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Neuronal Glutamate Transporters Mediate Stereotypic Reward-Based Behaviors

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Neuronal glutamate transporters mediate stereotypic reward-based behaviors

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Abstract

Obsessive-compulsive Disorder (OCD) is a common neuropsychiatric disorder characterized by obsessions (uncontrollable and recurring thoughts), and compulsions (behaviors that one has the urge to repeat several times). One of the genes carrying non-functional mutations in OCD is *Slc1a1*, the gene that encodes the neuronal glutamate transporter EAAC1. However, we still have an incomplete understanding of how EAAC1 contributes to the onset of compulsivity in OCD-like behaviors. EAAC1 is abundantly expressed in the striatum, the input nucleus of the basal ganglia implicated with compulsivity and reward. Here, we use a series of behavioral assays to determine whether and how reward-based behaviors vary between wild type and EAAC1$^{-/-}$ mice. We found that EAAC1$^{-/-}$ mice have an increased propensity to engage in reward-based behaviors. Together, these findings suggest that EAAC1 may be critical to limit hyperactivity in the striatum and its ability to integrate reward and sensory information.

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Table of Contents

Introduction

Obsessions and compulsions can be debilitating and have negative impacts on the on the quality of life, as it happens for individuals affected by OCD. A major factor contributing to the psychopathology of OCD is the processing of reward and reward-seeking behaviors, as patients with OCD often behave in a repetitive manner that is perceived as being able to reduce anxiety (Alves-Pinto, 2019).

One of the genes that genetic studies found to be associated with OCD is *Slc1a1*. This gene encodes glutamate transporter EAAC1 (Arnold et al., 2006), which shows loss-of-function mutations in OCD (Dickel et al., 2006). Some of the behavioral effects that have been observed with the loss of EAAC1 are anxiety-like behaviors like excessive grooming (Bellini et al., 2018). Loss-of-function of EAAC1 may also lead to neuronal hyperactivity, but this hypothesis remains to be tested (Porton et al., 2013).

EAAC1 is primarily expressed post-synaptically at excitatory synapses onto medium spiny neurons (MSNs) in the striatum. In addition, EAAC1 is expressed pre-synaptically at inhibitory synapses in the striatum. By transporting glutamate, a precursor of GABA, in pre-synaptic terminals, EAAC1 strengthens inhibition onto MSNs. Since the execution of stereotyped behaviors falls under the control of the striatum, and depends on dopamine release, we asked whether behaviors associated with dopamine release could also be altered in the absence of EAAC1. Dopamine plays an important role in motivation, executive function, motor control, and that it is correlated with reward value. Specifically, it is thought to encode the temporal difference between expected and actual reward time, also known as reward prediction error (Schultz, 2016). The striatum is the main input nucleus of the basal ganglia, which controls the execution of voluntary movements, including stereotyped movements and reward-based behaviors (Bellini, 2018). In the striatum, projection neurons receive modulation via dopaminergic projections. Dopaminergic motor and reward inputs are then used to select action outputs. Previously, it has been found that 1 year old EAAC1$^{-/-}$ mice substantially lack dopaminergic neurons in the substantia nigra pars compacta (Berman, 2011). The substantia nigra pars compacta (SNc) plays a large role in dopamine production and is a projection to the basal ganglia circuitry (Bolam, 2000). The basal ganglia is a group of subcortical nuclei including the putamen, globus pallidus externus, and internus), the subthalamic nucleus (STN), and the substantia nigra (SN). These structures function together to regulate movement (Young, 2022). Patients with OCD show disturbances in the function in the basal ganglia circuit as well as the orbitofrontal cortex and anterior cingulate cortex (Middleton, 2000). The striatum is the primary nucleus input of the basal ganglia. Alterations in dopaminergic projection to the striatum promote the development of compulsive behaviors especially those present in OCD. OCD is associated with disrupted striatal activity, and previous studies have shown altered activation in

fronto-striatal networks in association with the processing of reward (Sha, 2020). The striatum is associated with hyperactivity as seen in patients with OCD as well as reward processing.

Current research on OCD and EAAC1 has not yet explored how EAAC1 functions within striatal neurons to encode behaviors, such as the stereotyped movements and reward-based behaviors. In previous research, $EAAC1^{-/-}$ mice have shown an impulsive behavioral phenotype (Bennink, 2020). In our previous behavioral tests, $EAAC1^{-/-}$ mice perform better when given a repetitive reward-based lever-press task. In a previous test in which the mice pressed a lever one time for a water reward (FR1), wild type (WT) mice pressed the lever more than the $EAAC1^{-/-}$ mice in later sessions. When tasked with pressing a lever eight times for a water reward (FR8), there was no significance found in differences between lever presses and rewards received between WT and $EAAC1^{-/-}$ mice. Considering the important role of dopamine in the temporal difference in reward processing and the lack of dopaminergic neurons in the SNc, we introduce a timed component in which both groups of mice will press the lever eight times within six seconds to receive the water reward to assess any differences in temporal processing of reward between WT and $EAAC1^{-/-}$ mice.

Materials & Methods

Ethics Statement.

All experimental procedures have been approved by the Institutional Animal Care and Use Committee at the State University of New York at Albany and guidelines described in the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

Mice.

All experiments were be performed on cohorts of 16 (8 male and 8 female) C57BL6/J (WT) and EAAC1$^{-/-}$ mice aged 2-6 months.

One week before the beginning of our tests, the home cage water of all mice was be replaced with 2% (w/v) citric acid water, the purpose being to provide more of an incentive for the mice to work for a freshwater reward. Citric acid is a food-grade, colorless, weak organic acid. By creating a 2% w/v solution for each home cage, the mice still had access to adequate hydration without complete water deprivation or depleting motivation for a reward.

Behavioral Tests.

Each 10-min behavior session took place between 8:00 AM and 7:00 PM in a behavioral chamber with two adjacent suites separated by an opaque wall. The mice were free to detect their body odors and vocalize. Each behavior session was video recorded with Free2X Webcam Recorder. The chambers were cleaned with 70% isopropyl alcohol between each session.

Control for Social Interaction.

Both groups of mice (WT and EAAC1$^{-/-}$) first explored an empty behavioral chamber with no lever or water valve. Each subject underwent two 10-min sessions (one individual and one paired) of the Control task every day for a total of 18 sessions. In individual sessions, each mouse explored one suite of the behavioral chamber alone for 10 min. In a separate session, each mouse was paired with one of their littermates, and both mice explored their respective suite of the behavioral chamber at the same time for 10 minutes. Because each suite was separated by an opaque divider, the mice were not able to see each other, but they were still able to detect each other's body odors and vocalize. Our objective was to familiarize the mice with the behavior chamber and observe possible differences in hyperactivity between the WT and EAAC1$^{-/-}$ mice.

The mice were then placed in a behavioral chamber with a lever and a transparent divider. Each subject underwent two 10-min sessions (one individual and one paired) of this task every day for a total of 18 sessions. Here, we measured intermouse difference and observed the mice's behavior for any indication of differences in social interaction between WT and EAAC1$^{-/-}$ mice. The purpose of this experiment was to determine whether the desire for social interaction is a

potential confounding variable in the experiment, or if mice were more interested in the factors of enrichment present in the behavioral chamber.

FR-1 Lever Press Training task.

Both groups of mice were trained to press a lever once to receive a 7 µl water reward. Lever and valve control was accomplished using a B-pod state machine, an open-control system for measurement of animal behavior. Each subject underwent a 10-min session of the FR-1 Lever Press Training task every day for a total of 9 sessions. Each mouse was paired with one of their littermates, and both mice completed the task in their respective suite of the behavioral chamber at the same time. The goal of this experiment was to familiarize the mice with pressing a lever for a reward.

FR-8 Sequence Training task.

In both groups of mice, the reward was delivered to the mouse only if they pressed the lever in an eight time sequence. Lever and valve control was accomplished using a B-pod state machine. Each subject underwent a 10-minute session of the FR-8 Sequence Training task every day for a total of 9 sessions. Each mouse was paired with one of their littermates, and both mice completed the task in their respective suite of the behavioral chamber at the same time. The goal of this experiment was to examine behavioral differences in reward processing between the two groups of mice during the completion of this task.

Timed FR-8 Sequence Training task.

In both groups of mice, the reward was delivered to the mouse only if they pressed the lever eight times within a six-second interval. If a mouse failed to press the lever eight times within a six-second interval, the counter would restart, and the mouse would need to restart the sequence. Lever and valve control was accomplished using a B-pod state machine. Each subject underwent a 10-minute session of the Timed FR-8 Sequence Training task every day for a total of 9 sessions. Each mouse was paired with one of their littermates, and both mice completed the task in their respective suite of the behavioral chamber at the same time. The goal of this experiment was to further examine any behavioral differences during reward processing between the two groups of mice during the completion of this task. Through this task, we can analyze the total presses of each mouse plus their total rewards and out-of-sequence presses.

Analysis.

ezTrack is an open-source video analysis program that uses Python code to allow the selection of a particular region of interest and uses an animal's center of mass to accurately track the animal's activity. With this information, we were able to determine each mouse's distance traveled as well as intermouse distance (IMD) and generate heat maps to display mouse localization within the behavioral suite.

MATLAB is a programming and computing platform most used to analyze data and create algorithms. We used a custom code written in MATLAB to operate a B-pod state machine, which is an open-source system used to measure animal behavior in multiple experimental trials. Three separate protocols were written for the FR-1, FR-8, and Timed FR-8 conditions for the lever and reward delivery to operate effectively in each of the tasks. MATLAB generates Excel files of data from each experiment, all of which include the number of lever presses and the time point in which each lever press occurred. The FR-8 experimental data also includes the number of rewards, and the Timed FR-8 experimental data includes all of the above as well as the number of unrewarded presses.

IgorPro (Wavemetrics) is a commonly used scientific data analysis software program. Data from the Excel files generated from MATLAB were entered and analyzed through this program. ANOVAs were conducted with SPSS software to assess statistical significance ($p<0.05$). Data is shown as mean ± SEM.

Immunohistochemistry.

c-Fos is an immediate early gene used as a marker for neuronal activity (Velazquez, 2015). GAD67 is an enzyme that is involved in metabolizing glutamate into GABA. We use an immunohistochemistry staining technique to look for markers of cellular activation with c-Fos and GAD67. An hour after completing the final phase of the lever press experiments, the mice were perfused, and their brains were post-fixated 4% PFA in PBS at 4°C overnight. Then, after overnight post-fixation, the brains were washed with cold PBS (3 x 30 min). They were then placed in 30% sucrose in PBS and stored at 4°C for 1-2 days. Afterwards, the brain was washed again with cold PBS (3 x 30 min), and stored in PBS at 4°C. The brains were then sliced and stained with a 1:1000primary antibody concentration of rabbit anti c-Fos and mouse anti-GAD67, then incubated overnight with motion at 4°C. The next day, we treated them with secondary antibodies goat anti-rabbit AlexaFluor 594, goat anti-mouse AlexaFluor 488, and goat anti-mouse AlexaFluor 647 at a concentration of 1:1000. They were then incubated for 2-3 hrs at room temperature, stained with DAPI, then mounted and imaged with both a fluorescence and confocal microscope. Stained cells from the images captured by the microscopes were counted and density was calculated in cells per mm^3.

Results

Locomotor activity is similar in WT and EAAC1$^{-/-}$ mice

In the first control phase of our experiment, the mice underwent a control task in which they each received nine individual 10-minute behavior session in a 3D printed behavioral chamber with no lever or enrichment factors present (**Fig. 1A**). Then, the mice underwent nine paired behavior sessions in the chamber, separated by an opaque divider. Our ezTrack analysis showed that WT and EAAC1$^{-/-}$ mice traveled similar distances across all paired sessions (**Fig. 1B-1C**). There are additionally no apparent sex differences present between male and female WT mice or male and female EAAC1$^{-/-}$ mice in distance traveled during these sessions (**Fig. 1D-1G**). Thus, we conclude that there is no indication that the loss of EAAC1 has a significant impact on hyperactivity.

Social interactions are similar in WT and EAAC1$^{-/-}$ mice

During the lever press phase of our experiment, our mice were placed in a behavioral chamber with a transparent divider and a lever present inside the box. We observed that the mice tended to localize mostly near the lever by the ninth session (**Fig. 2B, F, J**). There was no significant difference in intermouse distance (IMD) throughout all sessions between the WT (n=46) and EAAC1$^{-/-}$ mice (n=50) (**Fig. 2C, G, K**). There was also no significant difference between the WT and EAAC1$^{-/-}$ mice in distance traveled across all nine sessions (**Fig. 2D, H, L**).

Additionally, we detected similar patterns across sexes both between WT and EAAC1$^{-/-}$ mice, indicating that both groups showed similar social interaction behavior with their partner on the other side of the divider. We also observed through our analysis that the patterns of exploration exhibited by all the mice are similar from the first to the ninth session, in which they tend to confine to the areas surrounding the lever. We thereby conclude that with the presence of enrichment factors in the behavioral chamber, the loss of EAAC1 has little to no effect on social interaction behavior.

WT mice show more reward-based behaviors in single-lever press task

In this phase of the experiment, both groups of mice were tasked with pressing a lever once for a 7 μl water reward (**Fig. 3A**). Here, we observed that in session 1 for both male and female WT and EAAC1$^{-/-}$ mice, both groups received similar numbers of rewards in the first few sessions, but later showed differences. In the first session, EAAC1$^{-/-}$ and WT mice received similar amounts of rewards. By the ninth session, the number of cumulative rewards ($p=2.0e^{-4}$) was greater in WT mice. However, the difference in the total number of presses between WT mice and EAAC1$^{-/-}$ mice showed no significance (**Fig. 3B**). In WT and EAAC1$^{-/-}$ male mice, similar numbers of rewards were received across the nine sessions. In session 9, some significance can be seen in that the total number ($p=0.03$) and cumulative number of rewards ($p=6.2e^{-5}$) received was greater in male WT mice than EAAC1$^{-/-}$ mice (**Fig. 3C**). In WT and EAAC1$^{-/-}$ female mice

in session 1, EAAC1$^{-/-}$ mice had significantly greater total ($p=0.01$) and cumulative presses ($p=2.0e^{-4}$) than WT mice (**Fig. 3D**).

Behavior of WT and EAAC1$^{-/-}$ mice is similar in an FR8 sequence-lever press task

During this experiment, the mice were again given a lever press task, but in these sessions needed to press the lever eight times instead of once for the same 7 µl water reward (**Fig. 4A**). Here, we observed that in session 1 for both male and female WT and EAAC1$^{-/-}$ mice, both groups pressed the lever a similar number of times and received similar numbers of rewards from session 1 to 9. (**Fig. 4C**). In the initial session, there is some significance in that WT mice in total pressed the lever more ($p=1.1e^{-8}$) and received more rewards ($p=4.6e^{-6}$) than EAAC1$^{-/-}$ mice (**Fig. 4D**). By session 9, these numbers were about the same (**Fig. 4E**). We found no significant differences in sequence duration and the amount of time in inter-sequence intervals between WT and EAAC1$^{-/-}$ mice (**Fig. 4F**).

In session 1 for male WT and EAAC1$^{-/-}$ mice, WT mice pressed the lever more ($p=6.1e^{-4}$) but received similar numbers of rewards from session 1 to 9. (**Fig. 5B**). In the initial session, there is some significance in that WT mice in total pressed the lever more ($p=1.1e^{-8}$) and received more rewards ($p=2.0e^{-4}$) than EAAC1$^{-/-}$ mice (**Fig. 5C**). By session 9, there was still some significance in total amounts of presses ($p=6.2e^{-5}$) and rewards ($p=1.7e^{-3}$), but not in cumulative presses and rewards (**Fig. 5D**). We found no significant differences the amount of time in inter-sequence intervals between WT and EAAC1$^{-/-}$ mice but found that WT male mice had shorter sequence durations (**Fig. 5E**).

In session 1 for female WT and EAAC1$^{-/-}$ mice, both groups pressed the lever a similar number of times and received similar numbers of rewards from session 1 to 9. (**Fig. 6B**). In the initial session, there is some significance in that WT mice in total pressed the lever more ($p=6.2e^{-5}$) and received more rewards ($p=4.6e^{-6}$) than EAAC1$^{-/-}$ mice (**Fig. 6C**). By session 9, it appeared that EAAC1$^{-/-}$ mice pressed the lever more ($p=5.6e^{-6}$) and received more rewards ($p=2.6e^{-7}$) (**Fig. 6D**). We found no significant differences the amount of time in inter-sequence intervals between WT and EAAC1$^{-/-}$ mice but found that EAAC1$^{-/-}$ female mice had shorter sequence durations (**Fig. 6E**).

EAAC1$^{-/-}$ mice execute more reward-based behaviors in timed sequence task

To assess the temporal differences in reward-based behavior between WT and EAAC1$^{-/-}$ mice, we examined the mice perform a timed sequence behavioral task (**Fig. 7A**). Here, we found that EAAC1$^{-/-}$ mice perform better in this task than their wildtype counterparts. In this task, 14% of EAAC1$^{-/-}$ mice (n=21) performed in this task compared to 0% of WT mice (n=31) performers. We also found that the WT mice had a higher percentage of improvers in the task (8% of WT improvers compared to 2% of EAAC1$^{-/-}$ improvers). (**Fig. 7B**). In session 1, we observed that EAAC1$^{-/-}$ mice pressed the lever significantly more times than the WT mice ($p=1.1e^{-5}$), as well as received more rewards ($p=2.3e^{-6}$). EAAC1$^{-/-}$ mice also had significantly more cumulative lever presses and rewards. By session 9, although the WT mice had improved at the task, the

amount of lever presses ($p=4.6^{-3}$) and rewards ($p=2.6e^{-7}$) in EAAC1$^{-/-}$ mice was still significantly higher. We found no significant difference in out sequenced presses between WT and EAAC1$^{-/-}$ mice (**Fig. 7C**). We observed a significantly greater number of lever presses ($p=5.6e^{-4}$) and rewards ($p=1.7e^{-10}$) in EAAC1$^{-/-}$ mice across all sessions, and similar trends between WT and EAAC1$^{-/-}$ mice in number of out sequenced presses (**Fig. 7D**).

In male WT and EAAC1$^{-/-}$ mice, we found that EAAC1$^{-/-}$ mice perform better in this task than their wildtype counterparts. 11% of male EAAC1$^{-/-}$ mice (n=9) performed in this task compared to 0% of WT mice (n=13) performers. We also found that the male WT mice had a higher percentage of improvers in the task (4% of WT improvers compared to 0% of EAAC1$^{-/-}$ improvers) (**Fig. 8B**). In session 1, we observed no significant differences between male WT mice and male EAAC1$^{-/-}$ mice in lever presses. Male EAAC1$^{-/-}$ mice had significantly more rewards ($p=1.8e^{-5}$) and cumulative rewards ($p=4.6e^{-3}$) towards the end of session 1 and less cumulative out-of-sequence presses ($p=1.7e^{-3}$). By session 9, there was no significant difference between male WT mice and EAAC1$^{-/-}$ mice in lever presses and rewards received. Male EAAC1$^{-/-}$ mice again had less out-of-sequence presses ($p=4.6e^{-6}$) and less cumulative out-of-sequence presses ($p=6.1e^{-5}$) (**Fig. 8C**). We observed a greater number of rewards in EAAC1$^{-/-}$ mice across all sessions, and similar trends between WT and EAAC1$^{-/-}$ mice in number of lever presses. Male WT mice also had significantly more out of sequence presses ($p=0.027$) (**Fig. 8D**).

In female WT and EAAC1$^{-/-}$ mice, we found that EAAC1$^{-/-}$ mice perform much better in this task than their wildtype counterparts. 17% of female EAAC1$^{-/-}$ mice (n=12) performed in this task compared to 0% of WT mice (n=18) performers. We also found that the female WT mice had a higher percentage of improvers in the task (11% of WT improvers compared to 4% of EAAC1$^{-/-}$ improvers) (**Fig. 9B**). In session 1, we found that female EAAC1$^{-/-}$ mice had significantly greater total ($p=4.6e^{-6}$) and cumulative lever presses ($p=1.8e^{-5}$). Female EAAC1$^{-/-}$ mice had significantly more total ($p=2.0e^{-9}$) and cumulative rewards ($p=8.4e^{-12}$), but also more total ($p=1.7e^{-3}$) and cumulative ($p=1.7e^{-3}$) out-of-sequence presses. By session 9, female WT mice had improved at the task but still had significantly less presses ($p=1.5e^{-13}$) than the female EAAC1$^{-/-}$ mice and less rewards received ($p=1.8e^{-14}$). Female EAAC1$^{-/-}$ mice this time had less out-of-sequence presses ($p=2.5e^{-2}$) (**Fig. 9C**). We observed a greater number of rewards in EAAC1$^{-/-}$ mice across all sessions, and similar trends between WT and EAAC1$^{-/-}$ mice in number of lever presses. (**Fig. 9D**).

Together, these findings indicate that although WT mice can learn the task and improve their temporal processing of reward, EAAC1$^{-/-}$ mice have greater abilities for temporal processing in reward-based behavior.

Increase in neuronal activity in the primary somatosensory cortex in EAAC1$^{-/-}$ mice

From our immunohistochemistry staining procedure, we found that WT and EAAC1$^{-/-}$ mice have differential c-Fos expression (**Fig. 10A**). One EAAC1$^{-/-}$ and one WT mouse was sacrified 1hr after they had completed nine sessions of the timed sequence task. We analyzed specifically mice who performed/improved at the task. We calculated c-Fos cell density (cells/mm^3) in

coronal slices and found that c-Fos expression differed greatly in the EAAC1$^{-/-}$ performer mouse compared to the WT improver mouse (**Fig. 10B**). Specifically, we found that EAAC1$^{-/-}$ mice show the greatest c-Fos expression in cortical layer IV of the direct tectospinal pathway (>60,000 cells/mm^3), the primary somatosensory cortex (>40,000 cells/mm^3), and the barrel cortex (>40,000 cells/mm^3) compared to WT mice (**Fig. 10E**). The direct tectospinal pathway regulates head movement in response to auditory and visual stimuli (Rea, 2015). The primary somatosensory cortex plays a major role in processing afferent somatosensory input (primarily from the thalamus) and the sensory and motor signals involved in the execution of skilled movement (Borich, 2015). The barrel cortex is another region of the somatosensory cortex that is heavily involved in sensory processing. EAAC1$^{-/-}$ mice showed the greatest increase in c-Fos expression compared to WT mice in the primary somatosensory cortex, layer IV (**Fig. 10F**). These findings suggest that the behavioral differences between WT and EAAC1$^{-/-}$ mice may be due to differential neuronal activity in the primary somatosensory cortex. Because this region as well as the direct tectospinal pathway and barrel cortex are associated with sensory perception, we hypothesize that differences in sensory perception may play a role in modulating reward-based behaviors.

Discussion

EAAC1 only composes a very small amount of all glutamate transporters, as well as varies in function across different areas of the brain. Thus, it is difficult to pinpoint an overall functional role of EAAC1 in the brain. In the striatum, which is the primary input nucleus of the basal ganglia, EAAC1 is expressed abundantly and functions in excitation and increased reward-based behavior (Bellini et al., 2018). EAAC1 is also abundantly expressed in the cortex, thalamus, and hippocampus (Holmseth, 2012). In the hippocampus, however, behavioral effects are diminished (Bennink, 2020). For example, it has been found that EAAC1$^{-/-}$ mice have significant learning and memory impairments (Lee, 2012) showing worse contextual and tone-related learning and memory as well as less expression of activity-regulated cytoskeleton-associated protein (Arc) in the CA1 regions and the entorhinal cortices (Wang, 2014). As mentioned before, EAAC1$^{-/-}$ mice have also shown anxiety-like behavior and repetitive habitual behaviors (Bellini, 2018) and have shown an impulsive behavioral phenotype (Bennink, 2020).

So far, we have not found any significant differences in the hyperactivity or social interaction aspects of OCD between WT and EAAC1$^{-/-}$ mice. Although our findings here do not support the findings of previous work in which the loss of EAAC1 contributes to patterns of hyperactivity, the lack of effect seen here may have different causes. While WT mice may not express hyperactivity during these trials, similar observed behaviors may be due to anxiety-like behaviors previously found in EAAC1$^{-/-}$ mice, in which they are more apprehensive to new stimuli. In future experiments, it may be useful to study this further through different measures of hyperactivity. In order to further investigate sociability, a measure such as the Automated Three-Chamber Social Approach Task can be used in which the subject is given the option of interacting with either another mouse or a novel object, and sociability is measured depending on whether the subject spends more time with the other mouse or the novel object (Yang, 2011). We could also further study hyperactivity using lengthier and larger open-field tests to obtain a more comprehensive analysis of locomotor activity and other behavioral indicators such as defecation (Seibenhener, 2015).

The timed sequence experiment we conducted can provide us with some insight into the temporal processing of reward-based behavior and how those abilities can substantially increase with the loss of EAAC1. As of right now, we do not have enough data nor a large enough sample size to generalize this result, but we plan to study this aspect of reward-based behavior further to determine the role of EAAC1 in temporal processing of reward.

Currently, treatments for OCD only consist of selective serotonin reuptake inhibitors (SSRIs) and cognitive behavioral therapy (CBT), however, these treatments are largely ineffective as symptoms of OCD tend to reoccur and persist. (Dougherty et al., 2004; Zike et al., 2017). However, the development of novel, more effective pharmaceutical treatments has been directed

by the use of dopaminergic and glutamatergic transmission (Sujith & Lane, 2009). Being able to gain a more thorough understanding of EAAC1 and its functional role can provide greater insight into the development of new treatments for human OCD patients, as well as the treatment of patients with other neuropsychiatric disorders with similar characteristics.

Limitations

It is important to note that this study is not a full model of OCD. Because we are using an animal model to study these behavioral effects, we can only draw insight from the behavior patterns of these mice rather than make a generalized statement about OCD patients. There are several other aspects of OCD that we have been unable to account for through our study with mice, such as the presence of recurring uncontrollable thoughts. We also only operationalized "anxiety-like behaviors" through locomotor activity and grooming behaviors. This study did not account for other measures such as defensive behaviors or responses to stressors.

Future directions

Our future directions with these experiments would be to use *in vivo* optogenetics to further observe the differences in neuronal activity in the primary sensory cortex in real time. Our hypothesis is that these differences may be responsible for hyperactivity previously seen in the striatum. We will analyze *in vivo* recordings in the primary somatosensory cortex that will take place during the timed sequence task so that we can interpret the function of EAAC1 in reward-based behaviors at a cellular level.

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Figures and legends

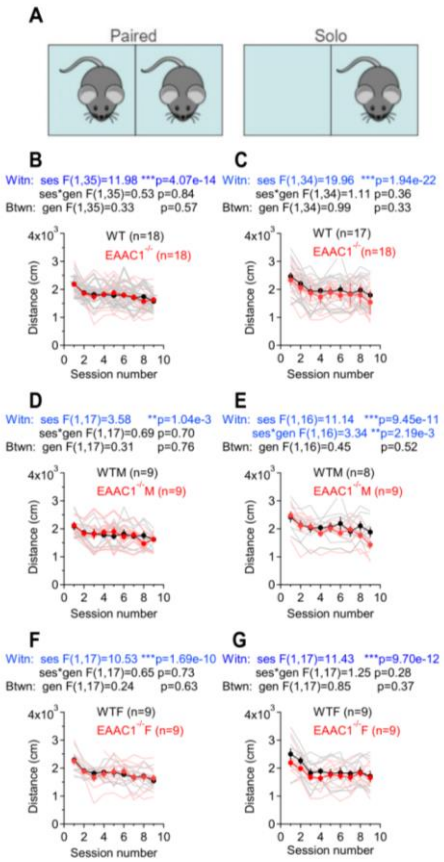

Figure 1. EAAC1$^{-/-}$ and WT mice travel similar distances when separated by opaque divider. In the control behavior sessions measuring hyperactivity, **(A)** WT and EAAC1$^{-/-}$ mice both underwent nine 10-minute paired sessions and nine 10-minute solo sessions in which they explored an empty chamber with an opaque divider and no factors of enrichment present. **(B)** Distance traveled (cm) by WT (n=18) and EAAC1$^{-/-}$ mice (n=18) across nine paired sessions. **(C)** Distance traveled (cm) by WT (n=17) and EAAC1$^{-/-}$ mice (n=18) across nine solo sessions. **(D)** Distance traveled (cm) by male WT (n=9) and EAAC1$^{-/-}$ mice (n=9) across nine paired sessions. **(E)** Distance traveled (cm) by male WT (n=8) and EAAC1$^{-/-}$ mice (n=9) across nine solo sessions. **(F)** Distance traveled (cm) by female WT (n=9) and EAAC1$^{-/-}$ mice (n=9) across nine paired sessions. **(G)** Distance traveled (cm) by female WT (n=9) and EAAC1$^{-/-}$ mice (n=9) across nine solo sessions.

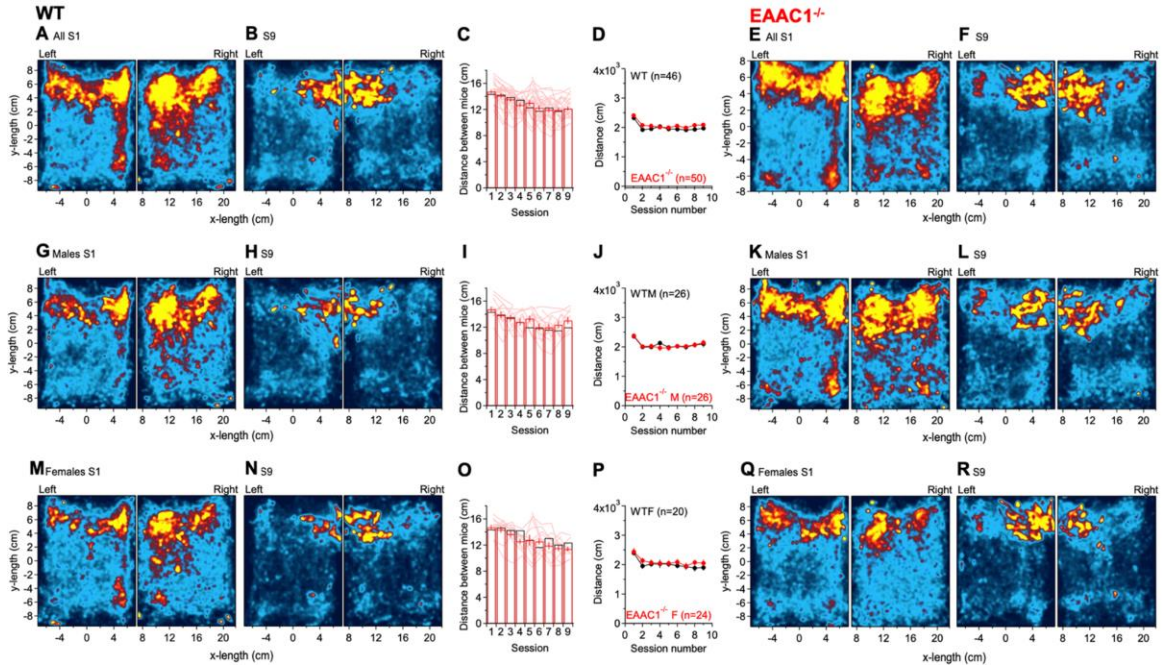

Figure 2. EAAC1$^{-/-}$ and WT mice interact similarly during single-lever press task. In the single-lever press task, **(A)** Map showing where WT mice spent most of their time during S1. **(B)** Map showing where WT mice spent most of their time during S9. **(C)** Inter-mouse distances (IMDs) of EAAC1$^{-/-}$ and WT mice across nine sessions. **(D)** Total distance traveled across nine sessions by EAAC1$^{-/-}$ and WT mice (WT n=46; EAAC1$^{-/-}$ n=50). **(E)** Map showing where EAAC1$^{-/-}$ mice spent most of their time during S1. **(F)** Map showing where EAAC1$^{-/-}$ mice spent most of their time during S9. **(G)** Map showing where male WT mice spent most of their time during S1. **(H)** Map showing where male WT mice spent most of their time during S9. **(I)** Inter-mouse distances (IMDs) of EAAC1$^{-/-}$ and WT male mice across nine sessions. **(J)** Total distance traveled across nine sessions by EAAC1$^{-/-}$ and WT male mice (WTM n=26; EAAC1$^{-/-}$M n=26). **(K)** Map showing where male EAAC1$^{-/-}$ mice spent most of their time during S1. **(L)** Map showing where male EAAC1$^{-/-}$ mice spent most of their time during S9. **(M)** Map showing where female WT mice spent most of their time during S1. **(N)** Map showing where female WT mice spent most of their time during S9. **(O)** Inter-mouse distances (IMDs) of EAAC1$^{-/-}$ and WT female mice across nine sessions. **(P)** Total distance traveled across nine sessions by EAAC1$^{-/-}$ and WT female mice (WTF n=20; EAAC1$^{-/-}$F n=24). **(Q)** Map showing where female EAAC1$^{-/-}$ mice spent most of their time during S1. **(R)** Map showing where female EAAC1$^{-/-}$ mice spent most of their time during S9.

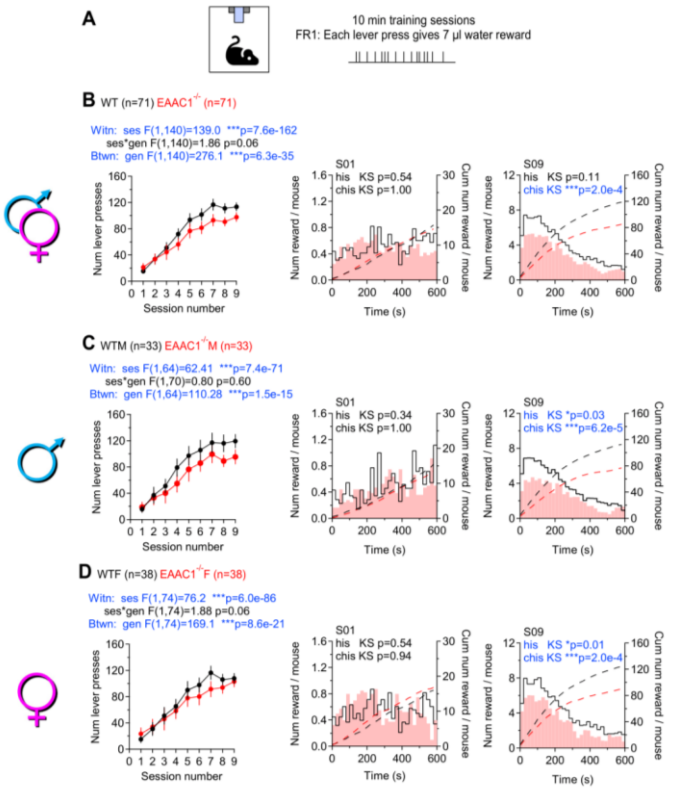

Figure 3. EAAC1$^{-/-}$ and WT mice initially receive similar numbers of rewards in single-lever press task with some differences in last session. In the single lever press behavior sessions, **(A)** A 7µl water reward is given each time the mouse presses a lever during each 10-minute training session. **(B)** Number of rewards received for all mice (WT n=71; EAAC1$^{-/-}$M n=71) across nine sessions; total number and cumulative number of rewards received in S1; total number and cumulative number of rewards received in S9. **(C)** Number of rewards received for male mice (WTM n=33; EAAC1$^{-/-}$M n=33) across nine sessions; total number and cumulative number of rewards received by male mice in S1; total number and cumulative number of rewards received by male mice in S9. **(D)** Number of rewards received for female mice (WTF n=17; EAAC1$^{-/-}$F n=16) across nine sessions; total number and cumulative number of rewards received by female mice in S1; total number and cumulative number of rewards received by female mice in S9.

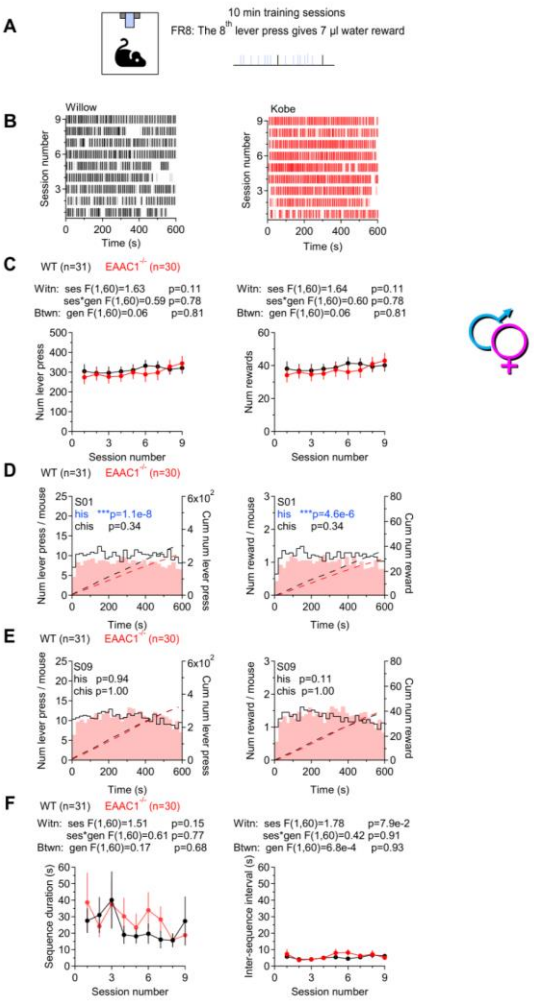

Figure 4. EAAC1$^{-/-}$ and WT mice press lever similarly and receive similar rewards in sequence-lever press task. In the sequence lever press behavior sessions, **(A)** A 7µl water reward is given when the mouse presses a lever eight times during each 10-minute training session. **(B)** Times in which a sample WT and a sample EAAC1$^{-/-}$ mouse pressed the lever in each session. **(C)** Number of lever presses for all mice (WT n=31; EAAC1$^{-/-}$ n=30) and number of rewards received by all mice across nine sessions. **(D)** Total number and cumulative number of lever presses done by mice in S1; total and cumulative number of rewards received by mice in S1 **(E)** Total number and cumulative number of lever presses done by mice in S9; total and cumulative number of rewards received by mice in S9. **(F)** Sequence durations of all mice across nine sessions; inter-sequence intervals of all mice across nine sessions.

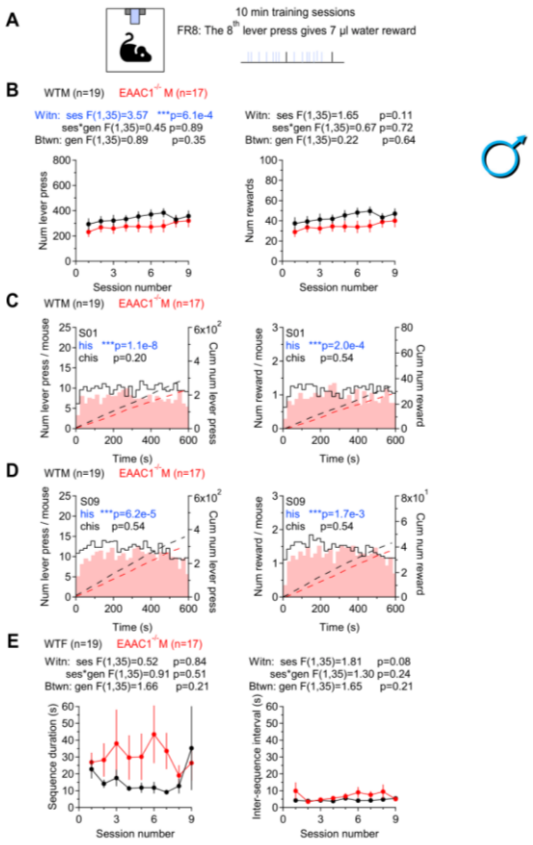

Figure 5. Male EAAC1$^{-/-}$ and WT mice press lever similarly and receive similar rewards in sequence-lever press task. In the single lever press behavior sessions, **(A)** A 7µl water reward is given when the mouse presses a lever eight times during each 10-minute training session. **(B)** Number of lever presses for male mice (WTM n=19; EAAC1$^{-/-}$M n=17) and number of rewards received by male mice across nine sessions. **(C)** Total number and cumulative number of lever presses done by male mice in S1; total and cumulative number of rewards received by male mice in S1 **(D)** Total number and cumulative number of lever presses done by male mice in S9; total and cumulative number of rewards received by male mice in S9. **(E)** Sequence durations of male mice across nine sessions; inter-sequence intervals of male mice across nine sessions.

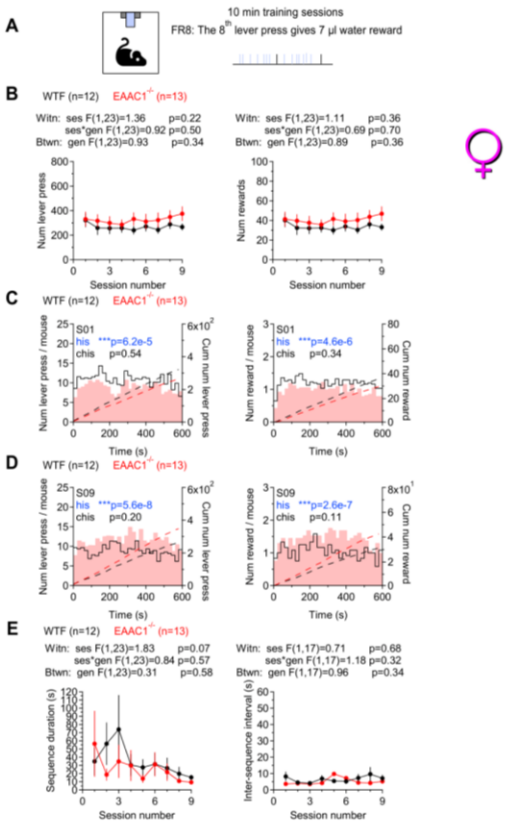

Figure 6. Female EAAC1$^{-/-}$ and WT mice press lever similarly and receive similar rewards in sequence-lever press task. In the sequence lever press behavior sessions, **(A)** A 7µl water reward is given when the mouse presses a lever eight times during each 10-minute training session. **(B)** Number of lever presses for female mice (WTF n=12; EAAC1$^{-/-}$F n=13) and number of rewards received by female mice across nine sessions. **(C)** Total number and cumulative number of lever presses done by female mice in S1; total and cumulative number of rewards received by female mice in S1 **(D)** Total number and cumulative number of lever presses done by female mice in S9; total and cumulative number of rewards received by female mice in S9. **(E)** Sequence durations of female mice across nine sessions; inter-sequence intervals of female mice across nine sessions.

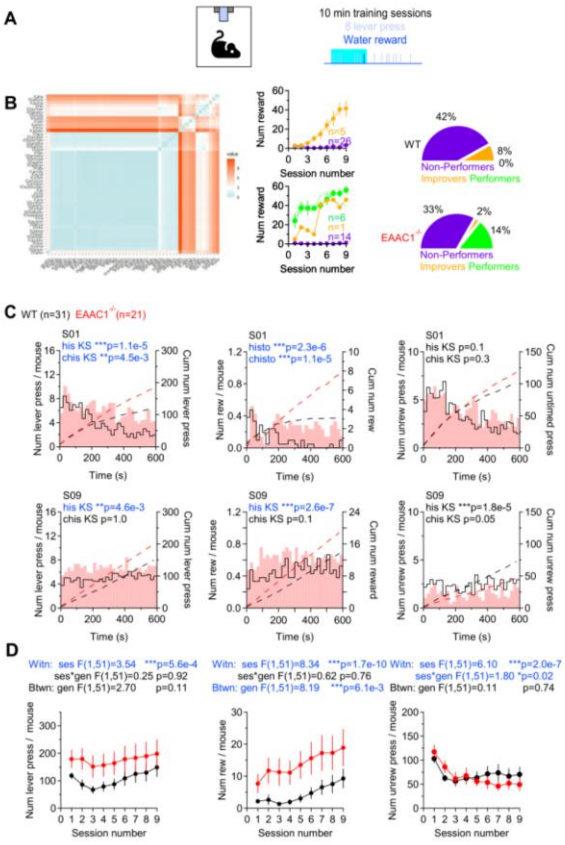

Figure 7. EAAC1$^{-/-}$ mice press lever quicker and receive more rewards in timed lever press task than WT mice. In the lever press behavior sessions, **(A)** A 7µl water reward is given every time the mouse presses the lever eight times within six seconds during each 10-minute training session. **(B)** Correlation between numbers of rewards for each mouse (WT n=31; EAAC1$^{-/-}$ n=21); number of rewards across nine sessions for non-performers, improvers, and performers; percentage of WT and EAAC1$^{-/-}$ non-performers, improvers, and performers. **(C)** Total numbers and cumulative numbers of lever presses, rewards, and unrewarded presses in S1 and S9. **(D)** Total numbers of lever presses, rewards, and unrewarded presses across nine sessions.

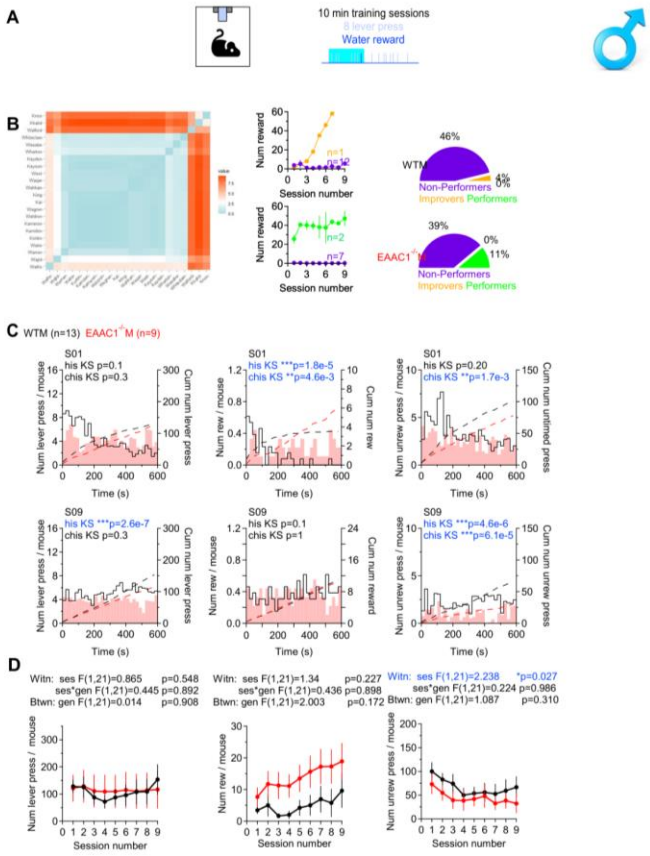

Figure 8. Male EAAC1$^{-/-}$ mice receive more rewards than WT mice. In the lever press behavior sessions, **(A)** A 7µl water reward is given every time the mouse presses the lever eight times within six seconds during each 10-minute training session. **(B)** Correlation between numbers of rewards for each male mouse (WTM n=13; EAAC1$^{-/-}$M n=9); number of rewards across nine sessions for non-performers, improvers, and performers; percentage of WT and EAAC1$^{-/-}$ male non-performers, improvers, and performers. **(C)** Total numbers and cumulative numbers of lever presses, rewards, and unrewarded presses in S1 and S9. **(D)** Total numbers of lever presses, rewards, and unrewarded presses across nine sessions.

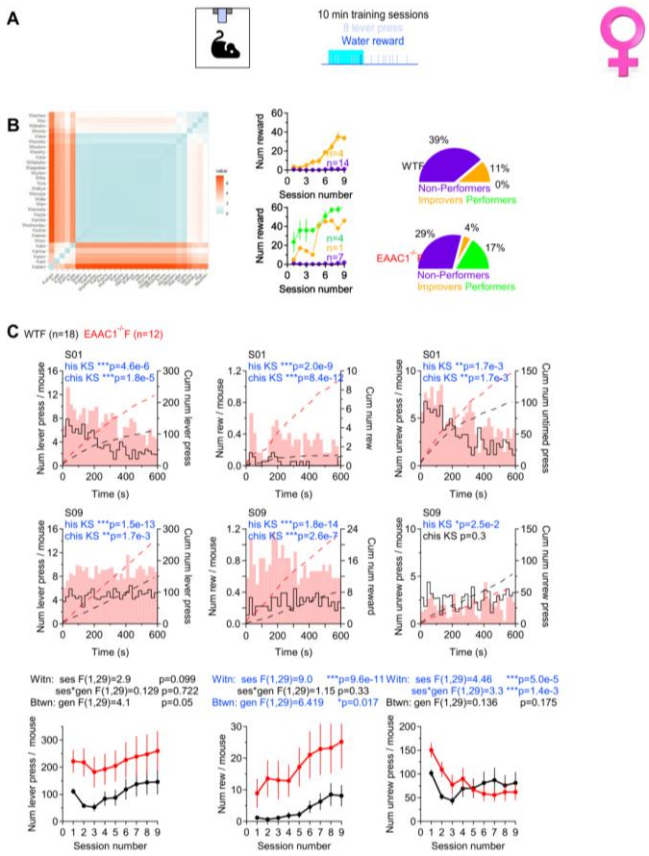

Figure 9. Female EAAC1$^{-/-}$ mice press lever quicker and receive more rewards than WT mice. In the lever press behavior sessions, **(A)** A 7µl water reward is given every time the mouse presses the lever eight times within six seconds during each 10-minute training session. **(B)** Correlation between numbers of rewards for each female mouse (WTF n=18; EAAC1$^{-/-}$F n=12); number of rewards across nine sessions for non-performers, improvers, and performers; percentage of WT and EAAC1$^{-/-}$ female non-performers, improvers, and performers. **(C)** Total numbers and cumulative numbers of lever presses, rewards, and unrewarded presses in S1 and S9. **(D)** Total numbers of lever presses, rewards, and unrewarded presses across nine sessions.

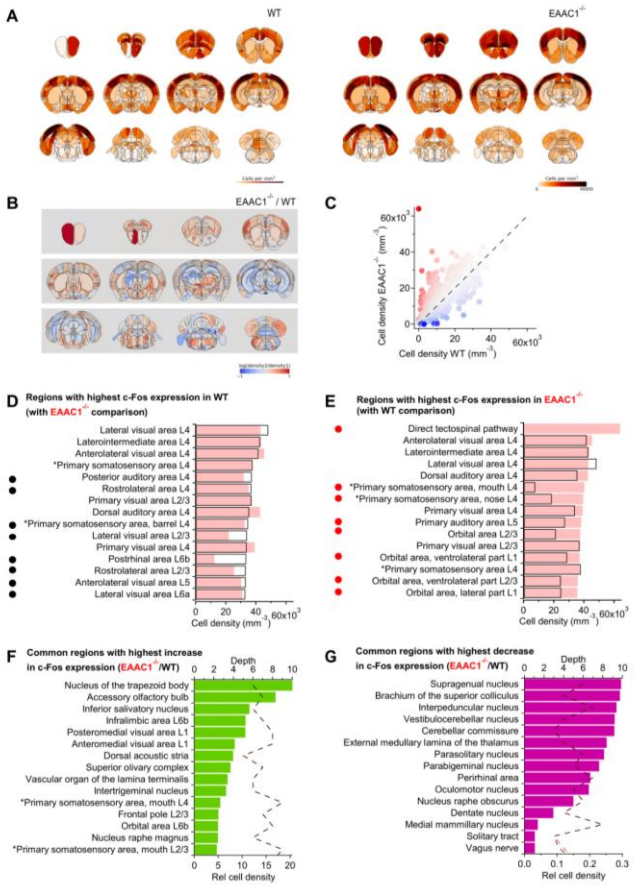

Figure 10. EAAC1$^{-/-}$ and WT mice show differential c-Fos expression. In the immunohistochemistry staining procedure, **(A)** c-Fos expression differences in cells of WT and EAAC1$^{-/-}$ mice indicated in coronal view. **(B)** Cell density in WT vs. EAAC1$^{-/-}$ brain regions. **(C)** Relationship of cell density in WT vs. EAAC1$^{-/-}$. **(D)** WT brain regions showing highest c-Fos expression compared to EAAC1$^{-/-}$ mice. **(E)** EAAC1$^{-/-}$ brain regions showing highest c-Fos expression compared to WT mice. **(F)** Regions showing increase in c-Fos expression in EAAC1$^{-/-}$ compared to WT mice (relative cell density). **(G)** Regions showing decrease in c-Fos expression in EAAC1$^{-/-}$ compared to WT mice (relative cell density).