The role of the KCC2 in substance use and abuse: A systematic review [Protocol]

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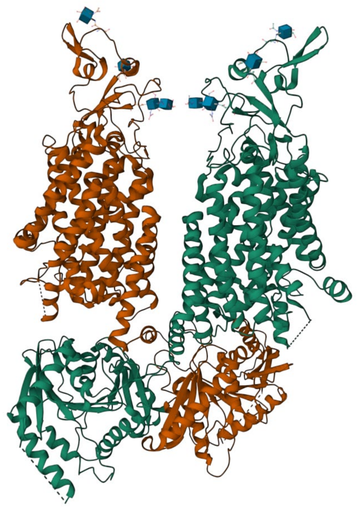

Figure 1. 3-D structure of human KCC2. Accessed from RCSB Protein Databank [1]

Purpose

KCC2 and substance use

- Potassium-Chloride Cotransporter 2 (KCC2) is a **neuronal membrane protein** specific to the central nervous system.
- It is responsible for removing Cl^- ions from the intracellular space, **maintaining a normal Cl^- gradient**. This is critical to the function of certain inhibitory synapses.
- Dysregulation causes an **upward shift in the Cl^- reversal potential** resulting in a hyperexcitable state of the postsynaptic neuron (see Fig. 3).
- Existing literature shows **intra-VTA administration of furosemide**, a non-selective KCC2 inhibitor, produces effects **similar** to those found **in chronic opioid dependence**. This points to a possible link between the hyperexcitability resulting from KCC2 dysregulation and opioid dependance.
- Substance use disorder is an aggressive disease that affects aspects of brain matter and ultimately changing behavior.

KCC2 and GABA modulation

- Prior work indicates **KCC2 is also involved in neuropathic pain and seizures**
- KCC2 appears to play **an important role in modulating GABA**
- Inhibitory synapses such as **gamma-aminobutyric acid** (GABA), play a crucial role in inhibition of neuronal excitability, and are **heavily influenced by a Cl- gradient.**
- KCC2 **may provide a target for pain and seizure therapies.**

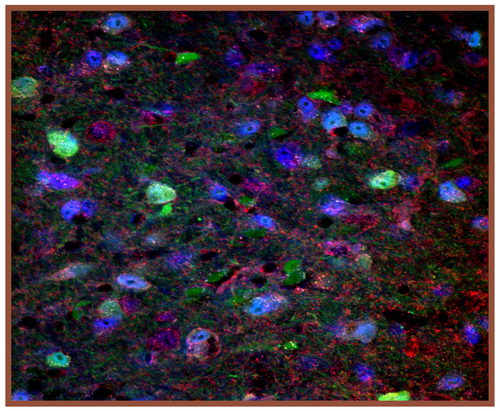

Figure 2. Composite immunohistochemistry image**.** The **Green channel** shows GAD67 GFP, the **blue channel** shows NeuN antibody stain, and the **red channel** shows KCC2 antibody stain.

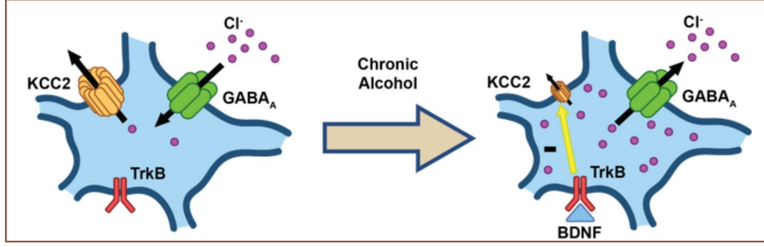

Figure 3. It is hypothesized that modulation of effector proteins that are upstream of KCC2 cause a decrease in KCC2 expression in the VTA and mediate some aspects of substance use disorders.

Methods

Study Design: Systematic review following PRISMA guidelines

Data Collection: Data will be collected from PubMed/MEDLINE, SciFinder, Embase, PsychInfo, and Cochrane Library. Scopus will be used for an aggregate search. A supplemental search will be conducted using Google Scholar.

Stop Criteria: Approximately 95% of relevant literature captured as calculated by the equation:

$$\frac{R_f}{R_t} \geq 95\%$$

Where R_f = the found relevant literature and R_t = the estimated true relevant literature [2].

Outcomes:

1. Evidence for or against the involvement of KCC2 in the effects of, use of, abuse of, and/or dependence on commonly used drugs.
2. Evidence for or against the efficacy of KCC2 manipulation in modulating the effects of, addiction to, or substance use behavior of any given substance.
3. Evidence for or against the involvement of KCC2 in consumption, craving, withdrawal, relapse or any other feature of substance use.
4. Evidence for or against the involvement of KCC2 gene expression or mutation in any of the above parameters.

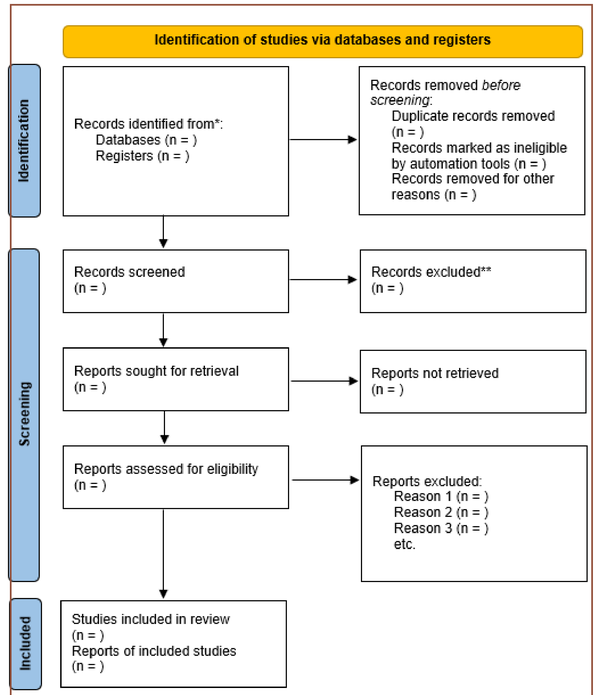

Figure 4. Example Prisma flowchart [3]

Analysis: Summary of article results without meta-analysis.

Search data: February 15, 2022

EndNote will be used as a reference manager and to de-duplicate the retrieved articles.

ASReview will be used to screen articles for relevance.

- ASReview utilizes machine learning to aid in the screening process in order to reduce screening time.
- According to a 2021 Nature paper, the average work saved over sampling is 83%.
- A variety of algorithms are available to help find and mark relevant papers [4].

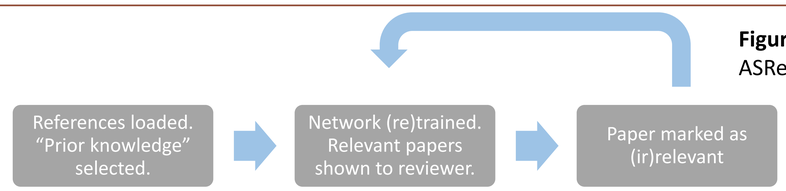

Figure 5. Flowchart depicting ASReview workflow

References
[1] Chi, X., Li, X., Chen, Y., Zhang, Y., Su, Q., & Zhou, Q. (2021). Cryo-EM structures of the full-length human KCC2 and KCC3 cation-chloride cotransporters. Cell Research, 31(4), 482-484.
[2] van Haastrecht, M., Sarhan, I., Yigit Ozkan, B., Brinkhuis, M., & Spruit, M. (2021). SYMBALS: a systematic review methodology blending active learning and snowballing. Frontiers in research metrics and analytics, 6, 33.
[3] Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., ... & Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. International journal of surgery, 88, 105906.
[4] Van De Schoot, R., De Bruin, J., Schram, R., Zahedi, P., De Boer, J., Weijdema, F., ... & Oberski, D. L. (2021). An open source machine learning framework for efficient and transparent systematic reviews. Nature machine intelligence, 3(2), 125-133.