Intracellular chloride accumulation impairs GABAergic signaling and memory in Down syndrome

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Down syndrome (DS) is a genetic disorder caused by the presence of a third copy of chromosome 21 and is characterized by altered brain development and varying degrees of intellectual disability. Trisomic mouse models of DS reproduce the main cognitive disabilities of the human syndrome. In particular, DS mice show structural and functional synaptic alterations as well as learning and memory deficits, largely determined by altered GABAergic transmission through chloride-permeable $GABA_A$ receptors ($GABA_A$Rs). Specifically, we have found that intracellular chloride accumulation shifts the chloride reversal potential and $GABA_A$R-mediated signaling from hyperpolarizing to depolarizing in the brain of the Ts65Dn mouse model of DS. Accordingly, the expression of the chloride importer NKCC1 is increased in the brains of both Ts65Dn mice and DS patients. Additionally, in DS neurons GABA developmental switch is incomplete and responses to pharmacological manipulation of $GABA_A$Rs are profoundly altered at the network and behavioral level, consistent with depolarizing actions of GABA.

Notably, both pharmacological inhibition by the FDA-approved NKCC1 inhibitor Bumetanide as well as knockdown of NKCC1 expression by RNA interference rescued intracellular chloride accumulation and $GABA_A$R-mediated inhibition in trisomic neurons. Most importantly, Bumetanide treatment and AAV-mediated neuron-specific NKCC1 knockdown *in vivo* rescued behavioral performance on different learning and memory tests in Ts65Dn mice.

Our findings demonstrate that NKCC1 overexpression drives depolarizing $GABA_A$R signaling in trisomic cells, leading to behavioral impairments in DS mice. Moreover, our study identifies a new molecular target for pharmacological treatments aimed at rescuing cognitive disabilities in persons with DS.