

# Manipulating a brain circuit associated with sociability by a two-recombinase system

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## Aim

### Forceps minor

involved in social behavior?

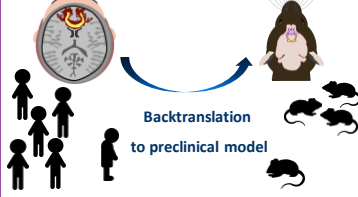


Figure 1: PRISM2-project: biological relationship between Forceps minor and social dysfunction; demonstration in animal model

## Introduction

Across various neuropsychiatric diseases, social withdrawal is often diagnosed as an early symptom and can therefore be considered as a transdiagnostic marker. Clinical data suggest that people exhibiting low social functioning show reduced white matter integrity, specifically in the Forceps minor (FM) a fiber tract connecting both orbital frontal cortices (OFC). As part of the PRISM2 (Psychiatric Ratings using Intermediate Stratified Markers) consortium, we developed the Two-Recombinase-System based on DREADDs. The combination between Cre/dlox and Flp/FRT system should allow us to bilaterally manipulate the interhemispheric projection neurons and back-translate those human findings into preclinical research.

## Methods

### Two-Recombinase-System:

- Combination between Cre/dlox and Flpo/FRT system
- Recombinases will retrogradely travel towards the contralateral side *via* Forceps minor and Corpus callosum

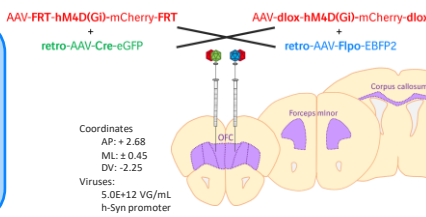


Figure 2: Schematic representation of Two-Recombinase-System injection side and travel theory

### RFID-assisted SocialScan

- Synchronization between RFID-data and video tracking
- 24 male mice (12x CNO, 12 controls)
- Acute: 5mg/kg CNO intraperitoneally

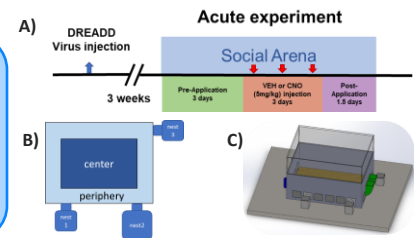


Figure 3: (A) Experimental timeline; (B-C) RFID-assisted 2D/3D version of Social Arena; 3 nests, 2 food hoppers, 2 water bottles

## Results

### In vivo validation: Two-Recombinase-System

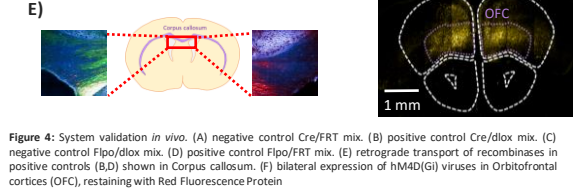
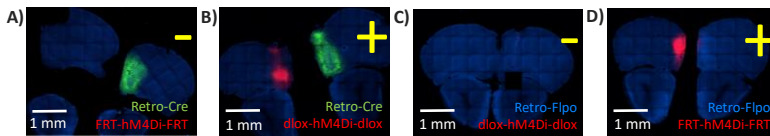


Figure 4: System validation *in vivo*. (A) negative control Cre/FRT mix. (B) negative control Cre/dlox mix. (C) negative control Flpo/FRT mix. (D) positive control Flpo/dlox mix. (E) retrograde transport of recombinases in positive controls (B,D) shown in Corpus callosum. (F) bilateral expression of hM4D(Gi) viruses in Orbitofrontal cortices (OFC), retaining with Red Fluorescence Protein

### Acute experiment

#### Periphery

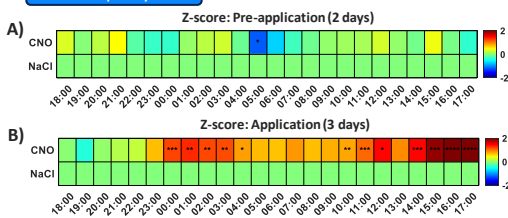


Figure 5: Z-score analysis of time spent in periphery. (A) Pre-application phase. (B) Application phase. Mean values (days, animals), Two-way ANOVA, Bonferroni posthoc

#### Distance > 10 cm

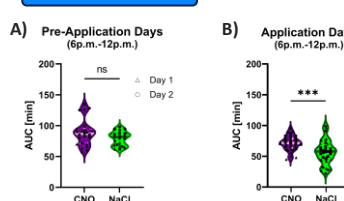


Figure 6: Interindividual distances >10 cm as mean Area under the curve (AUC) values ( $\pm$  SEM) for six-hour phase. (A) Pre-Application days, unpaired t-test;  $p = 0.0727$ . (B) Application days, unpaired t-test;  $p = 0.0004$

#### Locomotion

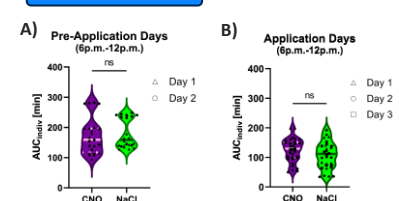


Figure 7: Locomotion of each animal as mean area under the curve (AUC) values ( $\pm$  SEM) for six-hour phase. (A) Pre-Application days, unpaired t-test;  $p = 0.6033$ . (B) Application days, unpaired t-test;  $p = 0.1166$

### Social nesting

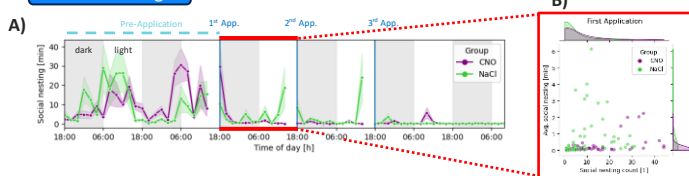


Figure 8: Social nesting behavior. (A) Social nesting time in Pre-Application phase (2 days) and Application phase (3 days) as mean values ( $\pm$  SEM); (B) scatter plot of first Application phase: Social nesting counts against average social nesting time. (C) pie chart shows percentage of total social nesting time across different phases of the experiment, values > 2.5% are not displayed

### Behavioral outcomes in CNO treated animals:

- prefer to stay in periphery
- increase their interindividual distances
- No difference in locomotor activity
- Reduce their social nesting time, whereas the social nesting count was increased

## Conclusion

The Two-Recombinase-System allows us to bilaterally inhibit the projection neurons running *via* Forceps minor in the animal model. After CNO injection those mice show **anxious-like** behavior by staying close to the walls compared to control groups. Additionally, they **increase** their **interindividual distance** which can be a sign of **social avoidance** behavior, indicated also by the fact that these mice reduce their social nesting times. Meanwhile, locomotion was unaffected during these conditions. By using our new technique, we were able to **back-translate** clinical PRISM-data into animal model. At the same time, we indicated an **involvement of Forceps minor**, part of the **Default Mode Network**, in social behavior.