Acetate Regulates Anxiety-Like Behavior Induced by Looming Visual Stimuli via CaMKII Neurons in the BNST

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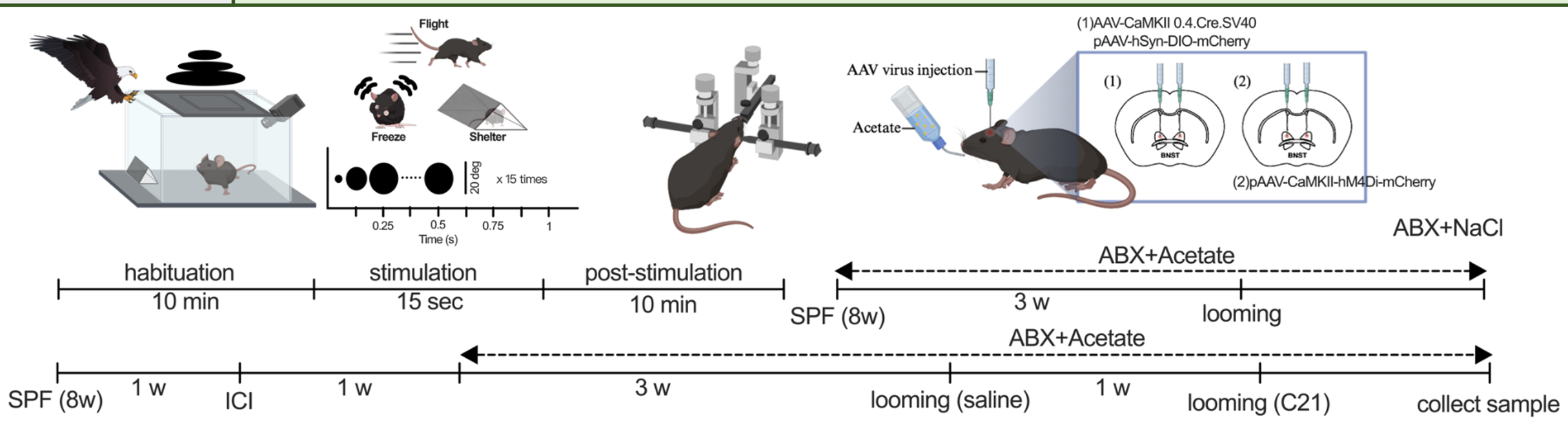
Introduction

Anxiety is an emotional state characterized by heightened tension and unease, often triggered by excessive vigilance toward the unknown. While moderate anxiety serves an adaptive role in avoiding danger and enhancing, excessive anxiety disrupts physiological homeostasis. Such an emotional state can be altered by stress or threat exposure. The looming visual stimuli test is a well-established method to elicit innate defensive behaviors in mice by simulating an approaching object mimicking aerial predatory threats. Defensive responses during stimulation include freezing, flighting, and hiding. Moreover, after the stimulation, rodents tend to avoid areas associated with the risk of approaching objects. Our prior works demonstrated that broad-spectrum antibiotic cocktail (ABX) treatment abolished the anxiety-like behavior in mice, while the administration of gut microbiota-derived metabolites, short-chain fatty acids (SCFAs), particularly acetate, restored the innate anxiety-like behavior to baseline level. In addition, we previously showed that acetate can alter behavior via calcium/calmodulin-dependent protein kinase II (CaMKII) neurons in the bed nucleus of the stria terminalis (BNST) through fatty acid oxidation. Based on these findings, we hypothesize that acetate-treated ABX mice display heightened anxiety-like behavior after the looming visual stimuli. First, ABX mice were administrated acetate orally for at least 3 weeks, using sodium chloride as the control. Behavioral responses following looming visual stimuli were analyzed. Interestingly, we observed that the acetate-treated ABX mice spent more time in the shelter compared to the control group during the post-stimuli period. Additionally, the acetate-treated group displayed the trends toward increased freezing and hiding behaviors compared to the control group during stimulation. Next, we employed a chemogenetic approach to selectively silence CaMKII neurons during looming visual stimuli by injecting AAV-CaMKII-hM4Di-mCherry into the BNST. Anxiety-like behavior induced by looming stimuli will be analyzed chemogenetically to understand the exact role of BNST CaMKII neurons in behavioral modulation. We anticipate that acetate elevates the looming visual stimuli-induced anxiety-like behavior via the activation of CaMKII neurons at the BNST. These findings provide insights into the gut-brain axis mechanisms underlying anxiety and offer potential therapeutic targets for managing anxiety disorders.

Materials and Methods



Results 1.

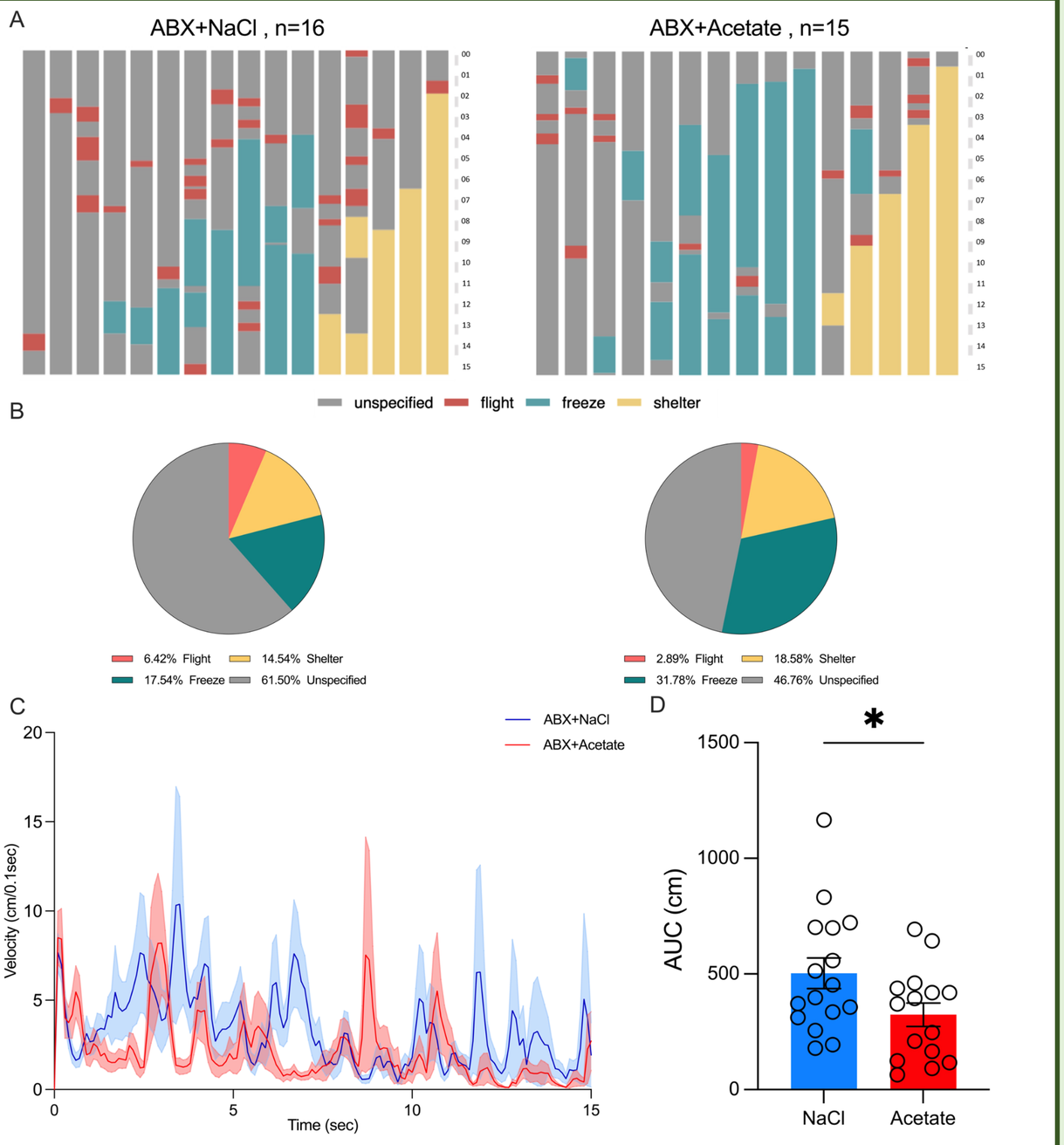


Fig1. Supplementation of acetate in antibiotic cocktail (ABX) mice (ABX+Acetate) alters innate defensive behavior evoked by looming visual stimuli.(A) Raster graph of defensive behavior in 15 seconds looming visual stimuli in control group (ABX+NaCl) and ABX+Acetate (n=16, 15). Each column represents a mouse and y axis represents time (second). Gray: unspecified; Red: flight; Blue: freeze; Yellow: shelter. (B) Pie chart for the percentage of behavioral responses to looming visual stimuli in ABX+NaCl (left) and ABX+Acetate mice (right) (n=16, 15). Gray: unspecified; Red: flight; Blue: freeze; Yellow: shelter. (C) Mean velocity of ABX+NaCl (blue) and ABX+Acetate mice (red) in 15 seconds during looming visual stimuli. (D) Area under the curve (AUC) of mean velocity for ABX+NaCl (blue) and ABX+Acetate mice. Data shown as individual points with ± SEM. Data analyzed by Two-tailed unpaired *t*-test. * $P < 0.05$.

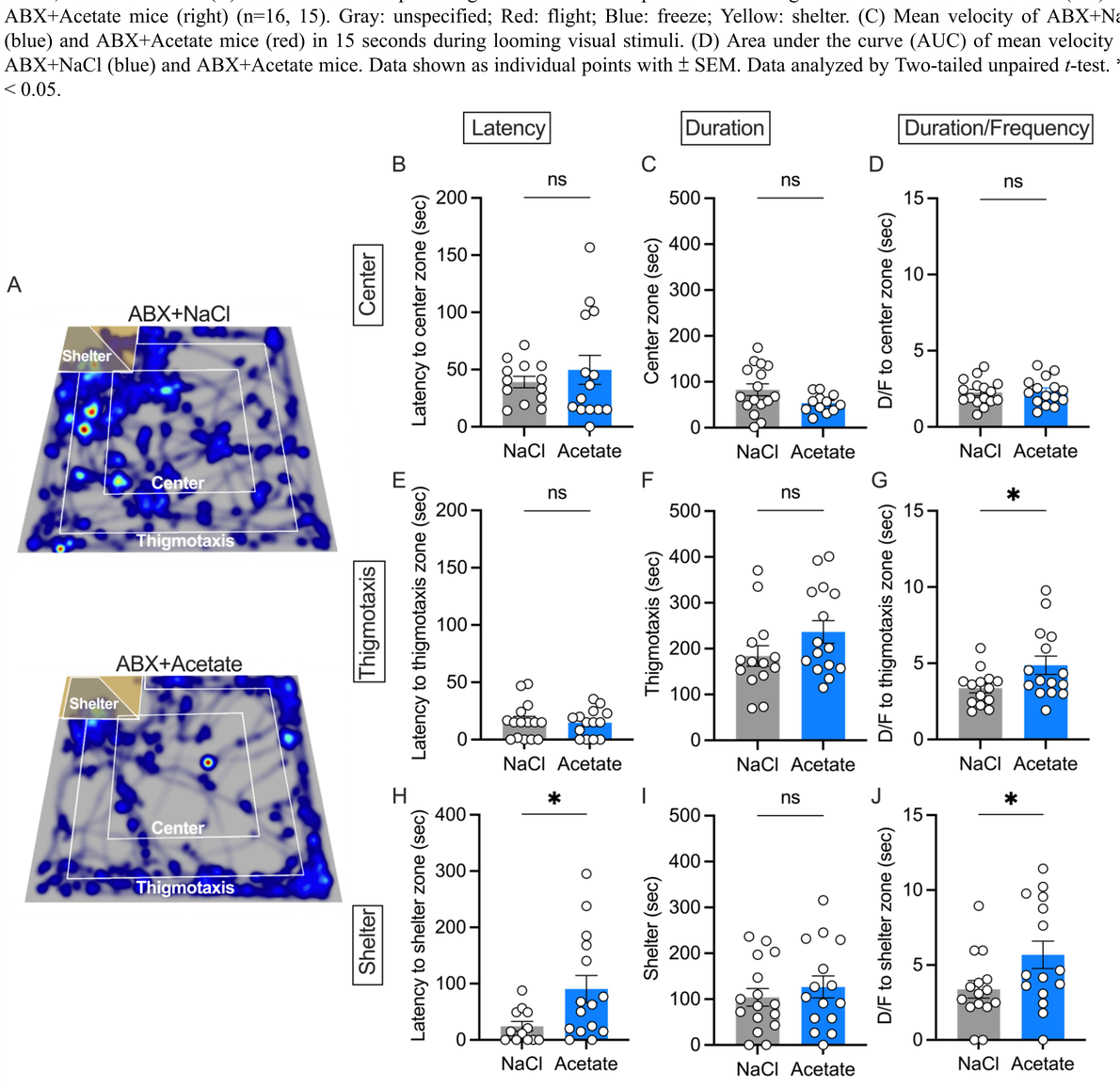


Fig2. Increased anxiety-like behavior in mice treated with ABX+Acetate after looming visual stimuli.(A) Representative image of heatmap of time spent in each zone in 10 minutes after looming visual stimuli in ABX+NaCl and ABX+Acetate-treated mice. (B, E, H) Latency, (C, F, I) time, (D, G, J) mean duration (Duration/Frequency, D/F) to/in center zone (B, C, D), thigmotaxis zone (E, F, G), and shelter (H, I, J) in ABX+NaCl and ABX+Acetate-treated mice (n=16, 15) in 10 minutes after looming visual stimuli. Data shown as individual points with ± SEM. Data analyzed by Two-tailed unpaired *t* test. * $P < 0.05$, ns: no significant.

Results 2.

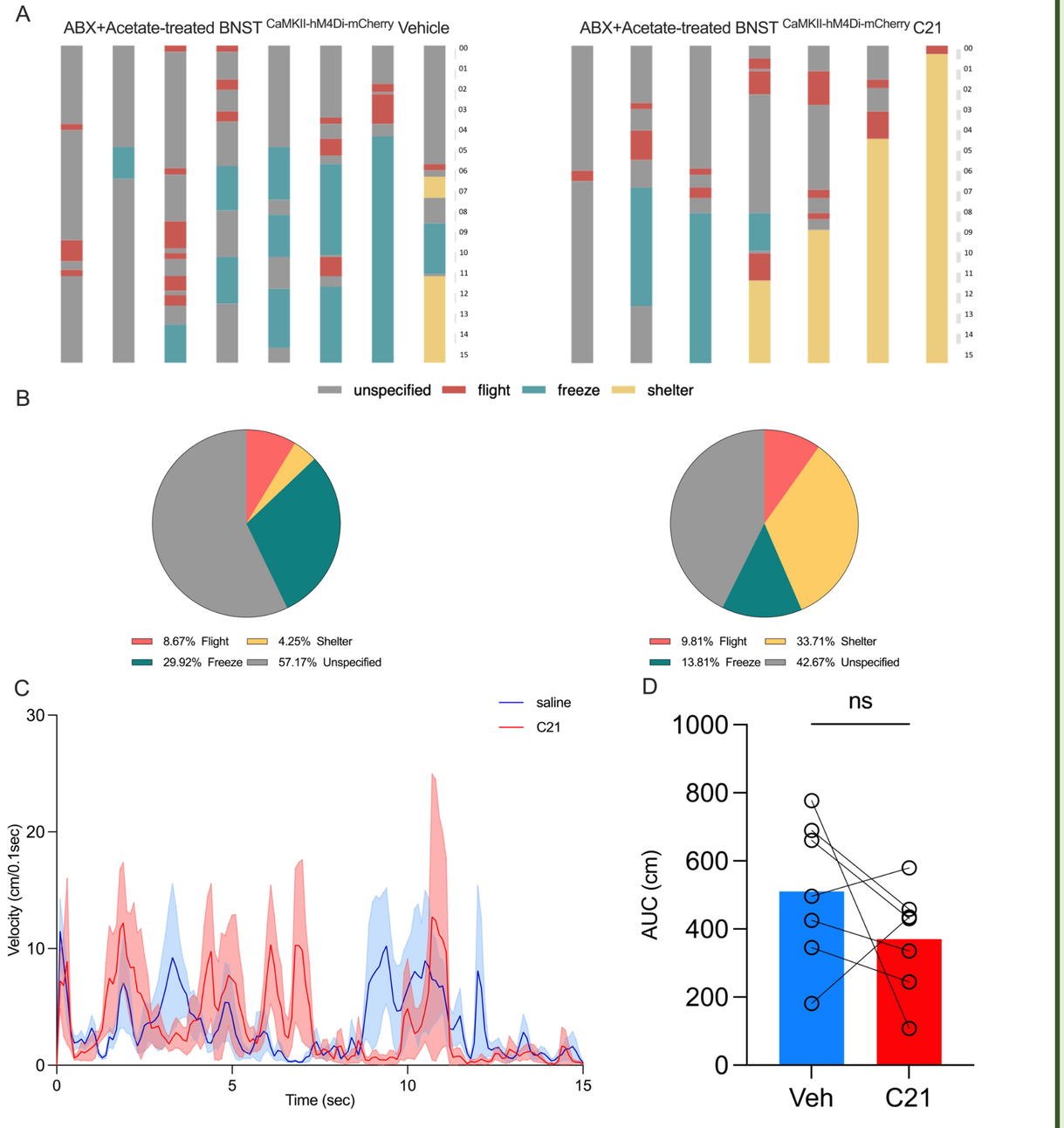


Fig3. Inhibition of CaMKII neurons in ABX+Acetate mice does not alter defensive behaviors evoked by looming visual stimuli.(A) Raster graph of defensive behavior in 15 seconds looming visual stimuli in vehicle- and C21- injected BNST$^{CaMKII\text{-}hM4Di\text{-}mCherry}$ mice (n=8, 7) with ABX+Acetate treatment. Each column represents a mouse and y axis represents time (second). Gray: unspecified; Red: flight; Blue: freeze; Yellow: shelter. (B) Pie chart for the percentage of behavioral responses to looming visual stimuli in vehicle- (left) and C21- (right) injected BNST$^{CaMKII\text{-}hM4Di\text{-}mCherry}$ mice (n=8, 7) with ABX+Acetate treatment. Gray: unspecified; Red: flight; Blue: freeze; Yellow: shelter. (C) Mean velocity of vehicle- (blue) and C21- (red) injected BNST$^{CaMKII\text{-}hM4Di\text{-}mCherry}$ mice (n=7) with ABX+Acetate treatment in 15 seconds during looming visual stimuli. (D) Area under the curve (AUC) of mean velocity for ABX+Acetate-treated BNST$^{CaMKII\text{-}hM4Di\text{-}mCherry}$ mice. Data shown as individual points with ± SEM. Data analyzed by Two-tailed paired *t*-test. ns: no significant.

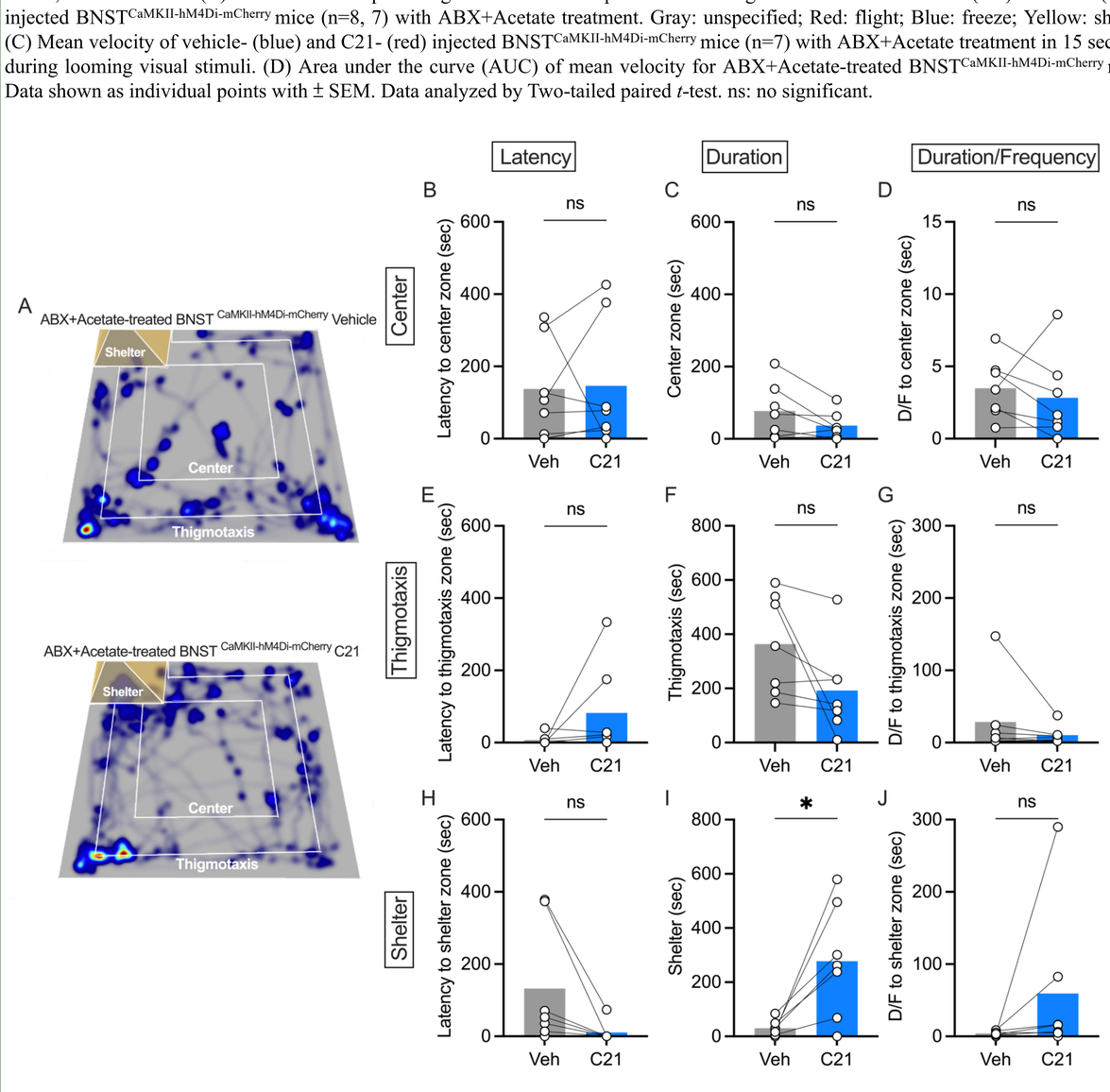


Fig4. Inhibition of CaMKII neurons in ABX+Acetate mice increases hiding behavior after looming visual stimuli.(A) Representative image of heatmap of time spent in each zone in 10 minutes after looming visual stimuli in ABX+Acetate-treated BNST$^{CaMKII\text{-}hM4Di\text{-}mCherry}$ mice. (B, E, H) Latency, (C, F, I) time, (D, G, J) mean duration (Duration/Frequency, D/F) to/in center zone (B, C, D), thigmotaxis zone (E, F, G), and shelter (H, I, J) in vehicle or C21 injected (n=7) ABX+Acetate-treated BNST$^{CaMKII\text{-}hM4Di\text{-}mCherry}$ mice after looming visual stimuli. Data shown as individual points with ± SEM. Data analyzed by Two-tailed unpaired *t* test. * $P < 0.05$, ns: no significant.