C1QL3 Facilitates Cognitively Challenging Behavior

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INTRODUCTION

Synaptic adhesion molecules (SAMs) make a specialized cell-cell junction across the synaptic cleft, and various complexes have been shown to control synapse formation and plasticity. Dysfunction of SAMs have been implicated in neuropsychiatric disorders such as autism and schizophrenia. Complement component 1, Q subcomponent-like 3 (C1QL3) is a novel potential SAM that is promising due to the behavioral deficits observed in mice that are correlated with impaired synaptic maintenance *in vivo*. SAMs are associated with neuropsychiatric disorders that exhibit impaired attention set shifting, a behavior that requires a subject to ignore stimuli that was previously relevant and instead respond to a previously irrelevant sensory modality. Previous studies have demonstrated *C1ql3* expression in the prefrontal cortex (PFC), a brain region implicated in attention deficits and cognitive flexibility. We aim to the investigate the role of C1QL3 in attention and cognitively flexibility by producing global KO and cKO (PFC) mice that were tested in attention set shifting behavior assays. As attention set shifting impairment is a common motif in neuropsychiatric disorders, these results could build toward understanding a new potential pathological process contributing to the symptoms in these disorders and identify novel targets for modulating synaptic function.

PFC C1QL3-HA Expression *In Vivo*

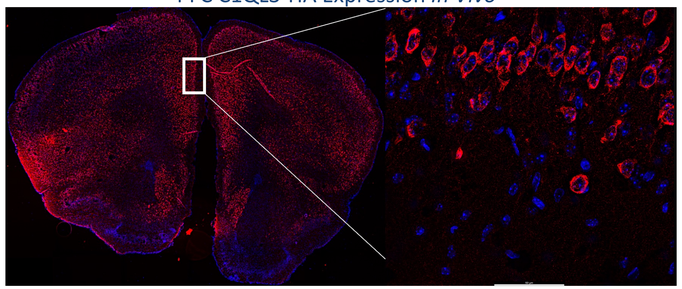


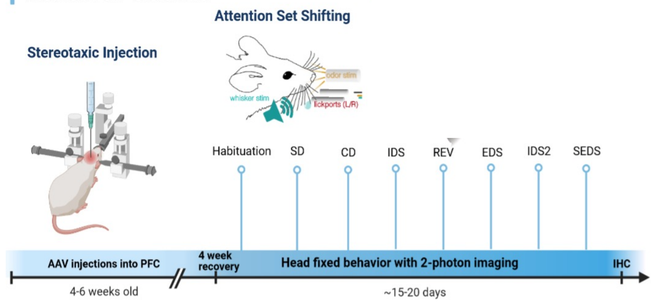
Figure 1. On left is a brain section of the PFC/ACA. On right is a magnified view revealing that a subset of cells express C1QL3-HA signal as well as puncta in the molecular layer, which is presumably revealing C1QL3 at synapses. Section taken from C1QL3-HA mouse, a novel line of mice with an HA epitope tag genetically knocked in to the *C1ql3* gene.

METHODS

Stereotaxic surgery and Knockout Model Creation: 4-6 week old mice are administered 1 AAV injection per hemisphere to target the infralimbic, prelimbic, and anterior cingulate portions of the PFC at -2mm, -1.5, and -0.75mm ventral, respectively. Injections were performed bilaterally. Conditional knockout (cKO) model was created using adeno associated viruses (AAVs) engineered to express Cre recombinase in neurons and mice homozygous for an allele with conditional knockout potential (Martinelli et al., 2016). The Cre AAV has a synapsin promoter for neuron specific expression. For the control group of the cKO a ΔCre AAV was used, which should not change the level of C1QL3 expression in the PFC. gKO mice were produced by interbreeding C1QL3 gKO heterozygotes to create wild type and homozygous KO littermates for comparisons.

Attention set shifting behavior: Mice will recover for 4 weeks post-surgery then begin behavior habituation and training. An equal numbers of each sex of mice were used. The mice are water restricted and, during trials, presented with two possible whisker vibration stimuli (35 versus 155 Hz bilateral vibration) and one of two possible odor stimuli (almond oil versus olive oil). They are cued to respond by licking a left or right lick spout to retrieve a water award upon termination of the 2.5 s compound stimulus[9] (Figure 2). We will be using the protocol from Spellman et al, (2021), in which mice are forced to learn incrementally more complex rules. Behavioral investigation begins with simple discrimination (SD), where animals are trained to discriminate between two stimuli within a single sensory modality (35 Hz whisker vibration signaling water from the left *vs* 155 Hz signaling water on the right). This is followed by compound discrimination (CD), where a distractor stimulus from the untrained sensory modality is added (mouse must still attend to whisker vibration, but now smells have been added that the mouse must ignore). Intradimensional shift (IDS), where stimuli from the relevant sensory modality are replaced with a new set of stimuli (shift the vibrations to 255 Hz for the left water spigot, clicks for the right spigot, while smell stays the same). This is followed by reversal (Rev) where the vibrations that previously signaled the left spigot are switched to signal for the right spigot and vice versa. Next is extradimensional shift (EDS), in which the pair of stimuli from the previously irrelevant sensory modality become the relevant stimuli (mice must now ignore whisker vibration and attend to olfactory stimuli to determine water location). Second IDS (IDS2) follows next, a pair of stimuli from the newly relevant sensory modality are replaced with a new pair of exemplars (shift olfactory stimuli from clove and almond scents to banana and citrus). The final task is serial extradimensional shift (SEDS), where the rules are switched automatically whenever the animal reaches criteria performance, which is 80% correct within a 30-trial moving window and >50% correct for both left and right trials. With this behavior task, one can tax the attention and executive function skills of a mouse similar to the human version of this task.

Behavior Outline



RESULTS

gKO and cKO mice Performance on Simple Discrimination (SD), Compound Discrimination (CD), Intradimensional Shift (IDS), and Reversal (REV) Tasks

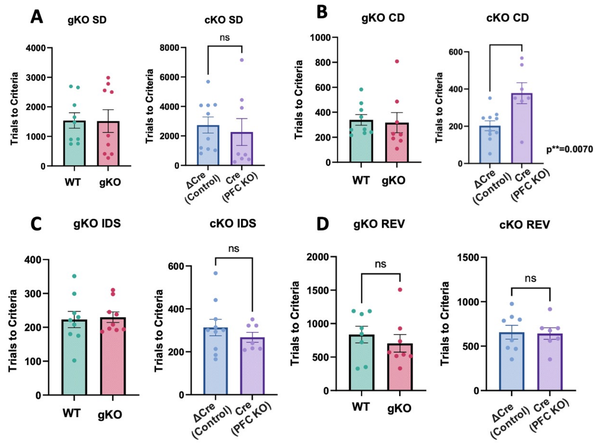


Figure 2. **(A)** Mean trial blocks completed for mice performing Simple Discrimination (SD) task in GKO and CKO cohorts of mice **(B)** Trials to criterion completed for mice performing Compound Discrimination (CD) task in GKO and CKO cohorts of mice. **(C)** Trials to criterion completed for mice performing Intradimensional Shift (IDS) task in GKO and CKO cohorts of mice. **(D)** Trials to criterion completed for mice performing Reversal (REV) task in GKO and CKO cohorts of mice.

gKO and cKO mice Performance on Extradimensional Shift (EDS), Intradimensional Shift 2 (IDS2), and Serial Extradimensional Shift (SEDS) Tasks

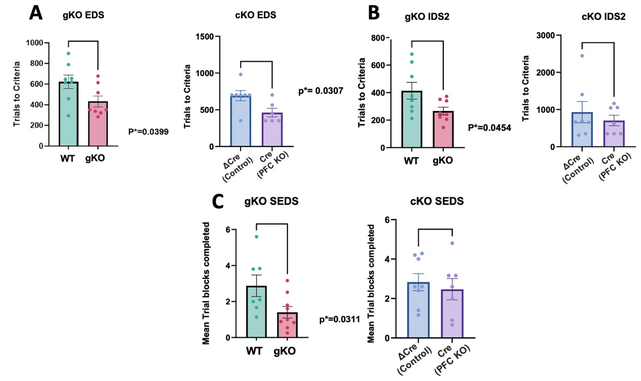


Figure 3. **(A)** Mean trial blocks completed for mice performing Extradimensional Shift (EDS) task in GKO and CKO cohorts of mice **(B)** Trials to criterion completed for mice performing Intradimensional Shift 2 (IDS2) task in GKO and CKO cohorts of mice. **(C)** Mean trial blocks completed for mice performing Serial Extradimensional Shift task in GKO and CKO cohorts of mice.

CONCLUSIONS

Results from Attentional Set Shifting Behavior Analysis supports the hypothesis that synaptic protein C1QL3 is important in affecting cognitively challenging behavior. This is bolstered by trends observed in the differing ability of mice from the gKO and cKO cohorts to attend to target sensory modalities (while ignoring distracting modalities) as well as adapting behavior when attention rules change. Trials to criterion measures the total number of trials completed by the mouse over each session they spend on a certain session/task. Once the mice finish a session with a passing percentage of correct trials (during a single session), they may proceed to the next behavior task. Thus, the more trials needed to reach criterion suggests that a mouse found a task more difficult, and vice versa. For the SEDS tasks, trial blocks measure how many times could adapt the rule shifts and succeed during the session. Higher mean trial blocks suggests a mouse found a task less difficult, and vice versa The ease or difficulty of these transitions in behavior could suggest that mice with impaired C1QL3 function may exhibit deficits in attention set shifting behavior, while others may exhibit increased aptitude for certain tasks.

- C1QL3 expressed in the brain of the gKO mice impacts aspects of cognition. Surprisingly it improves their performance when switching from whisker vibrations to detecting scents. This can be appreciated in Figure 2, where gKO mice showed considerable aptitude with the IDS2 task over the wild types. The IDS2 task focuses largely on attending to odor cues over previously relevant vibration cues.
- Behavior data for the gKO mice suggests that mice lacking C1QL3 protein had more difficulty completing tasks evaluating cognitive function. The EDS/SEDS tasks involves the stimuli (vibrations) that previously signaled the left spigot being switched to signal for the right spigot and vice versa. This shift in stimuli-reward mapping requires mice to exhibit a certain level of cognitive flexibility to adapt and learn the task. The CD task doesn't present this same level of cognitive duress. Instead, it is largely a measure of sensory/motor function and serves as a control experiment. gKO mice showed significant improvement when compared to the wild type group when it came to the SEDS and EDS tasks. This suggests that mice with impaired C1QL3 expression exhibited phenotypes of impaired cognitive function.

CITATIONS

- Martinelli, David C et al. "Expression of C1ql3 in Discrete Neuronal Populations Controls Efferent Synapse Numbers and Diverse Behaviors." Neuron vol. 91,5 (2016): 1034-1051. doi:10.1016/j.neuron.2016.07.002
- Spellman, Timothy et al. "Prefrontal deep projection neurons enable cognitive flexibility via persistent feedback monitoring." *Cell* vol. 184,10 (2021): 2750-2766.e17. doi:10.1016/j.cell.2021.03.047