RESEARCH PAPER

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The role of prefrontal somatostatin interneurons in emotional contagion

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The role of prefrontal somatostatin interneurons in emotion recognition is well characterized. Here, for the first time, we investigated the role of these neurons during remote transfer of emotional information in the safe environment of the home cage. To do that mice with fluorescently labelled somatostatin interneurons were housed in pairs for three weeks, one labelled an Observer, and the other a Demonstrator. In the test session, the Demonstrator was subjected to aversive stimuli outside of the home cage, while the Observer remained there undisturbed. Upon the return of the Demonstrator to the home cage, we recorded the interactions of the two animals. The behavior of both partners, assessed and classified with machine learning algorithms, was clearly affected by the emotional state of the Demonstrator. To assess the role of prefrontal somatostatin interneurons in this process we chemogenetically manipulated their activity in the Observers and found that activation of these cells abolishes the enhanced social investigation of a stressed Demonstrator. This is associated with disinhibition of the prefrontal cortex. The manipulation also affects the neuronal activation patterns in Demonstrators, which seems to reflect the change in the behavior of the Observers.

Key words: somatostatin, prefrontal cortex, emotional contagion, empathy

INTRODUCTION

Emotional contagion is an ability to perceive and react to an altered emotional state of another individual. A major class of inhibitory prefrontal interneurons, namely somatostatin (SST) positive cells, were shown to modulate emotion recognition (Scheggia et al., 2020; Dautan et al., 2024) as well as formation of social fear (Xu et al., 2019; Wang et al., 2020). Their sensitivity to $GABA_B$ agonist was decreased during direct witnessing of the distress of a conspecific (Observational Fear Learning, OFL, Liu et al., 2017), while optoinhibition of ventromedial prefrontal (vmPFC) pyramidal cells during OFL decreased the activity of a cluster of vmPFC cells active during escape of the Observers and affected the escape response itself (but not the vicarious freezing, Huang et al., 2023). Selective removal of aryl hydrocarbon receptor nuclear translocator 2 (ARNT2) specifically from somatostatin (SST)-expressing interneurons in the anterior cingulate cortex on the other hand reduced vicarious freezing during OFL and emotion recognition in the three chamber apparatus. ARNT2 was identified through RNA sequencing and loss of function studies as a candidate gene critical for variability of behavioral readout in the observational fear paradigm. Its silencing in SST neurons resulted in higher spontaneous firing rates of these neurons and lower Ca^{2+} influx during vicarious freezing (Choi et al., 2024).

Most of these behavioral paradigms permit only limited social contact. The Remote Fear Transfer paradigm (Knapska et al., 2006) allows for studying emotional contagion during free interaction in the context of safe environment of the home cage. Unlike OFL (for review see Kitamura et al., 2024) it does not require Observers to have previous experience with the aversive stimulus, even though such experience generally promotes pro-

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social behavior towards conspecifics in distress (and is dependent on prefrontal CRH neuron activity, Maltese et al., 2025). Our previous study showed that normosocial C57BL/6J mice engage socially with a distressed familiar cagemate, which is then reflected in the neuronal activation pattern in their prefrontal cortex and several nuclei of the amygdala, while no such effect was observed in an idiopathic mouse model of autism, the BTBR T^{+}Itpr3^{tf}/J mice (these mice withdrew from social interaction and the subsequent neuronal activation was lower in the medial nucleus of the amygdala, Meyza et al., 2015). Such prosocial behaviors of the Observers are likely to affect the emotionally aroused Demonstrator, resulting with social buffering of stress responses. The neural circuitry behind that is functionally the same as the one activated during first encounter with a conspecific (and is located in the infralimbic cortex, Gutzeit et al., 2020) and requires nicotinic receptor activation (β2-nAChRs, Nosjean et al., 2018).

Little is known about the role of prefrontal SST interneurons in prosocial responding of naïve animals to the emotional arousal of a conspecific. Here we aimed at characterizing the activation patterns of these cells upon such exposure and at describing the effects of chemogenetic manipulation of the activity of Observers' prefrontal SST interneurons on the behavior and neuronal activation patterns in the prefrontal cortex and the amygdalar complex of both Observers and Demonstrators in the Remote Transfer of Fear paradigm.

METHODS

Subjects

The study was conducted using n=28 SST Ai14_tdTomato (heterozygous offspring of SST-Cre Tg/Tg, Jackson Laboratory # 013044 and Ai14 Jackson Laboratory #007908 lines), n=18 SST<tm2.1 (cre)Zjh>/J (SST-Cre Tg/0, Jackson Laboratory # 013044) and n=18 C57BL/6J male mice (3 months old) bred at the Nencki Institute Animal House. Animals were housed under 12 h light/dark cycle with *ad libitum* access to Labofeed chow and water with temperature of 21 ± 2°C and 45-65% humidity in standard Plexiglas cages. All experimental procedures were conducted during the light phase and were approved by the Local Ethical Committee (approval number 1376P1/2022).

Surgery

For chemogenetic activation and inhibition of SST cells 500nl of pAAV-hSyn-DIO-HA-hM3D(Gq)-IRES-mCitrine or pAAV-hSyn-DIO-HA-hM4D(Gi)-IRES-mCitrine viral vectors (>1x10^{13}vg/ml, Addgene 50454-AAV8 and 50455-AAV8) were bilaterally injected with a 10 µL NanoFil syringe (World Precision Instruments) to the prelimbic cortex (PrL, AP +1.8, LM +/- 0.76 at a 20^{0} angle, DV -1.9 from dura) of SST-Cre mice. The injection volume was optimized for transfection rate and distribution. Prior to the onset of the experiment we have tested four different injection volumes (200 nl, 300 nl, 400 nl, and 500 nl per side of either vector) to find that volumes lower than 500 nl marked fewer neurons and filled the PrL only partially. Analgesia was provided *via* subcutaneous injection of butorphanol (Butomidor, 3.3 mg/kg). Postoperative care included administration of enrofloxacin (Baytril, 7.5 mg/kg) and tolfenamic acid (Tolfedine, 4 mg/kg) for 3–4 days. Mice were allowed to recover, at first in single cages, then in pairs for three weeks before the onset of habituation.

Remote Transfer of Emotional Information

A 10-day habituation protocol ensured that mice got used to the experimenter, the experimental room, equipment and to transportation. On day 1, one mouse from each pair was randomly (in case of SST Ai tdTomato mice) assigned as the Demonstrator and marked on the tail for identification. In the chemogenetic study SST-Cre mice were assigned as Observers, while C57BL/6J mice were used as Demonstrators. Following 3 days of habituation to experimenter alone, through consecutive 7 days Demonstrators were removed from the home cage and placed into a clean cage placed for 5 min in the conditioning chamber, while the Observer was transferred to a separate room to prevent any auditory, olfactory or visual contact. For chemogenetic manipulation, the Observers were at that time habituated to intraperitoneal injection. After 5 minutes, the Demonstrator was returned to the home cage, and the interaction was recorded for 9 minutes.

On the test day, animals were randomly assigned to either the No-fear or Fear group. No-fear animals underwent the same procedures as during habituation, with no exposure to aversive stimuli. In the Fear group, the Demonstrator was placed into the conditioning chamber (MedAssociates) and exposed to ten 1-second foot shocks (0.6 mA) delivered at 30-second intervals. Otherwise the procedure was identical to that on a habituation days preceding the test.

In the chemogenetic study SST-Cre mice received an intraperitoneal injection of Compound 21 (C21); 3 mg/kg, dose was chosen based on our previous experience with this activator, Rojek-Sito et al., 2023) or saline solution 30 minutes prior to the separation phase.

Behavioral analysis

Multi-animal pose estimation was performed using DeepLabCut (Lauer et al., 2022), a markerless tracking tool capable of analysing freely moving animals. For behavioral classification, simple behavioral analysis (SimBA) was used (Goodwin et al., 2024). SimBA employs pose estimation data to train supervised machine learning models for automated detection of complex social behaviors. The analysis focused on the following behaviors: (1) anogenital sniffing of the Demonstrator by the Observer, (2) body sniffing of the Demonstrator by the Observer, (3) rearing, (4) digging in bedding, and (5) self-grooming—scored for both Demonstrators and Observers. Manually annotated datasets were used to train and validate two supervised models: one for pose estimation and another for automated behavior classification. This type of data analysis also allowed for second-by-second comparison of distribution of given behaviors in time (during the 9 minute recording session) as well as comparison of the number and duration of episodes of each type of behavior.

Tissue processing

Ninety minutes after the onset of the test session, animals were anesthetized with isoflurane (5% in air) and euthanized *via* intraperitoneal injection of Morbital (133.3 mg/ml sodium pentobarbital, 26.7 mg/ml pentobarbital). Perfusion was performed using ice-cold phosphate-buffered saline (PBS, pH 7.4) and 4% paraformaldehyde (PFA) in PBS. Brains were extracted, post-fixed in 4% PFA overnight at 4°C, and subsequently transferred to 30% sucrose in PBS at 4°C until sinking. The brains were then frozen using dry ice and stored at -80°C. Coronal sections (40 µm) were cut using a cryostat (Leica) and stored in PBS containing 0.1% sodium azide. For immunohistochemical analysis, sections encompassing the amygdala (AP –1.58) and prefrontal cortex (AP +1.70) were selected, based on the Paxinos and Franklin mouse brain atlas (2019).

For SST tdTomato mice, only c-Fos immunostaining was performed, as SST-expressing cells were endogenously labelled. Slices from SST-Cre mice required immunostaining for both c-Fos and mCitrine/GFP, to visualize transfected SST-cells. Free-floating coronal brain sections were incubated in PBS (pH 7.45, Gibco #18912014) overnight at 4°C, followed by three PBS washes. Sections were then blocked (5% normal goat serum [NGS], Vector #S-1000-20, in 0.02% Triton X-100 [Chempur #498418109] in PBS [PBST]) for 90 min at room temperature (RT). Primary antibody incubation (anti-c-Fos, 1:1000, Millipore #ABE457) was performed in PBST with 2% NGS for 48 h at 4°C. After washing (3×PBST), sections were incubated at RT for 2 hours with either Alexa Fluor 488 (for SST tdTomato mice) or Alexa Fluor 594-conjugated (SST-Cre and C57BL/6J mice) secondary antibody (anti-rabbit, 1:1000, Invitrogen #A32731TR and #A32740) and then washed 3×PBS. Then sections from SST tdTomato mice were mounted and cover-slipped using Fluoromount (Sigma #F4680-25ML), while sections from SST-Cre mice were incubated with primary anti-GFP antibody conjugated with Alexa Fluor 488 (Invitrogen #A21311) for 48 h at 4°C. Imaging was conducted using a Nikon Eclipse Ni microscope and Image-Pro Plus 7.0.1.658 software (Media Cybernetics).

Quantification of c-Fos expression and c-Fos and SST colocalization

A semi-automated ImageJ-based protocol was implemented. Images were duplicated, and c-Fos+ cells were removed from the duplicate using the "Remove Outliers" function to isolate background signal. The background image was then subtracted from the original using the Image Calculator, yielding images with c-Fos+ cells on a uniform background. A threshold was applied to reduce non-specific signal, ROIs were manually defined, and cell counts and ROI areas were extracted using ImageJ. A custom macro (developed with Dr. Kacper Łukasiewicz) automated all steps except ROI selection. ROI areas were converted to mm^2 based on image calibration. For colocalization images acquired from two spectral channels were merged to generate a composite image. The overlap in fluorescence was manually marked and counted using Cell Counter plugin.

Statistical analysis

All datasets were tested for normal distribution (Shapiro-Wilk test) and based on the outcome subjected to either parametric or non-parametric comparisons. Behavioral data from SST-tdTomato mice were compared with either Student t-test or Mann-Whitney U-test. Chemogenetic study data was analyzed with two-way ANOVA followed by Tukey's multiple comparison test. Distribution of behaviors in 1 sec bins was compared with Kolmogorov-Smirnov test.

Neuronal activation and colocalization data were analysed separately for mPFC (prelimbic, PrL and infralimbic, IL cortices) and amygdala (basolateral, BLA, medial, MeA, and central nuclei divided into medial CeM and lateral CeL parts) regions with ANOVA with

repeated measures followed by (in case of significant interaction between factors) Tukey's multiple comparison test. $P<0.05$ was considered statistically significant.

RESULTS

The remote fear transfer protocol (Fig. 1A) induced elevated self-grooming of experimental SST Ai14 tdTomato Demonstrators. Both the number and the duration of self-grooming bouts were higher (unpaired t-test, $P=0.0057$ and $p=0.038$ respectively, Fig. 1C) in the stressed individuals than in the control group. The exposure of a naive SST Ai14 tdTomato Observer to a stressed cagemate in the safe environment of the home cage resulted in an increase in social investigation of the Demonstrator by the Observer. The most pronounced differences were observed for the sniffing of the anogenital region, for which both the number and the duration of episodes were elevated (Mann-Whitney U, $P=0.0099$ and $P=0.0175$ respectively, Fig. 1D). In the experimental pairs the majority of sniffing bouts (both anogenital and whole body sniffing) happened during the first 3 minutes of interaction, which proves that the naïve Observers immediately noticed the changed affective state of the Demonstrators. No such intensification was observed for the control pairs (data not shown, the distribution was compared with Kolmogorov-Smirnov tests and yielded $P<0.0001$ and $P=0.0045$ respectively). The social interaction upon reunion in the home cage also altered the distribution of exploratory behavior (reduced rearing) in stressed Demonstrators (Kolmogorov-Smirnov test, $P=0.0045$) as well as reduced digging in the bedding performed by the Observers paired with stressed Demonstrators (Kolmogorov-Smirnov test, $P<0.0001$). The number of self-grooming bouts of Observer mice was unchanged and its distribution was not different between control and stressed conditions but the overall duration of this behavior was higher in Observers paired with stressed Demonstrators (unpaired t-test $P=0.0087$, Fig. 1E).

The neuronal activation pattern imaged in the prefrontal cortex (Fig. 1B and F) did not change significantly in SST Ai14 tdTomato Observers paired with stressed *vs.* control Demonstrators as well as the Demonstrators themselves. Two-way ANOVA did not yield main effects of brain structure ($F_{(1,48)}=1,570$; $P=0.2162$) or group (Fear *vs.* No-Fear $F_{(3,48)}=1.318$; $P=0.2794$) and no significant interaction of the two factors was found ($F_{(3,48)}=0.04879$; $P=0.9856$). For the amygdalar complex (Fig. 1G) ANOVA with repeated measures yielded main effects of brain structure ($F_{(3,91)}=4.037$; $P=0.0096$) and group ($F_{(3,91)}=6.861$; $P=0.00039$) but no significant interaction of the two factors ($F_{(9,91)}=0.8537$; $P=0.5692$).

The percent of prefrontal somatostatin cells that were activated during the interaction was similar in Observers exposed to control and stressed cagemates (Fig. 1H) even though two-way ANOVA yielded a main effects of brain structure ($F_{(1,48)}=8.952$; $P=0.0044$) and group ($F_{(3,48)}=3.039$; $P=0.0379$). No significant interaction of the two factors was reported ($F_{(3,48)}=0.6228$; $P=0.6037$). In the amygdalar complex (Fig. 1I) this proportion changed only depending on the brain structure ($F_{(3,95)}=9,050$; $P<0.0001$) while the group effect was not significant $F_{(3,95)}=0,4479$; $P=0.7194$). Even though the interaction between the two factors was at a trend level $F_{(9,95)}=1,729$; $P=0.0929$) Tukey's multiple comparisons test results did not show any within brain structure differences related to the stress level of the Demonstrator.

Seeing as SST+ interneurons in the prefrontal cortex were previously reported to be involved in emotion recognition (Scheggia et al., 2020; Dautan et al., 2024) as well as social fear (Xu et al., 2019) and fear memory (Cummings & Clem, 2020) we decided to chemogenetically manipulate their activity in the remote fear transfer paradigm.

Chemogenetic activation of SST+ interneurons in the prefrontal cortex of the Observers during remote transfer of fear (Fig. 2A) did not affect the amount of self-grooming of the Demonstrators (two-way ANOVA group (Fear-No-Fear) effect: $F_{(1,12)}=1.961$; $P=0.1867$, treatment (NaCl *vs.* C21) effect: $F_{(1,12)}=0.5643$; $P=0.4670$ and the interaction of the two factors: $F_{(1,12)}=1.961$; $P=0.1867$ (Fig. 2C) but resulted in a blockade of the increase in anogenital sniffing observed in SST Ai14 tdTomato Observers (Fig. 1D) and NaCl treated SST-Cre Observers in response to the distress of their cagemate (two-way ANOVA group effect: $F_{(1,12)}=2.480$; $P=0.1413$, treatment effect: $F_{(1,12)}=0.3819$; $P=0.5481$ and the interaction of the two factors: $F_{(1,12)}=6.820$; $P=0.0227$, Tukey's multiple comparison test reporting $P=0.0466$ for NaCl treated Fear *vs.* No-Fear group comparison, Fig. 2D). At the same time Observer self-grooming seemed unaffected (Tukey's multiple comparison test did not show any significant differences between groups and treatment even though two-way ANOVA yielded a significant interaction of group x treatment ($F_{(1,19)}=4.775$; $P=0.0416$ (Fig. 2E).

The excitatory effect of chemogenetic activation was confirmed with increased activity of SST+ cells in Observers' PrL regardless of the emotional status of the Demonstrator (two-way ANOVA yielded large effects of brain structure $F_{(1,32)}=59.73$; $P<0.0001$, chemogenetic treatment $F_{(3,32)}=17.73$; $P<0.0001$ and the interaction of the two factors $F_{(3,32)}=17.44$; $P<0.0001$ (Fig. 2B and F). The treatment had an effect of c-Fos expression in the Observers (two-way ANOVA yielded a treatment effect

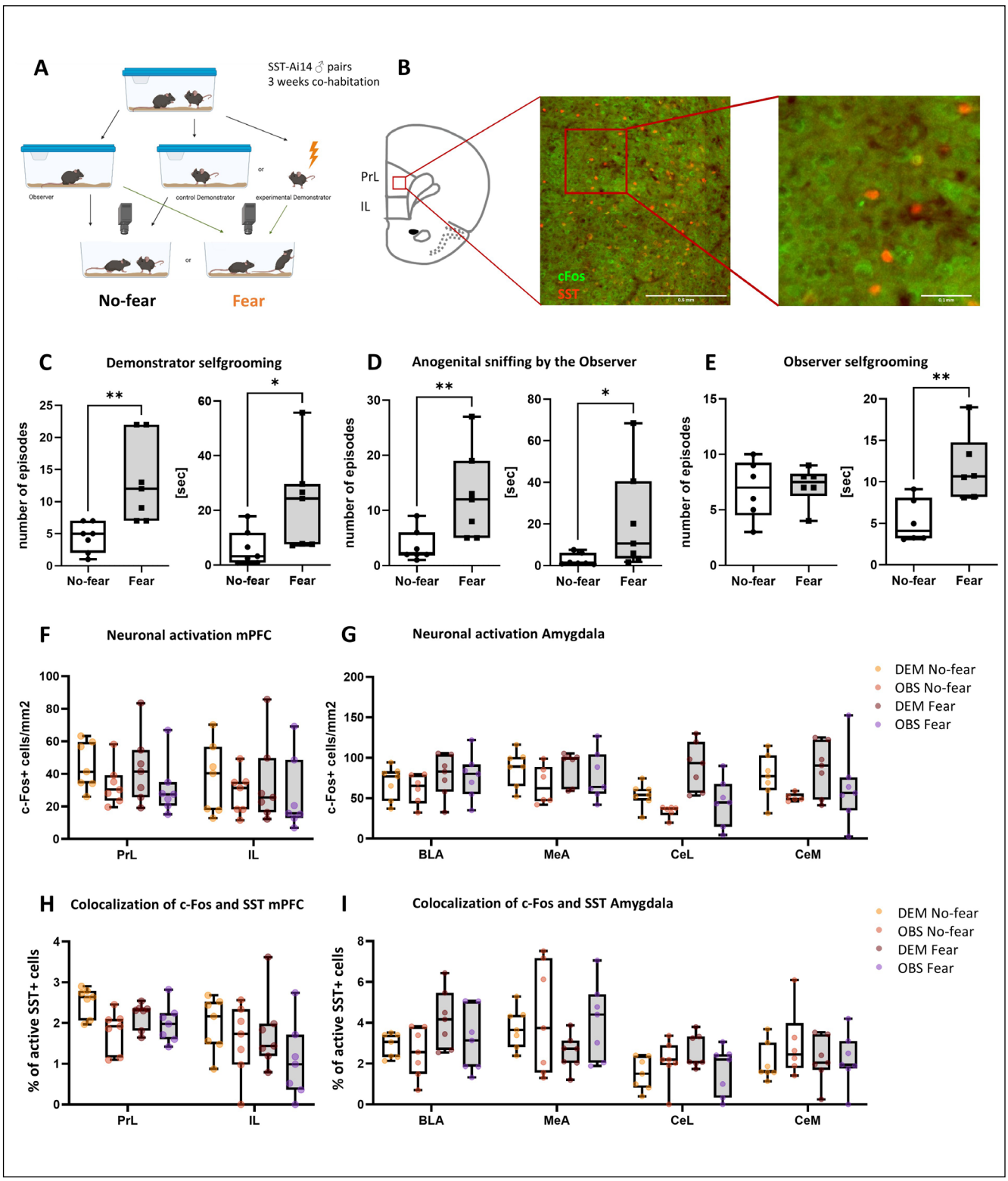

Fig. 1. Remote transfer of emotional information affects the behaviour of SST-tdTomato mice but is of little consequence to the neuronal activation pattern in their prefrontal cortex and amygdalar complex. (A) Experimental schematic (B) Exemplary microphotograph of c-Fos and SST colocalization [scale 0.5 mm and 0.1 mm for the zoom-in], (C) Demonstrator self-grooming, (D) Social investigation of the Demonstrator by the Observer, (E) Observer self-grooming, (F) c-Fos expression in the mPFC, (G) c-Fos expression in the amygdala, (H) The ratio of activated/all SST cells in the mPFC, (I) The ratio of activated/all SST cells in the amygdala. Results presented as min-max box plots with individual values, *P<0.05, **P<0.01. White bars represent animals from No-fear pairs, gray ones represent pairs where Demonstrator was subjected to fear conditioning.

in both the No-Fear group $F_{(3,24)}$=10.14; P=0.0002, and the Fear group $F_{(3,35)}$=11.51; P<0.0001 (Fig. 2G) which, despite habituation to injections, appeared to be related more to the injection itself than specifically to C21. The response was similar in both parts (PrL and IL) of the mPFC in the No-Fear group, but was less pronounced in IL during exposure to a stressed Demonstrator. The neuronal activation of the amygdala was dependent on the emotional status of the Demonstrator (Fig. 2H). In the No-Fear group ANOVA with repeated measure yielded a main effect of brain structure ($F_{(3, 47)}$=6.651; P=0.0008) as well as treatment ($F_{(3,47)}$=3.418; P=0.0247) but there was no interaction between the two factors ($F_{(9,47)}$=0.1996; P=0.9931). In the Fear group the main effects of brain structure ($F_{(3,64)}$=18.64; P<0.0001) and treatment ($F_{(3,64)}$=5.246; P=0.0027) were accompanied by a nearly significant brain structure x treatment interaction ($F_{(9,64)}$=1.873; P=0.0721). The main differences

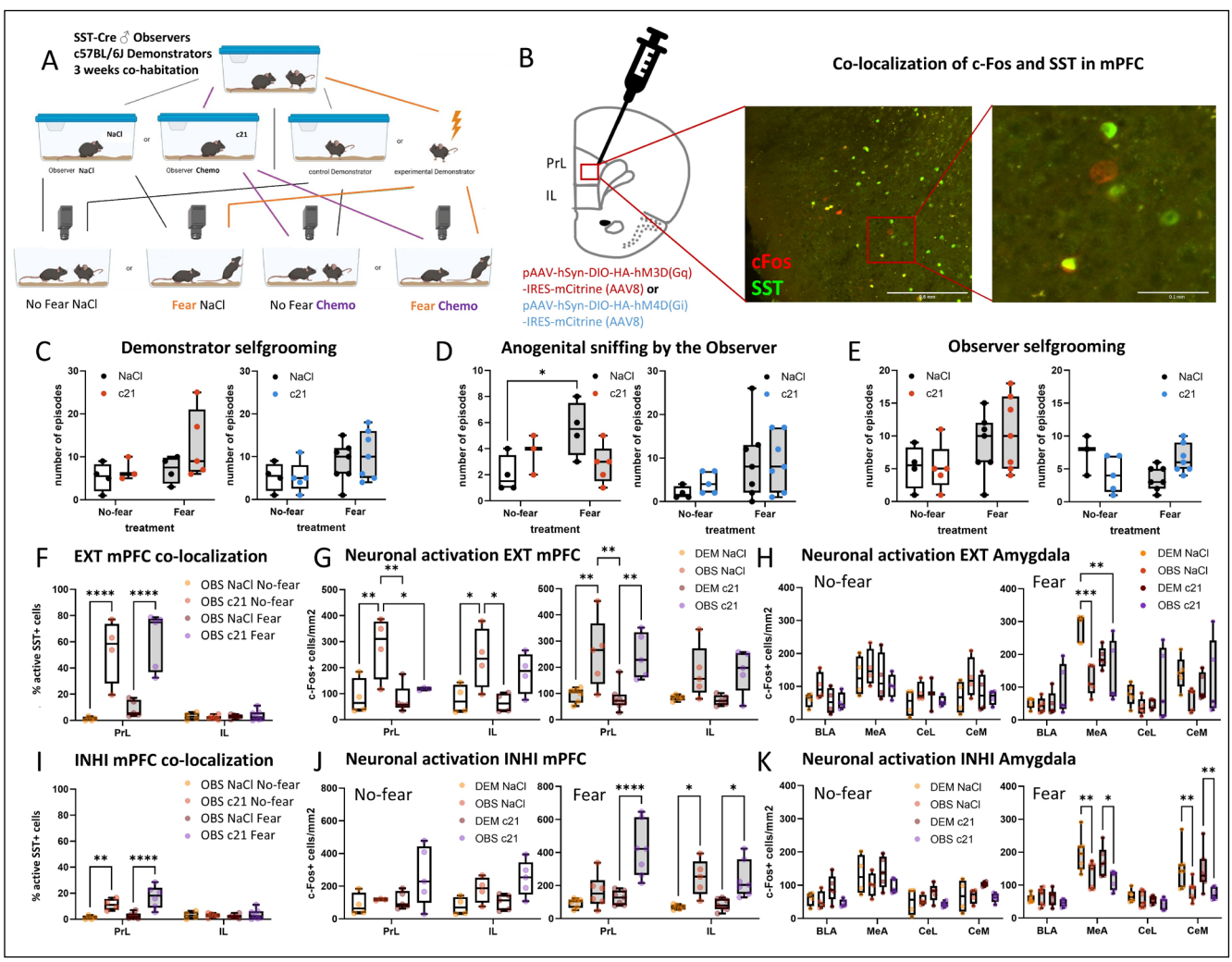

Fig. 2. Chemogenetic manipulation of prefrontal SST+ interneurons affects the behaviour of the SST-Cre Observers and neuronal activation patterns in both mice subjected to Remote Transfer of Fear. (A) Experimental schematic (B) Exemplary microphotograph of c-Fos and SST colocalization in the PrL [scale 0.5 mm and 0.1 mm in the zoom-in], (C) Demonstrator self-grooming, (D) Social investigation of the Demonstrator by the Observer, (E) Observer self-grooming, (F) The ratio of activated/all SST cells in Observers with chemoactivated SST+ cells in the prefrontal cortex (G) c-Fos expression in mPFC upon chemoactivation, (H) c-Fos expression in the amygdala upon chemoactivation, (I) The ratio of activated/all SST cells in Observers with chemoinhibited SST+ cells in the prefrontal cortex (J) c-Fos expression in the mPFC upon chemoinhibition, (K) c-Fos expression in the amygdala upon chemoinhibition. Results presented as min-max box plots with individual values, *P<0.05, **P<0.01, ***P<0.001, ****P<0.00001. In panels (C), (D) and (E) red and blue dots represent animals or partners of animals injected with pAAV-hSyn-DIO-HA-hM3D(Gq)-IRES-mCitrine (AAV8) or pAAV-hSyn-DIO-HA-hM4D(Gi)-IRES-mCitrine (AAV8) treated with C21. Black dots represent animals or partners of animals injected with these viruses but treated with NaCl. In panels (F) and (I) yellow and purple dots represent NaCl treated Observers, while orange and lilac represent C21 treated animals. In panels (G), (H), (J) and (K) yellow and purple dots represent Demonstrators while orange and lilac dots represent Observers. White bars represent animals from No-fear pairs, gray ones represent pairs where Demonstrator was subjected to fear conditioning.

here were related to the activation of the MeA of the Demonstrators' amygdala.

Inhibition of SST+ cells in the PrL did not affect either anogenital sniffing (even though group effect was significant $F_{(1,12)}$=6.820; *P*=0.0227, treatment effect $F_{(1,19)}$=0.1742; *P*=0.6811 and the interaction of the two were not $F_{(1,19)}$=0.2210; *P*=0.6436), Fig. 2D) or Demonstrators' self-grooming (group effect $F_{(1,19)}$=4.775; *P*=0.0416, treatment effect $F_{(1,19)}$=0.1750; *P*=0.6804, group x treatment $F_{(1,19)}$=0.1967; *P*=0.6624), (Fig. 2C). It mildly affected the number of self-grooming bouts in Observers exposed to a stressed cagemate (interaction of group x treatment $F_{(1,18)}$=8.360; *P*=0.0097 (Fig. 2E) although Tukey's multiple comparison test reported only a trend towards higher self-grooming in Observers paired with a stressed Demonstrator.

Chemogenetic inhibition of prefrontal SST+ cells resulted in a (possibly rebound) activation of these cells in the PrL (two-way ANOVA of colocalization of c-Fos and SST within the mPFC yielded the effect of brain structure $F_{(1,34)}$=14.76; *P*=0.0005 and chemogenetic treatment $F_{(3,34)}$=10.41; *P*<0.0001 and an interaction of the two factors $F_{(3, 34)}$=7.543; *P*=0.0005 (Fig. 2I). It was much lower than the activation obtained by administration of C21 in mice with chemoactivatory DREADD expression (Fig. 2F). It also affected the neuronal activation patterns in the prefrontal cortex (in the No-Fear condition two-way ANOVA yielded no effect of brain structure $F_{(1,27)}$=0.04450; *P*=0.8345 but an effect of treatment $F_{(3,27)}$=6.114; *P*=0.0026 and no interaction between the two factors $F_{(3,27)}$=0.2705; *P*=0.8461, while in the Fear condition the was no effect of brain structure $F_{(1,42)}$=2.241; *P*=0.1419, and a significant effect of treatment $F_{(3,42)}$=19,50 *P*<0.0001 with an brain structure x treatment interaction $F_{(3,42)}$=4.176; *P*=0.0112 (Fig. 2J). Tukey's multiple comparisons showed strong increase in c-Fos expression in C21 treated Observers in both PrL and IL cortices and while the activation in the IL seemed to be generalized to the injection procedure, in the PrL it only occurred in C21 treated animals. In the amygdalar complex the neuronal activation pattern changed in a stress dependent way. In No-Fear condition ANOVA with repeated measures yielded an effect of brain structure $F_{(3,55)}$=13.41; *P*<0.0001 and an effect of treatment $F_{(3,55)}$=6.052; *P*=0.0012 but no interaction of the two factors, while in the Fear condition it yielded an effect of brain structure $F_{(3,92)}$=50.40; *P*<0.0001, treatment $F_{(3,92)}$=11.44 *P*<0.0001 and a brain structure x treatment interaction $F_{(9,92)}$=2.215; *P*=0.0278 (Fig. 2K). Tukey's multiple comparisons showed that neuronal activation of the medial nucleus and the medial part of the central nucleus of amygdala was higher in Demonstrators as compared with Observers regardless of the chemogenetic status of the Observer they were paired with.

DISCUSSION

The ability to detect and respond to the altered emotional state of conspecifics is crucial for formation and maintenance of social bonds. It also provides information about environmental challenges, which often require a dynamic reaction. Here we observed an immediate response to the return of a stressed cagemate. Naive Observers displayed elevated social sniffing already in the early stages of the interaction. We also demonstrated, for the first time, that chemogenetic activation of prefrontal somatostatin interneurons abolishes this effect while inhibition of that circuitry had no effect on social aspects of behavior. No changes in other types of behavior (self-grooming, rearing and digging in the bedding), were induced by chemogenetic manipulation. The lack of behavioral change upon chemogenetic inhibition of SST neurons was unexpected as according to Scheggia et al. 2020 optosilencing of this population disrupts emotional state recognition in mice. In our paradigm the only behavior mildly affected by this manipulation was (interaction of group x treatment was significant, but *post hoc* test showed only a trend towards higher number of episodes of) the Observer self-grooming. Such effect (absent in SST Ai14 and NaCl treated SST-Cre mice) could indicate that the C21 treated mice with inhibitory DREADD expression perceived the emotional state of the stressed cagemate differently and thus employed a diverse coping strategy in this stressful situation. A somewhat similar effect of chemogenetic silencing of PrL SST neurons was observed during social fear conditioning. Mice (in that study treated with CNO) did not form aversion to the social compartment associated with an aversive stimulus, which the authors interpreted as decreased fear related to disinihibition of the prefrontal circuitry (Xu et al., 2019).

Neuronal activation patterns examined in the prefrontal cortex and the amygdalar complex of both SST tdTomato Observers and Demonstrators revealed that the remote fear transfer protocol used here was a mild stimulus for naive Observers. This is in line with a recent study showing that the magnitude of response to the stress of a partner is experience-dependent (and controlled by prefrontal CRH circuitry, Maltese et al., 2025). It is also partly in line with our previous study (Meyza et al., 2015) in which C57BL/6J Observers did not fully reproduce the neuronal activation pattern observed in Demonstrators. Here the induction of c-Fos expression in stressed SST tdTomato Demonstrators was quite modest, while in C57BL/6J Demonstrators tested before PrL, IL, BLA, MeA, CeL and CeM were affected. The results obtained here on C57BL/6J mice paired with NaCl treated SST-Cre mice were closer to

that result as they showed strong activation of MeA and CeM. This difference may stem from strain differences as SST Ai14 tdTomato are bred on mixed B6/129 genetic background known for diminished emotional contagion (Keum et al., 2016). Also, the habituation protocol used in the current study was more extensive than in our previous study, which could have affected c-Fos induction.

The colocalization of SST and c-Fos was quite low (average of 2% of SST cells were c-Fos+) and independent of behavioral treatment in SST Ai14 tdTomato mice. Seeing as similar values were observed in SST-Cre (Tg/0) mice treated with NaCl in the chemogenetic study it would speak for the validity of this observation. Whether this low activation rate is related to them being a heterogenous offspring of SST-Cre Tg/Tg, Jackson Laboratory # 013044 and Ai14 Jackson Laboratory #007908 lines remains to be clarified. The former line has been shown to have somatostatin expression deficits similar to that of a full SST knockout, while heterogenous SST-Cre (Tg/0) mice displayed intermediate values and only slightly lower SST protein levels in the cortex than WT mice (Viollet et al., 2017). Our SST-Cre mice used in the chemogenetic study were also heterozygous.

The choice of PrL for injection of viral vectors encoding Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) was dictated by previous reports on the importance of prefrontal somatostatin interneurons in emotional recognition (Scheggia et al., 2020) and social fear modulation (for review see Wang et al., 2020). Chemoactivation of this circuitry resulted in an enormous increase in activation of SST cells, which was specific to PrL and did not spill over to the IL. The general neuronal activation (measured with c-Fos expression alone) of both of these cortices was however not specific to C21 administration (despite prolonged habituation to injections). The neuronal activation pattern within the amygdalar complex depended on the brain structure and the emotional status of the Demonstrator. In Demonstrators exposed to fear conditioning, interaction with a C21 treated Observer reduced the activation of the medial nucleus of the amygdala. This could be related to the decrease in social interaction between these animals and is unlikely to be a result of social buffering (which requires pro-social approach from the Observer).

The increase in colocalization of c-Fos and SST within the PrL of animals infused with chemoinhibitory vector (pAAV-hSyn-DIO-HA-hM4D(Gi)-IRES-mCitrine) may seem counterintuitive. It was much smaller than that of the mice transfected with chemoactivatory DREADD (pAAV-hSyn-DIO-HA-hM3D(Gq)-IRES-mCitrine) but nonetheless it was significant. The reason for this could be the timing of tissue collection (optimized for c-Fos protein immunohistochemistry). In our setting C21 was administered half-an-hour before the onset of the interaction, an optimal point from chemomanipulation effectivity standpoint, while tissue was collected 2 hours later, when slow rebound of activity, including that of the SST population, was likely to happen (Graf et al., 2024). Direct measurement of the dynamics of SST interneuron inhibition would require live imaging based on co-tranfection of SST cells with two vectors (the virus encoding the DREADD and e.g., a Cre-dependent GCamP) as well as the use of a wireless detector (to allow for free social interaction). The transfection rate in such case might also be suboptimal as the two vectors would compete for Cre-locus in SST cells.

The neuronal activation pattern in the mPFC of chemogenetically inhibited Observers highly depended on the emotional status of the Demonstrator. Upon interaction with a stressed cage-mate the PrL of C21 treated and IL of both NaCl and C21 treated Observers got highly activated (an effect not present for animals paired with No-Fear Demonstrators). No differences in the activity of their amygdala was observed. In the fearful Demonstrators the chemoinhibition of Observers' PrL SST interneurons did not change the rate of neuronal activation in either of the mPFC regions or within the amygdalar complex. Instead their MeA and CeM were highly activated regardless of treatment of the Observer. This, together with relatively low impact on the behavior of the Observers questions the accuracy of chemogenetic targeting of the small population of SST+ neurons responsive to altered emotional status of the Demonstrators as disinhibition of such major circuitry was expected to affect emotion recognition (Scheggia et al., 2020).

In sum, while chemogenetic activation of PrL SST neurons altered social approach and interaction and by doing so affected neuronal activation within the amygdala of the Demonstrators paired with C21 treated animals, chemoinhibition of this circuitry had much less effect of emotional transfer and prosocial reactivity in the Remote Transfer of Fear paradigm.

Our study has several limitations. Chemogenetic manipulation does not have the time resolution of optogenetic techniques. Seeing as most of social investigation happens during first minutes after the return of the Demonstrator to the home cage future studies should use optomanipulation to verify our findings. The role of prefrontal somatostatin interneurons should also be confirmed in females, as there are significant sex differences in emotional recognition and contagion (Christov-Moore et al., 2014) although the effect might be species and paradigm specific (Han et al., 2020, Keum et al., 2016).

CONCLUSIONS

Prefrontal SST neurons modulate behavioral responses to the emotional arousal of stressed partners even in the safe environment of a home cage. Chemoactivation of this circuit affects the interaction and the resulting neuronal activation pattern of the stressed animal, while chemoinhibition does not.

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REFERENCES

Choi, J., Jung, S., Kim, J., So, D., Kim, A., Kim, S., Choi, S., Yoo, E., Kim, J. Y., Jang, Y. C., Lee, H., Kim, J., Shin, H. S., Chae, S., & Keum, S. (2024). ARNT2 controls prefrontal somatostatin interneurons mediating affective empathy. *Cell Reports, 43*(9), 114659. https://doi.org/10.1016/j.celrep.2024.114659

Christov-Moore, L., Simpson, E.A., Coudé, G., Grigaityte, K., Iacoboni, M., & Ferrari, P.F. (2014) Empathy: gender effects in brain and behavior. *Neurosci Biobehav Rev.* 46 Pt 4(Pt 4): 604–627. https://doi: 10.1016/j.neubiorev.2014.09.001.

Dautan, D., Monai, A., Maltese, F., Chang, X., Molent, C., Mauro, D., Galbusera, A., Vecchia, D., Antonelli, F., Benedetti, A., Drago, F., Leggio, G. M., Pagani, M., Fellin, T., Gozzi, A., Schumann, G., Managò, F., & Papaleo, F. (2024). Cortico-cortical transfer of socially derived information gates emotion recognition. *Nature Neuroscience, 27*(7), 1318–1332. https://doi.org/10.1038/s41593-024-01647-x

Goodwin, N. L., Choong, J. J., Hwang, S., Pitts, K., Bloom, L., Islam, A., Zhang, Y. Y., Szelenyi, E. R., Tong, X., Newman, E. L., Miczek, K., Wright, H. R., McLaughlin, R. J., Norville, Z. C., Eshel, N., Heshmati, M., Nilsson, S. R. O., & Golden, S. A. (2024). Simple Behavioral Analysis (SimBA) as a platform for explainable machine learning in behavioral neuroscience. *Nature Neuroscience, 27*(7), 1411–1424. https://doi.org/10.1038/s41593-024-01649-9

Graf, J., Samiee, A., Flossmann, T., Holthoff, K., & Kirmse, K. (2024). Chemogenetic silencing reveals presynaptic $G_{i/o}$ protein-mediated inhibition of developing hippocampal synchrony *in vivo*. *iScience.* 27(10): 110997. https://doi: 10.1016/j.isci.2024.110997.

Gutzeit, V. A., Ahuna, K., Santos, T. L., Cunningham, A. M., Sadsad Rooney, M., Muñoz Zamora, A., Denny, C. A., & Donaldson, Z. R. (2020). Optogenetic reactivation of prefrontal social neural ensembles mimics social buffering of fear. *Neuropsychopharmacology, 45*(6), 1068–1077. https://doi.org/10.1038/s41386-020-0631-1

Han, Y., Sichterman, B., Carrillo, M., Gazzola, V., & Keysers, C. (2020) Similar levels of emotional contagion in male and female rats. *Sci Rep*. 10(1): 2763. https://doi: 10.1038/s41598-020-59680-2.

Huang, Z., Chung, M., Tao, K., Watarai, A., Wang, M. Y., Ito, H., & Okuyama, T. (2023). Ventromedial prefrontal neurons represent self-states shaped by vicarious fear in male mice. *Nature Communications, 14*(1), 3458. https://doi.org/10.1038/s41467-023-39081-5

Keum, S., Park, J., Kim, A., Park, J., Kim, K. K., Jeong, J., & Shin, H. S. (2016). Variability in empathic fear response among 11 inbred strains of mice. *Genes, Brain and Behavior, 15*(2), 231–242. https://doi.org/10.1111/gbb.12278

Kitamura, T., Ramesh, K., & Terranova, J. I. (2024). Understanding others' distress through past experiences: The role of memory engram cells in observational fear. In *Advances in Neurobiology* (Vol. 38, pp. 215–234). Springer. https://doi.org/10.1007/978-3-031-62983-9_12

Knapska, E., Nikolaev, E., Boguszewski, P., Walasek, G., Blaszczyk, J., Kaczmarek, L., & Werka, T. (2006). Between-subject transfer of emotional information evokes specific pattern of amygdala activation. *Proceedings of the National Academy of Sciences of the United States of America, 103*(10), 3858–3862. https://doi.org/10.1073/pnas.0511302103

Lauer, J., Zhou, M., Ye, S., Menegas, W., Schneider, S., Nath, T., Rahman, M. M., Di Santo, V., Soberanes, D., Feng, G., Murthy, V. N., Lauder, G., Dulac, C., Mathis, M. W., & Mathis, A. (2022). Multi-animal pose estimation, identification, and tracking with DeepLabCut. *Nature Methods, 19*(4), 496–504. https://doi.org/10.1038/s41592-022-01443-0

Liu, L., Ito, W., & Morozov, A. (2017). GABAb receptor mediates opposing adaptations of GABA release from two types of prefrontal interneurons after observational fear. *Neuropsychopharmacology, 42*(6), 1272–1283. https://doi.org/10.1038/npp.2016.273

Maltese, F., Pacinelli, G., Monai, A., Bernardi, F., Capaz, A. M., Niello, M., Walle, R., de Leon, N., Managò, F., Leroy, F., & Papaleo, F. (2025). Self-experience of a negative event alters responses to others in similar states through prefrontal cortex CRF mechanisms. *Nature Neuroscience, 28*(1), 122–136. https://doi.org/10.1038/s41593-024-01816-y

Meyza, K., Nikolaev, T., Kondrakiewicz, K., Blanchard, D. C., Blanchard, R. J., & Knapska, E. (2015). Neuronal correlates of asocial behavior in a BTBR T (+) Itpr3(tf)/J mouse model of autism. *Frontiers in Behavioral Neuroscience, 9*, 199. https://doi.org/10.3389/fnbeh.2015.00199

Nosjean, A., de Chaumont, F., Olivo-Marin, J. C., & Granon, S. (2018). Stress-induced brain activation: Buffering role of social behavior and neuronal nicotinic receptors. *Brain Structure and Function, 223*(9), 4259–4274. https://doi.org/10.1007/s00429-018-1745-7

Rojek-Sito, K., Meyza, K., Ziegart-Sadowska, K., Nazaruk, K., Puścian, A., Hamed, A., Kiełbiński, M., Solecki, W., & Knapska, E. (2023). Optogenetic and chemogenetic approaches reveal differences in neuronal circuits that mediate initiation and maintenance of social interaction. *PLoS Biology, 21*(11), e3002343. https://doi.org/10.1371/journal.pbio.3002343

Scheggia, D., Managò, F., Maltese, F., Bruni, S., Nigro, M., Dautan, D., Latuske, P., Contarini, G., Gomez-Gonzalo, M., Requie, L. M., Ferretti, V., Castellani, G., Mauro, D., Bonavia, A., Carmignoto, G., Yizhar, O., & Papaleo, F. (2020). Somatostatin interneurons in the prefrontal cortex control affective state discrimination in mice. *Nature Neuroscience, 23*(1), 47–60. https://doi.org/10.1038/s41593-019-0551-8

Viollet, C., Simon, A., Tolle, V., Labarthe, A., Grouselle, D., Loe-Mie, Y., Simonneau, M., Martel, G., & Epelbaum, J. (2017). Somatostatin-IRES-Cre Mice: Between Knockout and Wild-Type? *Front Endocrinol* (Lausanne) 8: 131. https://doi: 10.3389/fendo.2017.00131.

Wang, J., Tian, Y., Zeng, L. H., & Xu, H. (2020). Prefrontal disinhibition in social fear: A vital action of somatostatin interneurons. *Frontiers in Cellular Neuroscience, 14*, 611732. https://doi.org/10.3389/fncel.2020.611732

Xu, H., Liu, L., Tian, Y., Wang, J., Li, J., Zheng, J., Zhao, H., He, M., Xu, T. L., Duan, S., & Xu, H. (2019). A disinhibitory microcircuit mediates conditioned social fear in the prefrontal cortex. *Neuron, 102*(3), 668–682.e5. https://doi.org/10.1016/j.neuron.2019.02.026