neuronal NKCC1 expression leads to depolarising post-synaptic GABA-A responses. As NKCC1 expression declines with maturation, GABA-A signalling shifts to hyperpolarising responses, establishing the appropriate E/I balance within synaptic circuits [4].

In contrast, several neurodegenerative and neurodevelopmental disorders such as Alzheimer’s disease [5], Autism Spectrum Disorder [6], Schizophrenia [7], and Down syndrome [8], exhibit abnormally elevated CNS NKCC1 expression, indicative of persistently depolarising GABAergic transmission.

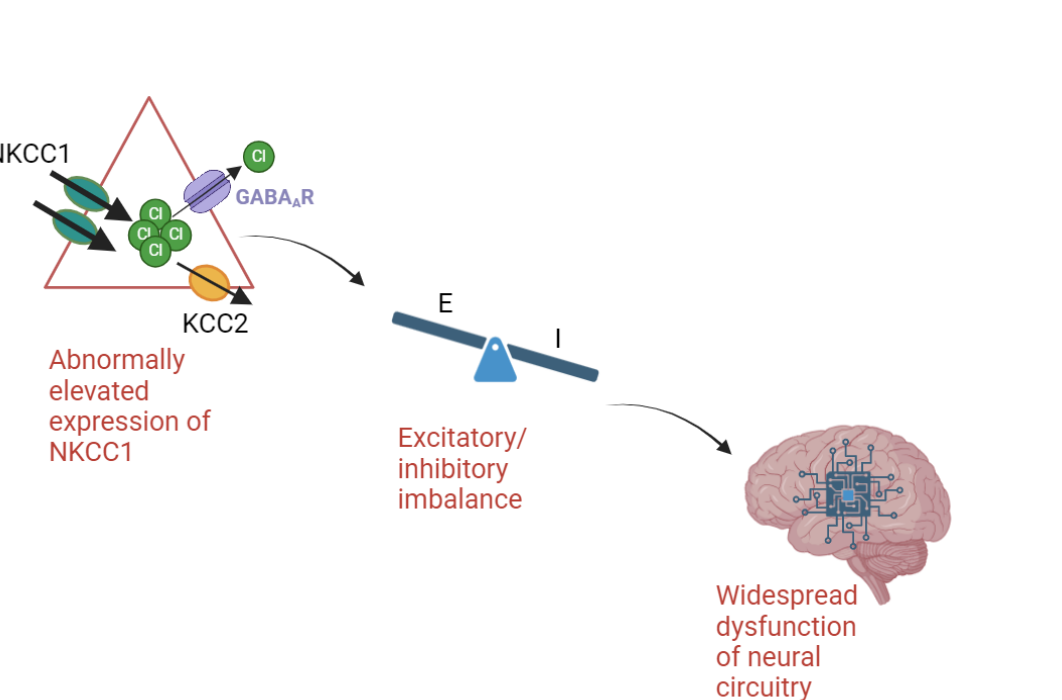


Fig. 2: Dysregulated NKCC1/KCC2 expression ratios induce dysfunction of neuronal network activity.

Bumetanide as a Therapy for Neurological Disorders

Bumetanide is a potent loop-diuretic that acts as an NKCC1 antagonist, inhibiting the influx of chloride ions across cell membranes [9].

More recently, bumetanide has been investigated as a potential therapeutic agent for several neurological conditions, including neurodegenerative diseases such as Alzheimer’s Disease [10], Parkinson’s Disease [11], and Huntington’s Disease [12], as well as neurodevelopmental disorders such as Autism Spectrum Disorder [13] and Down Syndrome [8].

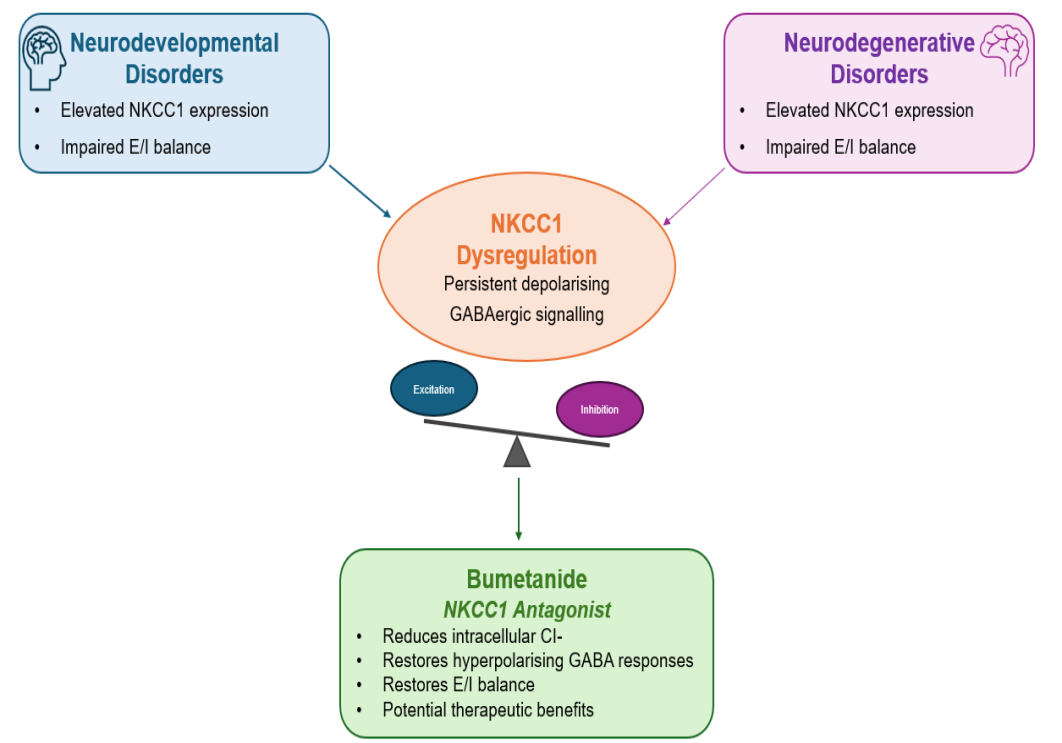


Fig. 3: Dysregulated NKCC1 activity is commonly observed across both neurodevelopmental and neurodegenerative disorders.

Methods: Bumetanide Systematic Review

A systematic literature search of primary research articles investigating the effect of bumetanide on neurological disorders was performed across PubMed, Scopus, and Web of Science databases

Hippocampi were dissected from P0 mice and cultured to 14 DIV. Cells were then exposed to treatment for 5 days, stained using a LIVE/DEAD Viability kit, and counted manually using ImageJ software

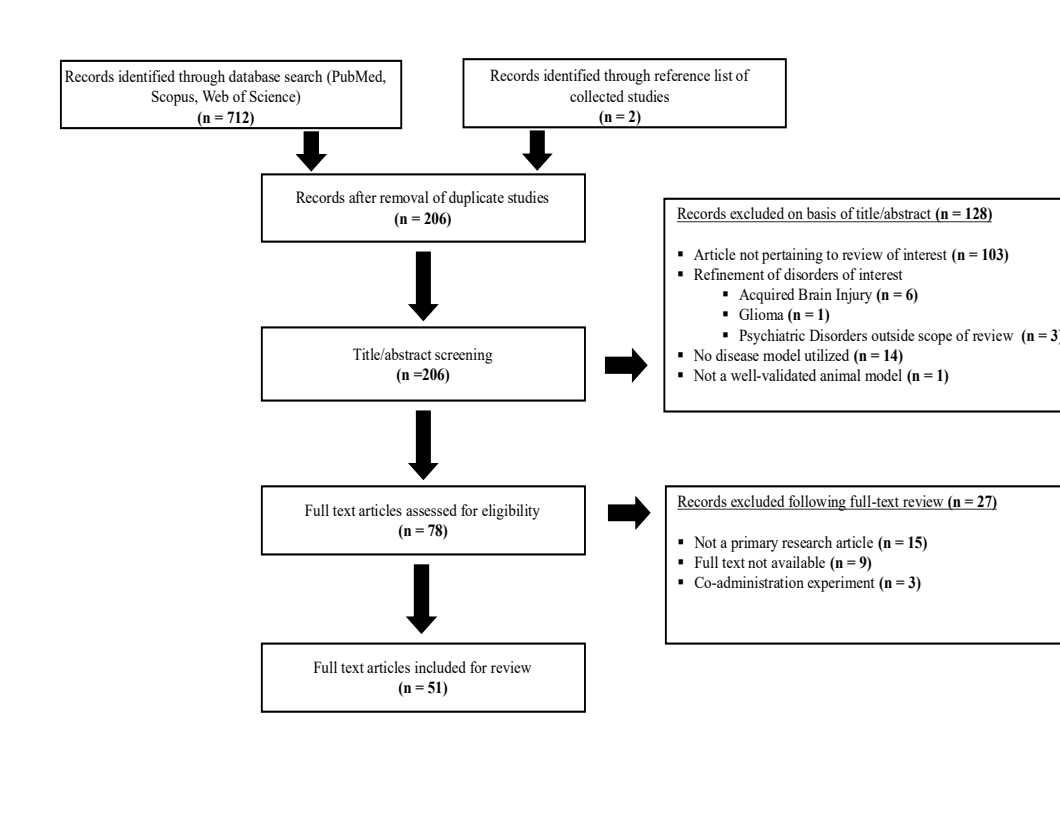


Fig. 4: PRISMA flowchart demonstrating the systematic review process. A total of 51 papers were deemed suitable for systematic review.

Methods: Bumetanide Cell Viability Assay

Primary hippocampal culture was utilised to determine the effect of bumetanide on neuronal cell viability and amyloid-β-induced neurodegeneration.

Hippocampi were dissected from P0 mice and cultured to 14 DIV. Cells were then exposed to treatment for 5 days, stained using a LIVE/DEAD Viability kit, and counted manually using ImageJ software.

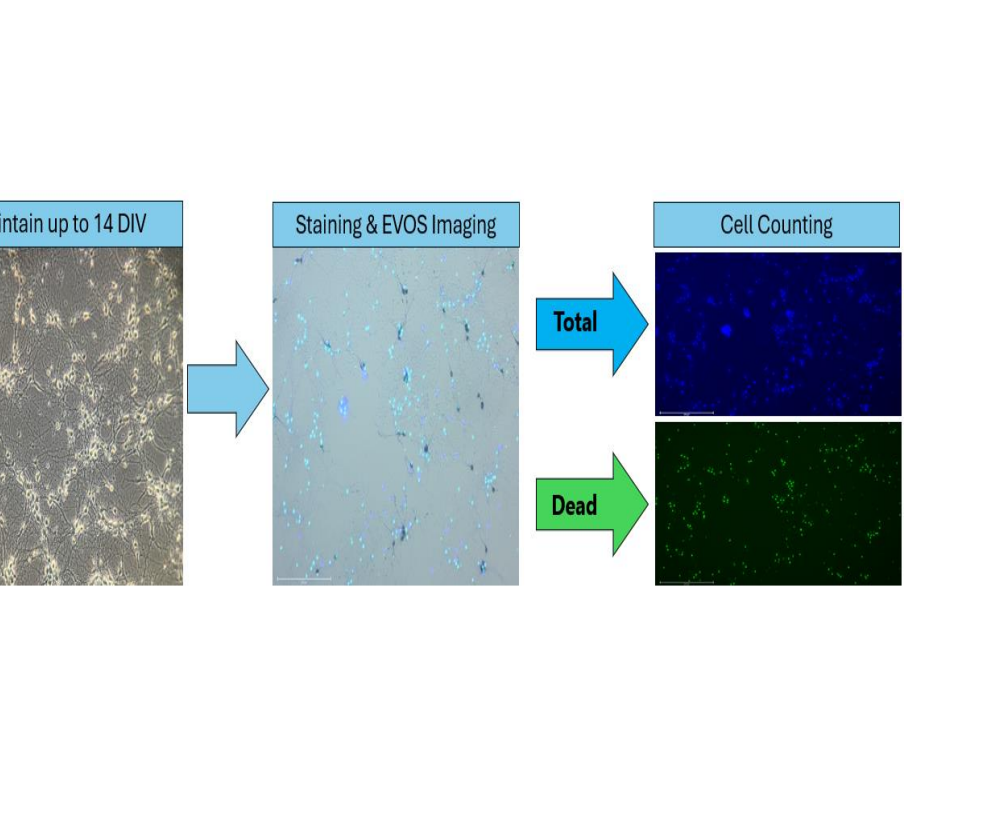


Fig. 5: Simplified primary hippocampal live/dead assay workflow schematic.

Methods: Acute Hippocampal Slice MEA

To assess CA1 hippocampal network activity, microelectrode array was performed on mouse acute hippocampal slices.

Stimulation of CA1 pyramidal layer was performed, and recordings of fEPSPs were recorded from the pyramidal layer and stratum radiatum.

Long-term potentiation was induced using a theta-burst stimulation following 30 minutes of baseline fEPSP response recording.

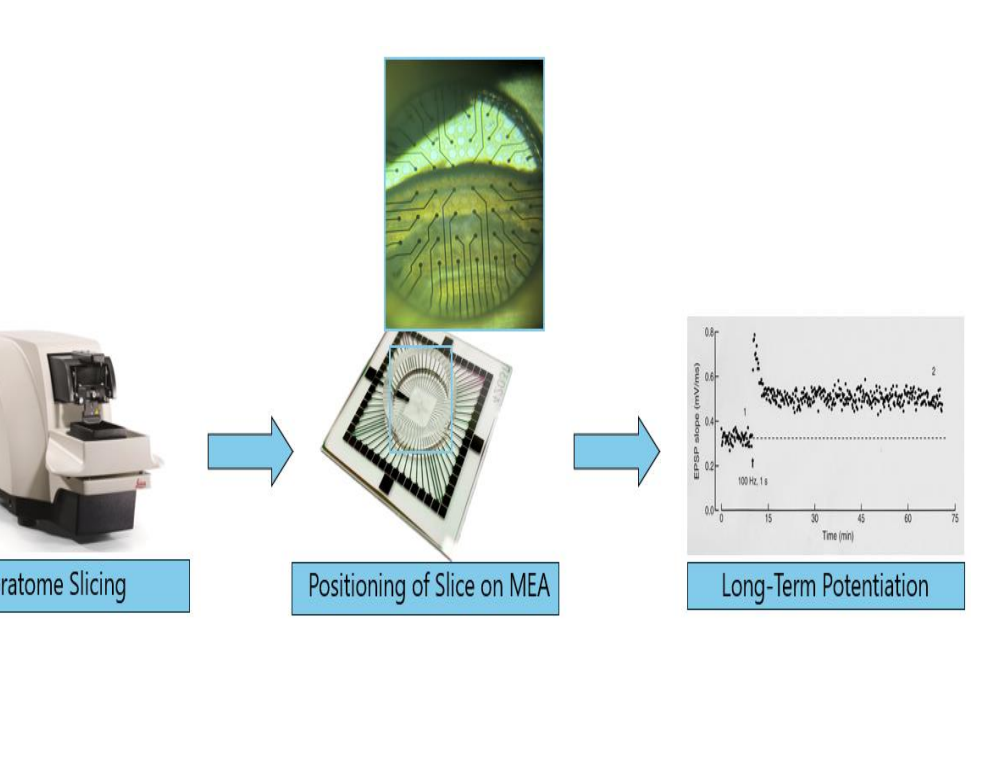


Fig. 6: Simplified microelectrode array (MEA) workflow schematic.

Results

Systematic Review: Bumetanide in Preclinical and Clinical Literature

Elevation of NKCC1:KCC2 expression ratios was consistently observed across multiple neurological disorders, including Alzheimer’s disease, Huntington’s disease, Autism spectrum disorder, and Down syndrome.

Bumetanide demonstrated beneficial effects in several preclinical models

Clinical findings demonstrated mixed results in Alzheimer’s disease and Autism spectrum disorder

Evidence supports NKCC1 inhibition as a promising therapeutic strategy despite concerns regarding CNS penetration.

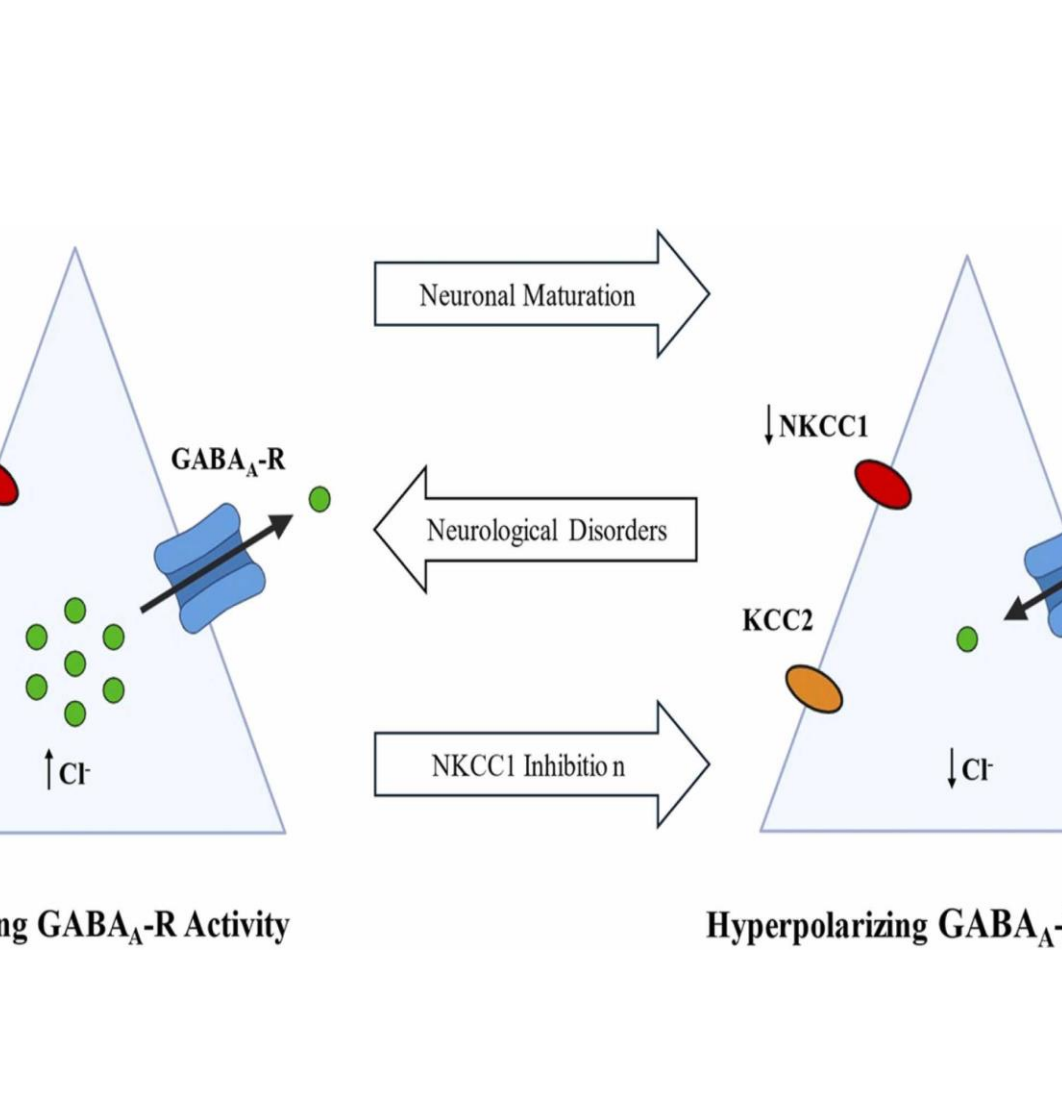


Fig. 7: Proposed mechanism of action of bumetanide in restoration of appropriate E/I balance.

Bumetanide is Unable to Attenuate Amyloid- β Induced Neurodegeneration

Bumetanide has previously demonstrated neurotoxicity in primary hippocampal neurons at 1 mM – 1 μM concentrations [14].

Bumetanide increased neurodegeneration at 1 μM – 10 nM concentrations (***p < 0.0001) and had no effect on neurodegeneration at 1 nM (p > 0.9999) compared to control cells.

Bumetanide was unable to rescue amyloid-β induced neurotoxicity across 1 μM – 1 nM concentrations.

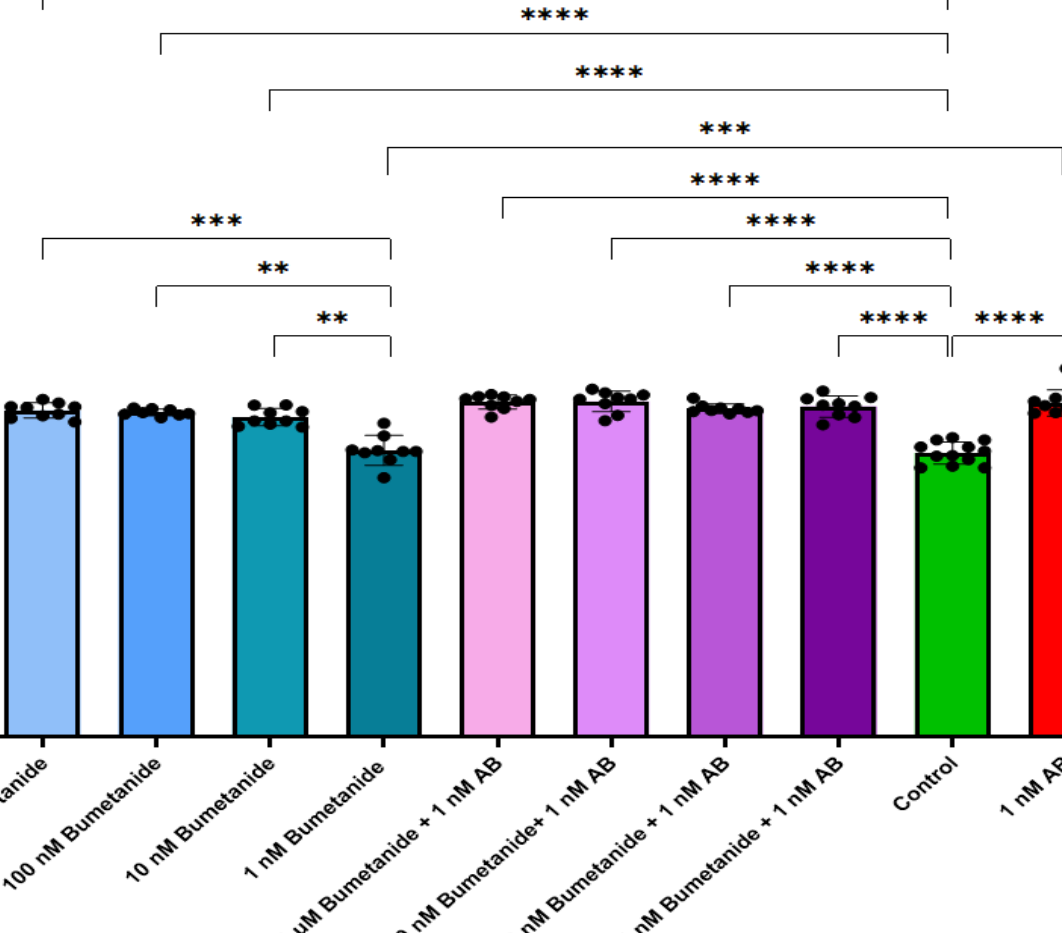


Figure 8: Bumetanide treatment increases neurotoxicity of primary hippocampal neurons at 1 μM (****), 100 nM (****), and 10 nM (****), but not 1 nM concentrations compared to control cultures. Data is represented as mean ± SD. n = 8-12 wells per group. Brown-Forsythe ANOVA test with Dunnett’s multiple comparisons post-hoc (F² = 41.65 (9,000, 54.82), ****p < 0.0001).

Effect of Bumetanide on Amyloid-β induced LTP deficits in CA1 Pyramidal Layer

A. Time Course of LTP Induction in Pyramidal Layer

Field potentials were evoked and recorded within the CA1 pyramidal layer using MEA electrodes. Baseline responses were stable for 30 minutes prior to LTP induction, after which TBS was used to induce LTP.

Control (green) slices demonstrated a robust increase in fEPSP amplitude for the remaining 90 minutes of recording, while slices treated with 100 nM amyloid-β (red) show markedly attenuated potentiation. 10 μM bumetanide (blue) and 10 μM bumetanide + 100 nM amyloid-β (purple) show sustained potentiation post-TBS.

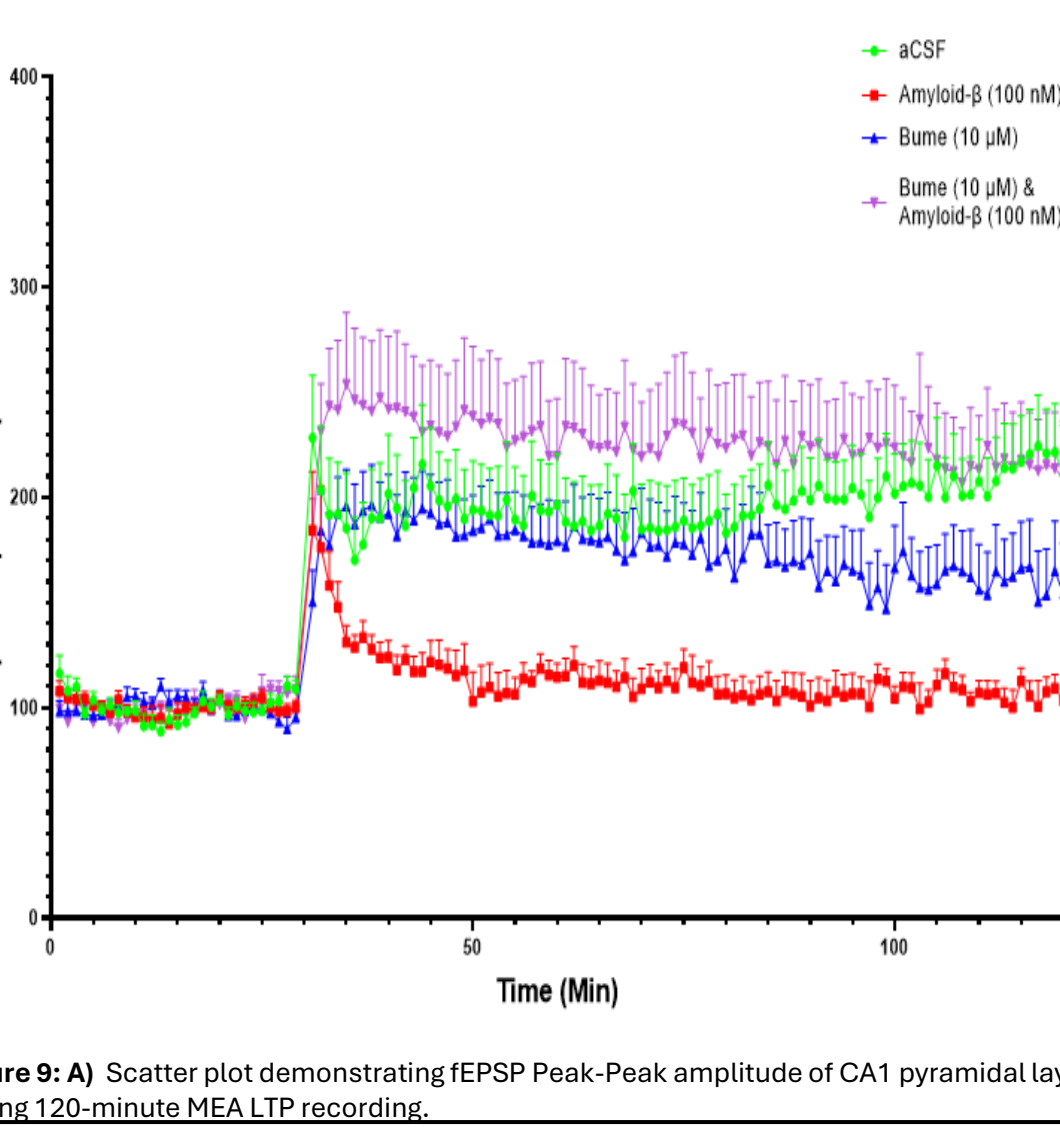


Figure 9: A) Scatter plot demonstrating fEPSP Peak-Peak amplitude of CA1 pyramidal layer during 120-minute MEA LTP recording.

B. Mean fEPSP Amplitude (100-110 min post-TBS) in Pyramidal Layer

Quantification of late-phase potentiation revealed a significant reduction between treatment groups (F* = 8.010 , *** p = 0.0007).

100 nM amyloid-β treated slices demonstrated a significantly decreased fEPSP amplitude compared to control slices (***p = 0.0010). 10 μM bumetanide significantly attenuated fEPSP deficits induced by amyloid-β compared to amyloid-β alone (*p = 0.0152), while having no effect on fEPSP when administered alone compared to control slices (p = 0.3467).

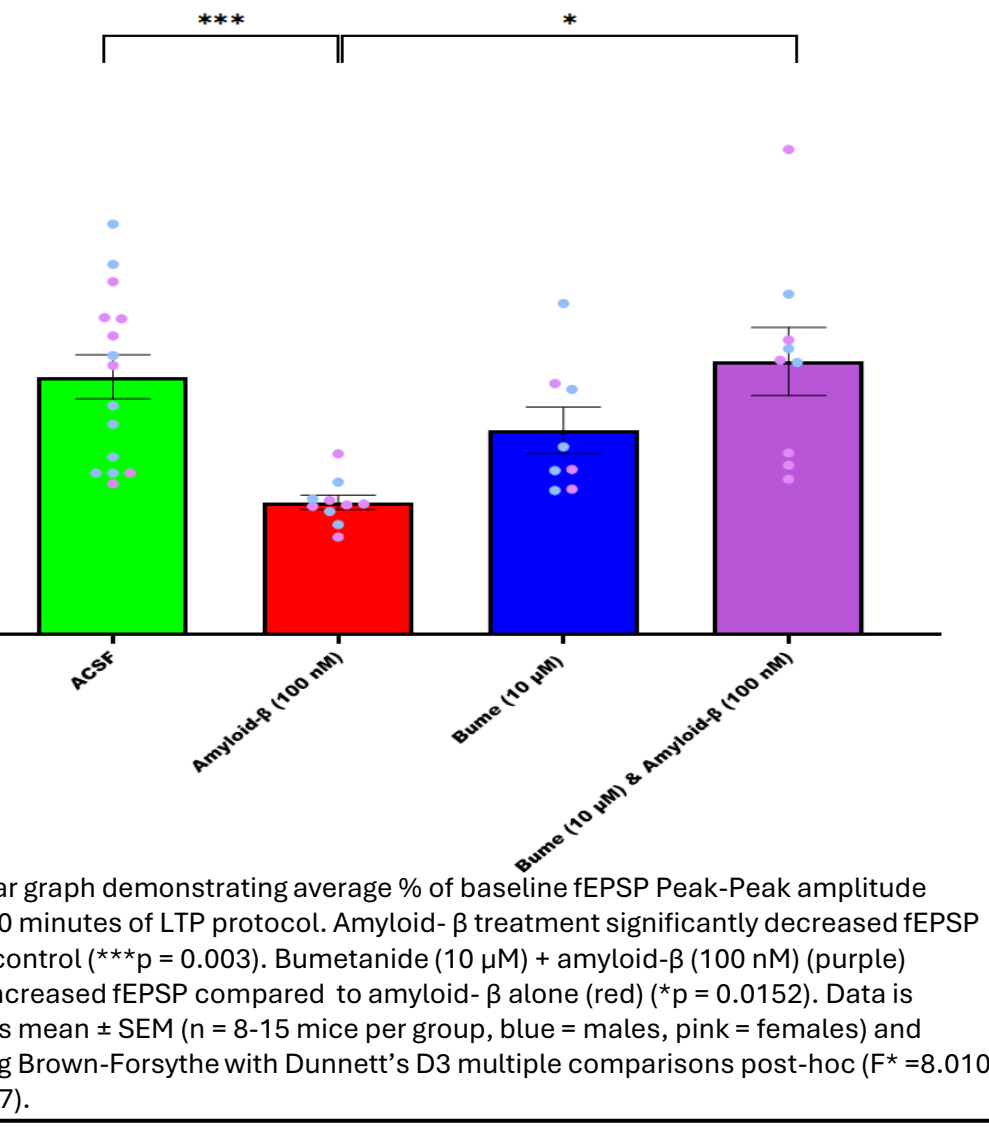


Figure 9: B) Bar graph demonstrating average % of baseline fEPSP Peak-Peak amplitude during 100-110 minutes of LTP protocol. Amyloid-β treatment significantly decreased fEPSP compared to control (***p = 0.003). Bumetanide (10 μM) + amyloid-β (100 nM) (purple) significantly increased fEPSP compared to amyloid-β alone (red) (*p = 0.0152). Data is represented as mean ± SEM (n = 8-15 mice per group, blue = males, pink = females) and analysed using Brown-Forsythe with Dunnett’s D3 multiple comparisons post-hoc (F³ = 8.010 , *** p = 0.0007).

Effect of Bumetanide on Amyloid-β induced LTP deficits in CA1 Stratum Radiatum

A. Time Course of LTP Induction in Stratum Radiatum

Field potentials were evoked and recorded within the CA1 pyramidal layer using MEA electrodes. Baseline responses were stable for 30 minutes prior to LTP induction, after which TBS was used to induce LTP.

Control (green) slices again demonstrated a robust increase in fEPSP amplitude post-LTP induction, while slices treated with 100 nM amyloid-β (red) showed markedly attenuated potentiation. 10 μM bumetanide (blue) and 10 μM bumetanide + 100 nM amyloid-β (purple) show sustained potentiation post-TBS.

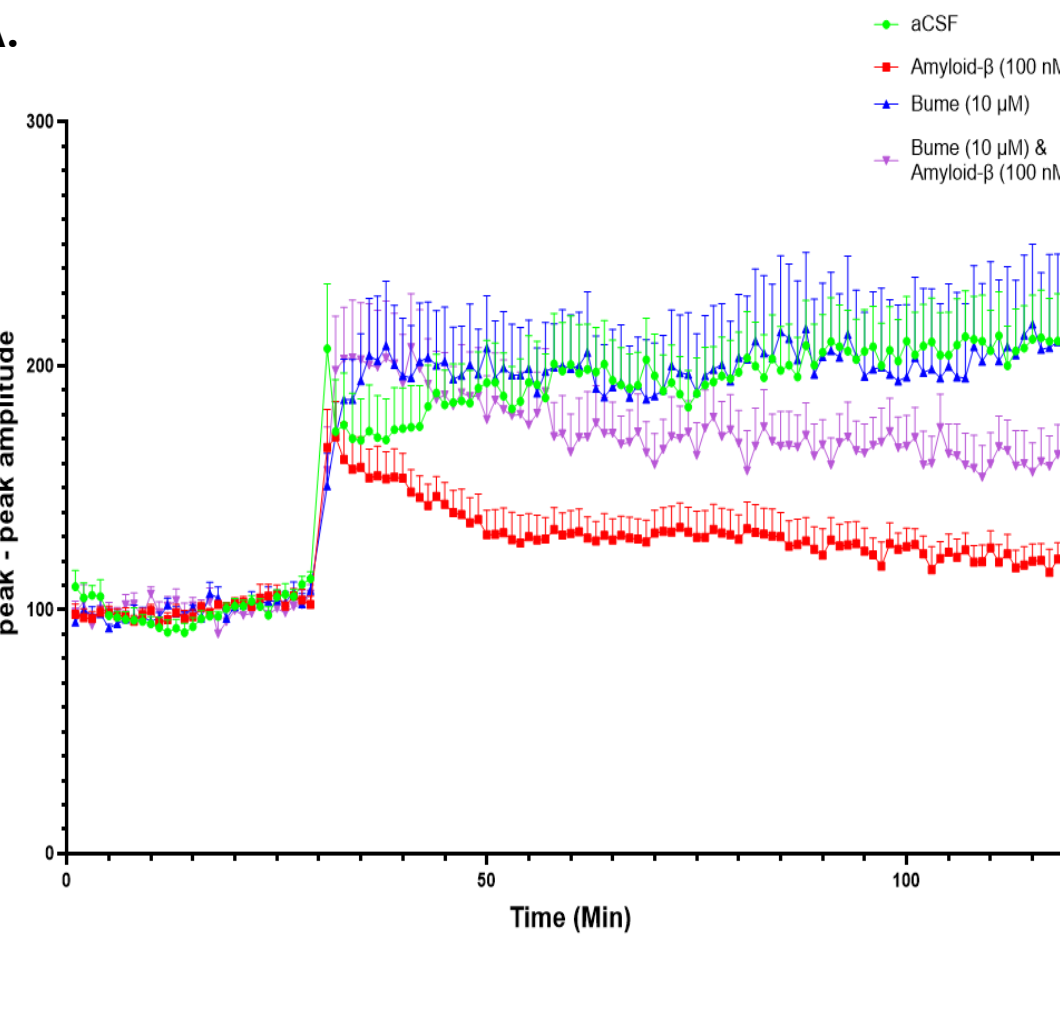


Figure 10: A) Scatter plot demonstrating fEPSP Peak-Peak amplitude of CA1 stratum radiatum layer during 120-minute MEA LTP recording.

B. Mean fEPSP Amplitude (100-110 min post-TBS) in Stratum Radiatum

Quantification of late-phase potentiation revealed a significant difference across all treatment groups (H = 18.79 , **** p = 0.0003).

100 nM amyloid-β treatment significantly reduced fEPSP amplitudes compared to control slices (***p = 0.0001). 10 μM bumetanide significantly attenuated fEPSP deficits induced by amyloid-β compared to amyloid-β alone (*p = 0.0436), while having no effect on fEPSP when administered alone compared to control slices (p > 0.9999)

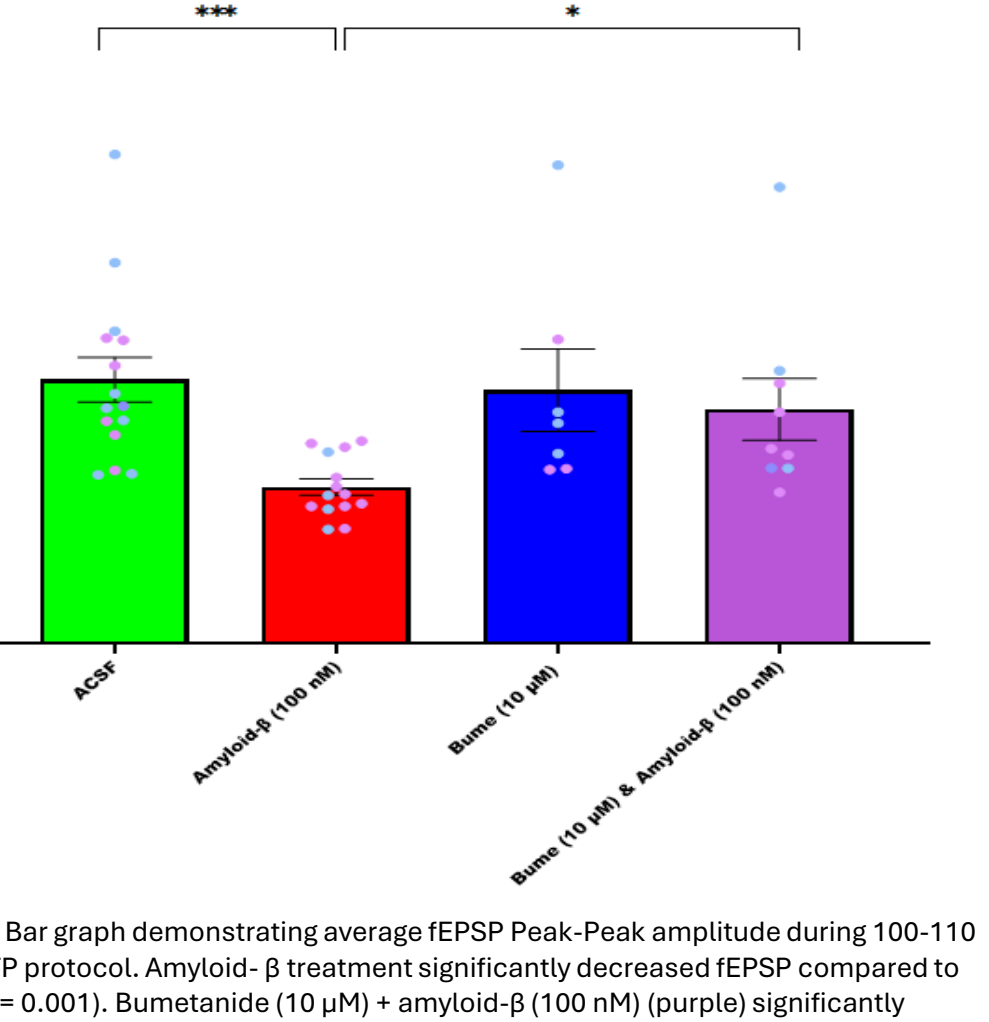


Figure 10: B) Bar graph demonstrating average fEPSP Peak-Peak amplitude during 100-110 minutes of LTP protocol. Amyloid-β treatment significantly decreased fEPSP compared to control (***p = 0.001). Bumetanide (10 μM) + amyloid-β (100 nM) (purple) significantly increased fEPSP compared to amyloid-β alone (red) (*p = 0.0436). Data is represented as median ± IQR (n = 6-15 mice per group, blue = males, pink = females) and analysed using Kruskal-Wallis test with Dunn’s multiple comparisons post-hoc (H = 18.79 , **** p = 0.0003).

Conclusions

NKCC1 activity is consistently dysregulated across neurological disorders:

Balance between chloride influx and chloride efflux is disrupted in several neurological disorders, causing the presence of depolarising GABAergic responses. Preclinically, bumetanide demonstrated the ability to restore hyperpolarising GABAergic activity, however clinical findings were inconsistent.

Bumetanide Demonstrates Dose-Dependent Cytotoxicity in Primary Hippocampal Neurons:

Findings from the primary hippocampal cell viability assay reveal a dose-dependent neurotoxic effect of bumetanide *in vitro*, while also failing to improve amyloid-β induced neurotoxicity across the tested concentration ranges following chronic exposure.

Bumetanide Restores Synaptic Plasticity Deficits Following Amyloid-β Exposure:

In acute hippocampal slices, 10 μM bumetanide treatment significantly restores LTP capacity following amyloid-β exposure in both axodendritic and somatic regions, suggesting improvements to both hippocampal signal processing and transmission. This indicates that bumetanide may be effective in mitigating early synaptic deficits associated with Alzheimer’s disease.

References

- 1.Lam, P., et al., Cation-Chloride Cotransporters KCC2 and NKCC1 as Therapeutic Targets in Neurological and Neuropsychiatric Disorders. Molecules, 2023. 28(3).
- 2.Nomura, T., Interneuron Dysfunction and Inhibitory Deficits in Autism and Fragile X Syndrome. Cells, 2021. 10(10).
- 3.Bi, D., et al., GABAergic dysfunction in excitatory and inhibitory (E/I) imbalance drives the pathogenesis of Alzheimer’s disease. Alzheimer’s Dement, 2020. 16(9): p. 1312-1329.
- 4.Ben-Ari, Y., et al., The GABA Excitatory/Inhibitory Shift in Brain Maturation and Neurological Disorders. The Neuroscientist, 2012. 18(5): p. 467-486.
- 5.Lam, P., et al., Beta-Amyloid (Aβ(1-42)) Increases the Expression of NKCC1 in the Mouse Hippocampus. Molecules, 2022. 27(8).
- 6.Zhang, H.L., et al., Inhibition of NKCC1 Ameliorates Anxiety and Autistic Behaviors Induced by Maternal Immune Activation in Mice. Curr Issues Mol Biol, 2024. 46(3): p. 1851-1864.
- 7.Hyde, T.M., et al., Expression of GABA signaling molecules KCC2, NKCC1, and GAD1 in cortical development and schizophrenia. J Neurosci, 2011. 31(30): p. 11088-95.
- 8.Deidda, G., et al., Reversing excitatory GABAAR signaling restores synaptic plasticity and memory in a mouse model of Down syndrome. Nature Medicine, 2015. 21(4): p. 318-326.
- 9.Feit, P.W., Aminobenzoic acid diuretics. 2. 4-Substituted-3-amino-5-sulfamoylbenzoic acid derivatives. Journal of Medicinal Chemistry, 1971. 14(5): p. 432-439.
- 10.Taubes, A., et al., Experimental and real-world evidence supporting the computational repurposing of bumetanide for APOE4-related Alzheimer’s disease. Nat Aging, 2021. 1(10): p. 932-947.
- 11.Damier, P., et al., A Double-Blind, Randomized, Placebo-Controlled Trial of Bumetanide in Parkinson’s Disease. Movement Disorders, 2024. 39(3): p. 618-622.
- 12.Dargaai, Z., et al., Restoring GABAergic inhibition rescues memory deficits in a Huntington’s disease mouse model. Proceedings of the National Academy of Sciences, 2018. 115(7): p. E1618-E1626.
- 13.Lemonnier, E., et al., A randomised controlled trial of bumetanide in the treatment of autism in children. Transl Psychiatry, 2012. 2(12): p. e202.
- 14.Lam, P., et al., Beta-Amyloid (Aβ(1-42)) Increases the Expression of NKCC1 in the Mouse Hippocampus. Molecules, 2022. 27(8).