Are you good at "Spot The Difference" games?
Understanding the neural substrates controlling behavioral pattern separation

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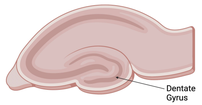
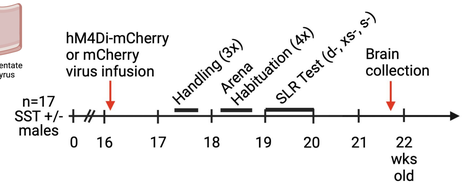
QUESTION

- **Does increasing memory load (more similar object locations) make novel object location discrimination harder?**
- **Does inhibition of an interneuron in the dentate gyrus (DG) decrease behavioral pattern separation?**

APPROACH



- **mCherry = control group**
- **hM4Di = inhibitory group**



RESULTS

- **Control group similarly discriminates novel object location regardless of various memory loads**
- **Inhibited DG somatostatin cells allow discrimination of dissimilar object location, but impair discrimination of similar object location**

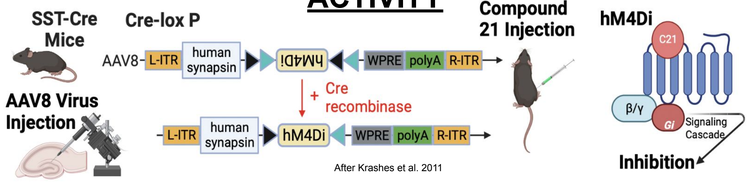
IMPLICATIONS

- **DG interneurons could play a role in pattern separation abilities**
- **DG interneurons can be targeted for individuals with impaired pattern separation due to aging or psychological disorders**

ABSTRACT

Behavioral pattern separation is the ability to distinguish between two very similar situations or stimuli. Dependent upon hippocampal function (especially the dentate gyrus, DG), this ability worsens with age and certain psychological/neurological disorders. It is critical to understand the neural substrates required for behavioral pattern separation in order to treat these individuals. Previous rodent studies propose that behavioral pattern separation requires sparse neuronal activity of granule cells. In short, interneuron somatostatin (SST) cells target DG granule cells directly or indirectly via other interneurons. Therefore, when SST cells are inhibited, the resulting disinhibition leads to overall excitation of granule cells and context discrimination is impaired. Still, a lot remains unknown. Study of interneuron SST cells more specifically confirms their role in context discrimination. However, as of yet, this research does not assess behavioral pattern separation with various memory loads. Here, we hypothesize that SST cells will impair behavioral pattern separation when inhibited. In our investigation, we used chemogenetics to inhibit SST cells in the DG. 4-month-old mature SST-Cre male mice (n=17) underwent bilateral viral infusion with Gi-coupled Cre-dependent human muscarinic receptor (hM4Di-mCherry, inhibitory) or control virus (mCherry). Then, 3 weeks post-infusion, we tested behavioral pattern separation with a spontaneous location recognition (SLR) task, consisting of a 10 minute sampling phase and a 5 minute testing phase. On test day, Compound 21 (C21, i.p.) was administered 30 minutes prior to the sampling phase. In our control group, we found similar performance in discrimination learning with various memory loads. More interestingly, while we did not find differences in discrimination of dissimilar and extremely similar object location between control and inhibition groups, DG inhibition did impair discrimination with similar object location. In the future, interneuron SST cells could be targeted to help individuals whose behavioral pattern separation abilities have been damaged by aging or psychological/neurological disorders.

CHEMOGENETICS: MANIPULATION OF INTERNEURON ACTIVITY



- **AAV8-hSyn-DIO-hM4Di-mCherry (n=10) and AAV8-hSyn-DIO-mCherry (n=7)**
- **hM4Di = DREADD (Designer Receptors Exclusively Activated by Designer Drugs)**
- **Designer drug (Compound 21) injection 30 minutes prior to sampling phase**

Similar weight change in inhibitory and control groups

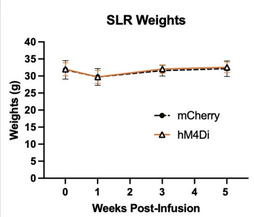


Figure 1. Weight change over the course of experimentation. Weights were recorded on the day of surgery/virus injection, the second day of handling, the first day of SLR testing, and the day of decapitation and brain collection. Type of virus treatment did not significantly impact weight. Two-way RM ANOVA, with virus having no significance p>0.05, and time having significance p<0.0001.

Similarly decreased total movement over the course of habituation in inhibition and control groups

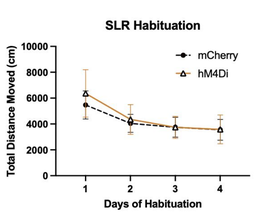
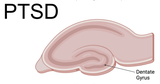


Figure 2. Total distance moved over the course of habituation to SLR arenas. The large amount of movement on Day 1 of habituation confirms the animals were not overly anxious. The trend for both mCherry and hM4Di animals shows a gradual decrease in total distance moved over the course of habituation. This suggests the animals, regardless of group, adjusted well to and became comfortable with in the arenas. Two-way RM ANOVA, with virus having no significance p>0.05, and time having significance p<0.0001. Post-hoc Tukey shows significant difference between distance moved on day 1 and day 4 for both mCherry, approximately p=0.001, and hM4Di, p=0.0003.

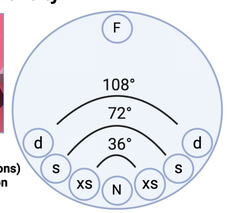
SPOT THE DIFFERENCE: BEHAVIORAL PATTERN SEPARATION (BPS)

- A cognitive function to differentiate between very similar experiences/memories
- Impaired in individuals with age, depression, AD, or PTSD (Reichelt et al. 2021)
- DG has a crucial role (Morales et al. 2021)
- Underlying mechanism involves DG interneurons inhibiting DG granule cells (Morales et al. 2021)



ASSESSING BPS IN MICE: SPONTANEOUS LOCATION RECOGNITION (SLR) TEST

Test relies on a mouse's innate interest in novelty.



Separation between objects decreases (d-, s-, xs-conditions) to increase pattern separation load.

- **F = familiar object**
- **N = novel object**

After Reichelt et al. 2021

BENEFITS

- **minimal training**
- **easier to distinguish between different memory processes**

MEMORY LOADS

- **low = dissimilar object location (d-SLR)**
- **medium = similar object location (s-SLR)**
- **high = extra similar object location (xs-SLR)**

Inhibited DG somatostatin cells allow discrimination of dissimilar object location, but impair discrimination of similar object location

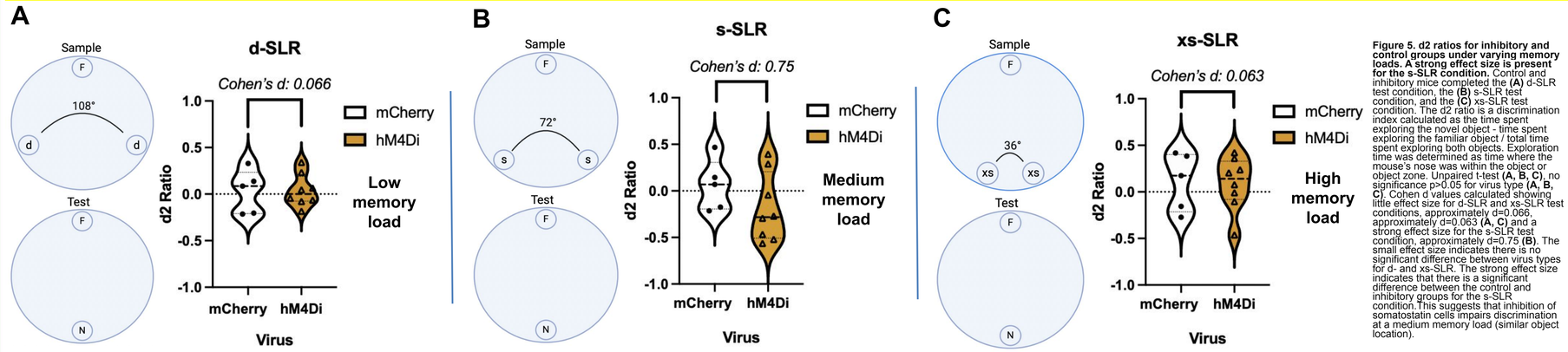


Figure 5. d2 ratios for inhibitory and control groups under varying memory loads. A strong effect size is present for the s-SLR condition. Control and inhibitory mice completed the **(A)** d-SLR test condition, the **(B)** s-SLR test condition, and the **(C)** xs-SLR test condition. The d2 ratio is a discrimination index calculated as the time spent exploring the novel object - time spent exploring the familiar object / total time spent exploring both objects. Exploration time was determined as time where the mouse's nose was within the object or object zone. Unpaired t-test **(A, B, C)**, no significance p>0.05 for virus type **(A, B, C)**. Cohen d values calculated showing little effect size for d-SLR and xs-SLR test conditions, approximately d=0.066, approximately d=0.063 **(A, C)** and a strong effect size for the s-SLR test condition, approximately d=0.75 **(B)**. The small effect size indicates there is no significant difference between virus types for d- and xs-SLR. The strong effect size indicates that there is a significant difference between the control and inhibitory groups for the s-SLR condition.This suggests that inhibition of somatostatin cells impairs discrimination at a medium memory load (similar object location).

Excluding data points and suitability of various memory loads

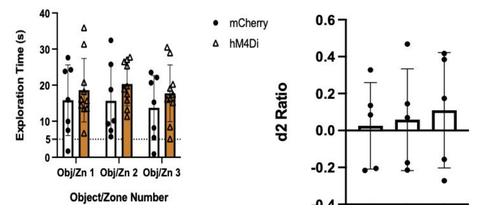


Figure 3. Exploration of object and object zones during the sample phase of xs-SLR condition. Animals failing to meet 5 s exploration time are excluded from Test data analysis. In this case, one animal failed to meet criteria twice, once for object and object zone 1, and again for object and object zone 3.

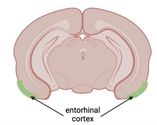
Figure 4. d2 ratios for the control group under varying memory loads. d2 ratios represent the capability to accurately discriminate between familiar and novel locations. It is calculated as the time spent exploring the novel object - time spent exploring the familiar object / total time spent exploring both objects. Exploration time was determined as time where the mouse's nose was within the object or object zone. Here, the d2 ratios reveal whether the various conditions are suitable for testing behavioral pattern separation. A positive d2 ratio signifies that more time was spent exploring the novel object; a negative d2 ratio means that more time was spent exploring the familiar object. One-way ANOVA, showing no significance p>0.05. Post-hoc Tukey's multiple comparisons test also shows no significance. This indicates that the control mice have similar levels of novel object location discrimination across all test conditions.

ONGOING/FUTURE DIRECTIONS

- Assess if virus appropriately targeted the DG. If not, remove those mice from their respective groups and reevaluate the data.
- Test with increased subject number (N)

Additionally, since the DG does not function in isolation, it is important to examine the broader neuronal circuit affecting behavioral pattern separation. The entorhinal cortex is of particular interest.

- Does stimulation/inhibition of the entorhinal cortex that provides direct input to the DG affect behavioral pattern separation?



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