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1. PROPOSAL DATA

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TITLE OF THE PROJECT: Regulation of social preferences by the lateral septum (SocialPref)

2. BACKGROUND, CURRENT STATUS AND JUSTIFICATION OF THE PROPOSAL

2.1 Background

Social preference, the decision to interact with one conspecific over another, is a feature displayed by gregarious animals which is critical to navigate their social space[1,2]. Thus, adult rodents prefer to interact with their kin[3,4], individuals from specific strains[5] and members of the opposite sex[6–9]. In addition to innate factors (e.g., kin, strain, and sex), social preference is also influenced by social memory[10], social hierarchy[9,11,12] and the affective state of the conspecific[13]. Thus, adult rodents display social novelty preference (SNP), choosing to interact with novel individuals over familiar ones[10]. For the last two decades, SNP has been used as a proxy to assess social memory[14–16] but the neuronal circuits mediating SNP and whether such circuits promote social interactions with the preferred individuals or prevent interactions with the non-preferred ones are unknown.

Memory-based social preferences, such as SNP, have a developmental window[17] and can change during the life of altricial animals. Young mice prefer their mother to an unfamiliar dam for the first few weeks[18]. After weaning, mice display reversed social preference for an unfamiliar dam over the mother[18]. Similarly, rat pups display a preference for their familiar siblings during the first 2 postnatal weeks, after which preference shifts toward novel pups[3,4]. Although the mechanisms that regulate these shifts remains elusive, the lateral septum (LS), a brain region associated with the regulation of motivated behaviors including social interactions[19], is necessary for kinship/familiarity preference in young rats[3] as well as for SNP in adult rodents[19–21]. Moreover, the ventral aspect of medial prefrontal cortex (mPFC), the infra-limbic area (ILA), is known for its involvement in decision-making and recent evidence shows it responds to social stimuli[22–24] and is necessary for SNP[25,26]. Although PFC is known to project to LS[27], how these regions integrate social memory cues and communicate to regulate social interactions and display a preference for the novel or familiar individual is still poorly understood.

Corticotropin-releasing hormone (CRH)[28], a 41 amino acid peptide, regulates diverse processes ranging from homeostatic neuroendocrine mechanisms to memory[29], including social behaviors in non-stressful context[30,31]. In human, CRH has been involved in psychiatric disorders associated with social deficits such as depression[32,33] or social phobia[34]. In rodents, knocking-out the gene encoding CRH in mouse forebrain inhibitory neurons decreases social interaction[35] and central administration of CRH inhibits social interactions in rats[36–40]. Moreover, application of the CRH receptor 1 agonist stressin-1 elicited the same effect[41] suggesting that CRH effects on social interactions are mediated by the CRH receptor 1 (CRHR1). CRH also plays an important role in SNP and social recognition[31,42,43]. Social recognition in rats is facilitated by over-expressing CRH[43] or increasing CRH tone by pharmacological targeting of CRH receptors or binding proteins[31]. Importantly, administration of a CRH-binding protein ligand inhibitor during social recognition did not affect social interaction during the initial exposure but impaired performance during recall[31].

Despite the evidence implicating CRH and its receptor in social interactions and SNP, the neural circuitry mediating its release and targeted by its action remained unknown. Given that CRH is expressed in ILA[44] and CRHR1 is expressed in LS[45], we hypothesized that CRH release from PFC to LS is involved in SNP.

2.2 Current status

Leveraging the *CRH-Cre* mouse model, we demonstrated through a combination of electrophysiological, chemogenetic, optogenetic, calcium recording and gene silencing techniques that the release of CRH from ILA neurons (ILA^{CRH} neurons) during familiar interactions into the rostro-dorsal region of LS (rdLS) suppresses social interaction between familiar mice. This circuit therefore controls familiarization (decrease in interaction as a novel rodent becomes familiar) and contributes to the SNP exhibited by adult mice. In addition, we showed that the increase in ILA^{CRH} neurons density during the second postnatal week is responsible for a shift in the social preference of young mice from familiar to novel conspecifics.

ILA^{CRH} cells project to rostro-dorsal lateral septum.

Using anterograde and retrograde tracing techniques we identified the rostral lateral septum as a target of CRH^{+} neurons located principally in the layer 2/3 of the infra-limbic area of the PFC. We thereafter refer to these neurons as ILA^{CRH} neurons. Based on immunohistochemistry and in situ hybridization labeling we established the molecular identity of these neurons as GABAergic. We also used channelrhodopsin recordings from post-synaptic LS neurons to confirm that ILA^{CRH} neurons make functional inhibitory connections in rLS (Fig. 1a). Rabies tracing revealed the many pre-synaptic neurons impinging on ILA^{CRH} neurons, including ventral CA1 neurons.

ILA^{CRH} neurons suppress social interactions with familiar mice.

Then, we used chemogenetic to silence ILA^{CRH} neurons and tested the mice for a barrage of behavioral tests. Silencing ILA^{CRH} neurons yielded no effect on anxiety, locomotion or sociability but abolished SNP measured during the 3-chamber test (Fig. 1f-j). Since mice are simultaneously confronted to a novel and a familiar mouse during the recall phase of this test, we decided to test the mice during the repetitive social presentation test. In control conditions, repetitive presentation of the same novel mouse leads to a steady decrease in social interaction (a process called familiarization thereafter). Mice with silenced ILA^{CRH} neurons however exhibited no familiarization (Fig. 1k-l). On the contrary, chemogenetic excitation of ILA^{CRH} neurons yielded to an increased facilitation (Fig. 1m), indicating that these neurons can bidirectionally regulate social interaction with familiar but not novel mice. Indeed, the amount of social interaction with novel mice (learning phase of the 3-chamber or first trial of the repetitive presentation test) is unchanged upon modulation of the ILA^{CRH} neuron activity. Control experiments with novel object preference or repetitive object presentation demonstrated that ILA^{CRH} neurons specifically regulated interaction with familiar conspecifics. Fiberphotometry of ILA^{CRH} neurons and c-fos labelling confirmed that these neurons were more active during familiar social encounter compared to novel social encounter or object interaction (Fig. 1n).

CRH release in rLS disinhibits rLS to suppresses social interactions with familiar mice and support SNP. We then proceeded to knocking-down *Crh* expression in ILA^{CRH} neurons using a shRNA and observed the same effect on behavior, suggesting that CRH release was necessary for familiarization and SNP (Fig. 1o-r). Optogenetic silencing of ILA^{CRH} neuron terminals in rLS or infusion of the CRH receptor antagonist Antalarmin also abolished familiarization and SNP, confirming that CRH release had to occur in rLS from ILA^{CRH} neuron terminals for the mice to exhibit SNP. CRH receptor 1 (CRHR1) is expressed in rLS and infusion of the CRHR1 agonist stressin-1 on acute brain slices while patching LS cells indicated that CRH released led to a disinhibition of rLS. Similar to CRH^{+} neurons in ILA, c-fos labelling and fiber-photometry in rLS showed a higher response during familiar social encounters compared to novel encounters (Fig. 1s-t). Finally, we leveraged a recent CRH biosensor developed by the lab of Yulong Li in order to measure CRH release in freely moving animals and confirmed that CRH release was higher during familiar encounters. Interestingly, c-fos of ILA^{CRH} neurons and rLS showed a strong correlation which could be disrupted when knocking-down *Crh* expression. Finally, optogenetic activation of rLS led to a decrease in social interaction with familiar conspecifics.

Overall, our data demonstrate that ILA^{CRH} neurons receive inputs from ventral CA1 which has been shown to contain engrams for social memory[46]. Activation of ILA^{CRH} neurons with familiarity cues

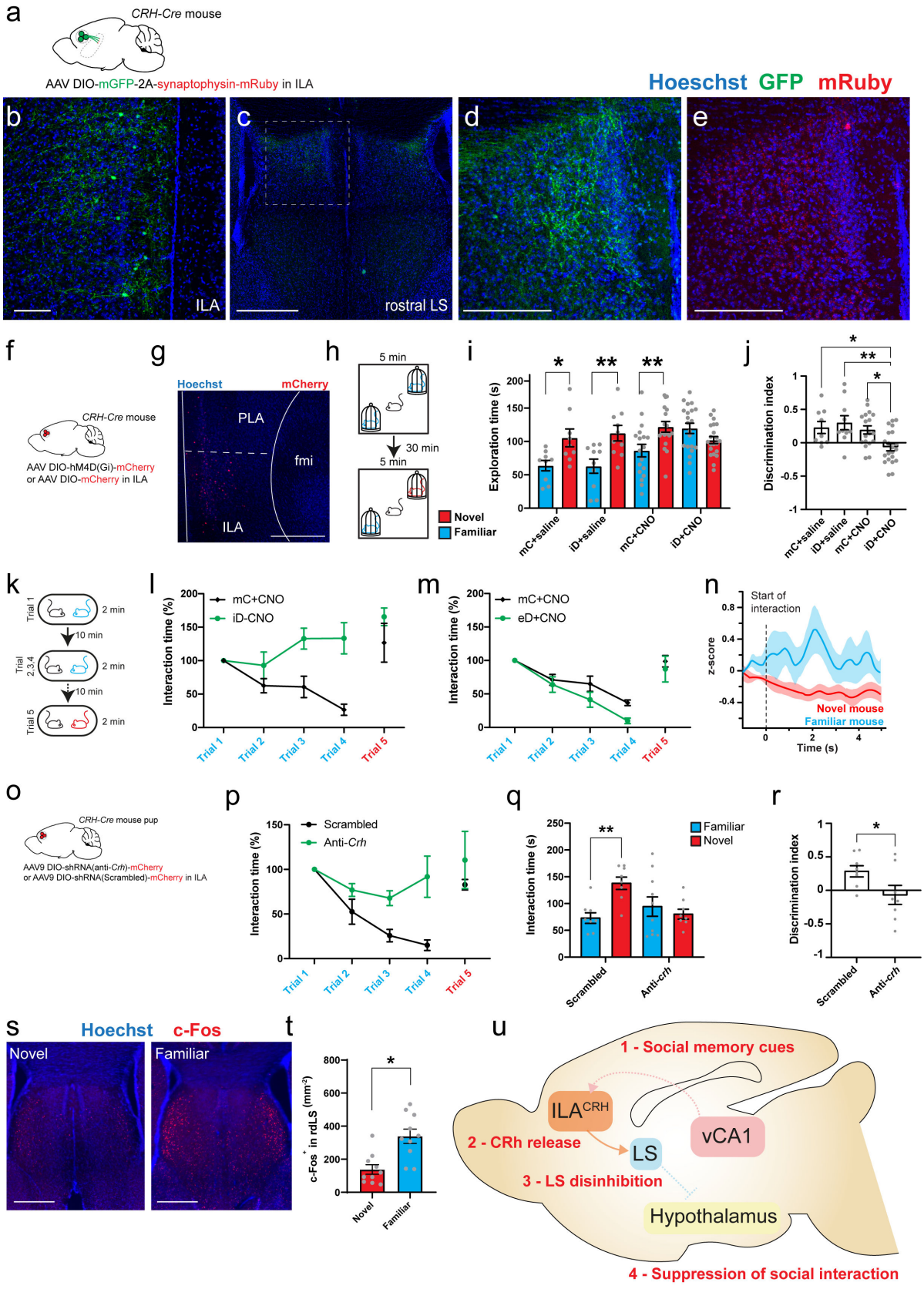

Fig. 1. Summary of previous results: a ILA to LS projection suppression social interaction with familiar mice. A-E. Anterograde tracing from ILA to rdLS. **F-L.** Chemogenetic silencing of ILA^{CRH} cells during social novelty preference test (F-J) and repetitive presentation test (K-L). **M.** Chemogenetic excitation of ILA^{CRH} cells during repetitive presentation test. **N.** Fiber-photometry recording of ILA^{CRH} cells during presentation of a novel or familiar mouse. **O-R.** shRNA-mediated silencing of crh during repetitive presentation (P) and social novelty preference tests (Q-R).S-T. c-fos labelling of rostral LS following interaction with a novel or familiar conspecific. **U.** Schematic of the propose circuit. See publication in BiorXiv for more statistics and detailed description.

triggers CRH release in rLS which activities CRHR1 and led to the disinhibition of rLS. Disinhibition of rLS in turn suppress social interaction with familiar mice and support SNP (Fig. 1u). A manuscript with these results and others results have been uploaded to BiorXiv[47] and the manuscript has been invited for resubmission at the journal *Cell*.

2.3 Justification of the proposal

Despite being proposed as early as 1949 as part of the limbic system for the regulation of mood, emotion and motivation[48,49], the lateral septum remained for many years a black box until the elegant studies by Risold and Swanson in the mid 90's demonstrating that cortical inputs to LS segregate to particular LS regions which in turn project to defined hypothalamic nuclei[50]. The topography of cortical inputs is therefore conserved and led to the proposal that LS is a collection of several parallel circuits regulating different goal-oriented behaviors[51]. Importantly, all LS neurons are GABAergic which led to the hypothesis that LS neurons provide tonic inhibition onto the hypothalamus nucleus promoting motivated behaviors, thereby providing a continuous brake on these behaviors until inhibition is lifted. Despite several recent studies investigating different cortical inputs to LS or specific LS outputs[27,52–55], very little progress has been made toward understanding LS internal circuitry, particularly in regards to parallel circuits working in synergy to promote a given behavior. Thanks to the support of our ERC starting grant, we previously investigated the function of separate cortico-hippocampal projections to LS in regulating distinct motivated behaviors. We also recently applied for a "consolidacion investigadora" grant proposing to investigate the interaction between two antagonistic LS circuits promoting feeding and social interaction respectively. None of the experiments described in these two grants overlap with the ones proposed below.

In this application, we propose to leverage our discovery of a novel PFC to LS circuit suppressing social interaction with familiar animals in order to continue unraveling LS circuitry and function. Although SNP has been used for decades as a hallmark of social memory, the mechanisms and circuits supporting this preference have remained elusive. We demonstrated how a circuit detecting social familiarity acts to suppress interactions with familiar individuals thus allowing the mice to display novelty preference. Our findings stand against the long-standing hypothesis that social novelty detection facilitates social exploration through dopaminergic signaling. Indeed, there are several evidences arguing for a LS circuit facilitating interaction with novel animals and working in synergy with the "familiarity" circuit described above to support SNP. Our study will fill important gaps regarding the neuronal circuits guiding social preferences and will serve as the basis to translational studies investigating LS dysregulation in mouse models of psychiatric disease with impaired SNP.

3. OBJECTIVES, METHODOLOGY AND WORK PLAN

3.1 General and specific aims

The overarching goal of our laboratory is to decipher LS circuits regulating goal-oriented behaviors and more particularly social preferences. We propose to continue our study of LS circuitry by investigating the following points. First, we will investigate how CRH release in rLS can suppress social interaction. We will also ask whether CRH release in LS can regulate other social preferences. Then, we will search for a LS circuit promoting sociability and more specifically interaction with novel conspecifics in order to support SNP. Evidence suggests that dopaminergic modulation of LS facilitates social interaction. Finally, will investigate how both circuits cooperate to support SNP.

We will first investigate how CRH release in rdLS leads to the disinhibition of a specific neuronal population in rLS and how disinhibition of these neurons can suppress social interaction with familiar mice (**Aim 1**). Are CRHR1^{+} neurons in rdLS excited by CRH release and familiar encounters (**Aim 1a**)? Can we use the *Fos*$^{2A\text{-}iCreERT2}$ mouse model to target viral expression and study LS neurons responding to social familiarity ("familiarity" neurons thereafter, **Aim 1b**). Where are familiarity cells projecting and how can they suppress social interaction (**Aim 1c**)? Once we have investigated CRHR1+ and familiarity cells we will be able to link them in one of the first identified intra-LS circuit (**Aim 1d**). Finally, we will ask whether CRH release in rdLS support other social preferences (**Aim 1e**)? Indeed, social

preferences in rodents rely not only on intrinsic factors such as memory but also extrinsic factors, such as sex, strain and kinship.

Second, we will delineate the LS circuit facilitating social interactions with novel mice (**Aim 2**). Previous hypotheses about the mechanisms underlying SNP supposed the existence of circuits promoting interaction with novel mice, probably under control of the rewarding properties of social novelty[56]. In addition, the kin preference toward mothers and siblings exhibited by young mice is disrupted following LS lesion which suggests that the circuits supporting this preference course through LS[18,57]. Very little is known however about the mechanisms supporting the rewarding properties of social cues. The lateral septum, which is heavily modulated by dopamine, vasopressin and oxytocin[19], may act as a hub and also integrate inputs promoting interaction with novel mice in order to regulate social preferences. Liu et al. (2022) showed that silencing dorsal LS (located posterior to rLS) suppresses social approach and facilitates avoidance with novel but not familiar mice. This suggest that dLS contains neurons facilitating social interactions with novel mice. We will identify dLS neurons activating during interaction with a novel mouse and promoting social interaction ("sociability" neurons, **aim 2a**) and will test whether they are modulated by dopamine, vasopressin or oxytocin (**Aim 2b**). Finally, we will establish how "familiarity" and "sociability" neurons regulate social interactions with familiar and novel conspecifics and establish how they cooperate to support SNP (**Aim 2c**).

3.2 Working plan and methodology

Aim 1: How does CRH release in rdLS lead to the disinhibition of "familiarity" neurons in rLS and suppression of social interaction with familiar mice? Our previous results demonstrate that CRH release in rdLS during familiar encounters disinhibit a specific population of rLS neurons which suppress social interactions with familiar mice. In addition, we showed that CRHR1 activation in rLS is necessary for SNP. CRHR1 activation in acute LS slices leads to disinhibition of rLS neurons and familiar encounters lead to the specific activation of a population of rLS neurons ("familiarity" neurons).

Aim 1a: Are CRHR1$^+$ cells in rdLS activated by CRH and familiar encounters in order to support SNP? We will prepare acute slices from the brains of CRHR1-Cre mice crossed with the *Ai9* mice. The *Ai9* mouse line is a TdTomato reporter mouse line so CRHR1 neurons will be labelled in red. We will obtain whole-cell patch-clamp recordings from of CRHR1$^+$ neurons in rLS expressing TdTomato and apply the CRHR1 agonist stressin-1 in current-clamp configuration in order to measure its effect on the cell excitability. We will measure changes in resting membrane potential, input resistance, rheobase and frequency-input curve. Similar studies on other neurons expressing the receptor suggests that activation of the receptor will increase the excitability of the neurons[59].

We will also record from identified CRHR1$^+$ neurons in voltage-clamp configuration and expect to see an inward current upon stressin-1 application[59]. In order to examine the receptor mechanisms underlying the stressin-1 response, we will repeat the application of the agonist in presence of blockers of second messengers in order to confirm the intra-cellular pathways leading to the increased excitability. Specifically, we will repeat the CRH application in presence of tetrodotoxin (TTX), 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f] quinoxaline-7-sulfonamide (NBQX), D-(-)-2-amino-5-phospho-nopentanoic acid (D-AP5), and gabazine. We do not expect these drugs to block the response. We apply the CRHR1 antagonist antalarmin as well as the CRHR2 antagonist antisauvagine-30 and expect the former but not the latter to block the response. Wang et al. found that CRH-dependent excitation of neurons in the cerebellar interpositus nucleus was due to closure of the inward rectifier K$^+$ channel and activation of the HCN1 channel. Therefore, we will test the CRH response in presence of the antagonist ZD7288 and tertiapin-Q.

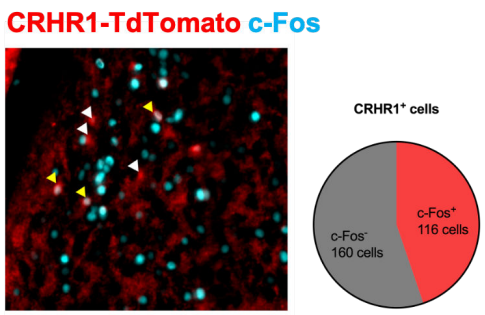

Fig. 2. CRHR1$^+$ neurons in rLS response to social interaction with a familiar conspecific. Left: Immunohistochemical labelling for c-Fos. CRHR1$^+$ neurons express TdTomato. Right: Percentage of CRHR1+ neurons expressing c-Fos (N = 3 mice).

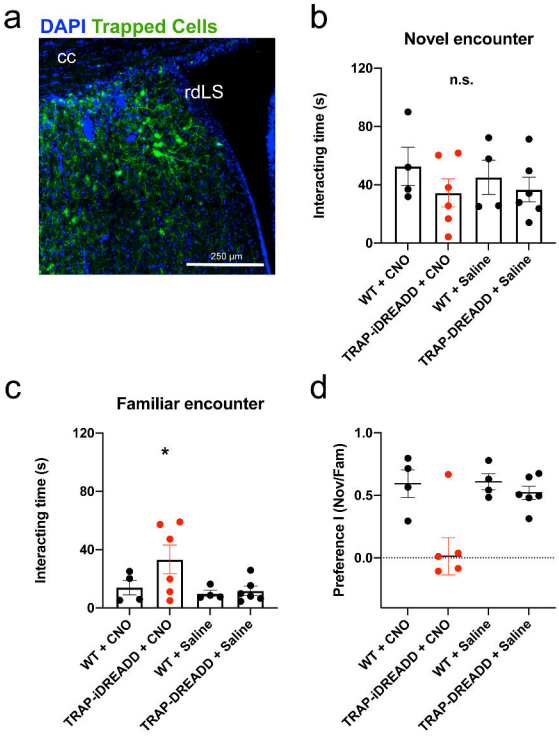

Fig. 3. Silencing "familiarity neurons" increase social interaction with familiar but not novel mice. a. "Familiarity" neurons were trapped using the Fos$^{2A\text{-}iCreERT2}$ mouse line and a Cre-dependent virus expressing GFP was injected in rLS. **b-c.** Inhibitory DREADD is expressed in "familiarity" neurons. Control mice are WT littermates receiving the same treatment. Mice received a CNO or saline injection before being presented with a novel (b) or familiar mice (c). One-way ANOVA $F_{3,16}$ = 2.92, p = 0.03. **d.** Preference index for social novelty vs. familiarity.

Then, we will measure the overlap between CRHR1-TdT$^+$ cells and c-fos$^+$ cells following a familiar encounter. We expect a significant portion of CRHR1$^+$ cells to be activated compared to presentation of a novel mice. Our preliminary results indicate that about 40% of CRHR1$^+$ cells in rLS co-express c-fos following a familiar encounter (Fig. 2).

Finally, we will use chemogenetic in CRHR1-Cre mice to silence LS^{CRHR1} cells. We will inject in rLS a Cre-dependent AAV expressing inhibitory DREADD or just mCherry as a control and perform the repetitive presentation test of familiarization and the 3-chamber test of SNP. We expect familiarization and SNP to be impaired. These results will confirm that CRHR1 cells are key neurons in relaying CRH signaling in LS in order to suppress social interaction with familiar mice. Overall, these results will confirm that: CRH activates CRHR1$^+$ neurons in rdLS, familiar social encounters recruit CRHR1$^+$ neurons and CRHR1$^+$ neurons are necessary to support familiarization and SNP.

Aim 1b: Can we target "familiarity" neurons in LS and modulate familiar encounters?

We will leverage the *Fos$^{2A\text{-}iCreERT2}$* mouse line in order to express Cre selectively in rLS cells responding to social familiarity. First, a Cre-dependent AAV-GFP virus is injected in rLS. One week later, 4-OHT (53 mg/kg) is administrated intra-peritoneally 5 minutes prior to an encounter with a familiar mouse. This triggers the activation and concomitant expression of c-Fos in "familiarity" neurons which allows the inducible Cre (iCreERT2) to be expressed. Due to the presence of 4-OHT, iCreERT2 becomes activated and recombine the Cre-dependent AAV-GFP specifically in the activated rLS cells upon familiarity detection. Two weeks later we can visualize "familiarity cells" (Fig. 3a). We repeated the trapping with a Cre-dependent AAV expressing the inhibitory DREADD in order to silence "familiarity" neurons during social interaction with familiar or novel mice. Interestingly, chemogenetic silencing of these cells increased the duration of social interaction with familiar (Fig. 3c,d) but not novel mice (Fig. 3b,d). Littermates which did not express Cre or DREADD-expressing mice that received saline injection showed no effect. We will repeat this experiment and test the mice during repetitive presentation and 3-chamber test as well.

Aim 1c: Where are familiarity cells projecting and how do they suppress social interaction?

After expressing Cre in "familiarity neurons", we injected a Cre-dependent AAV expressing membranous GFP and synaptophysin-mRuby (synaptotag virus) in order to trace fibers and axon terminals. We found terminals mostly in the lateral hypothalamic area (LHA) but also in the anterior hypothalamic area (AHA), and ventral periaqueductal grey (PAG) to a lesser degree (Fig. 4). All regions have been previously linked to the regulation of social interactions[58,60,61] but it is still unclear how increased inhibition from the" familiarity" neurons in rLS to these regions can suppress social interactions. Selective inhibition of Vglut2$^+$ neurons in dPAG increases social approach[60] which suggest that dPAG neurons suppress social interactions. Similarly, a remarkable increase of attention toward

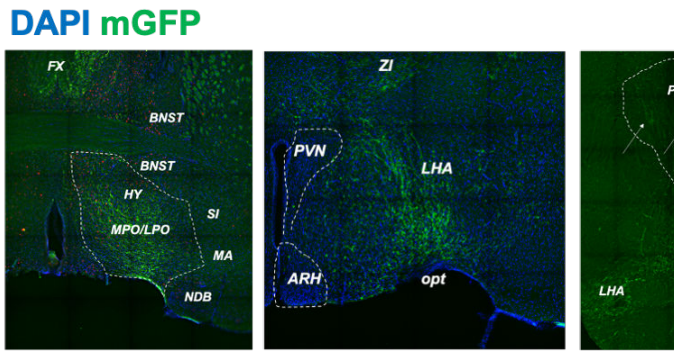

Fig. 4. Projections from the "familiarity" neurons in rLS. Familiarity neurons were trapped using the Fos$^{2A\text{-}iCreERT2}$ mouse line and a Cre-dependent virus expressing mGFP and synaptotagmin-mRuby was injected in rLS. Fibers and terminals can be traced in the medial and lateral preoptic areas (MPO/LPO), lateral hypothalamic area (LHA) and periaqueductal grey (PAG).

the object rather than the female was observed when LHA$^{CaMKII\alpha}$ neurons were activated[62]. Finally, activation of Foxb1^{+} neurons in the parvafox nucleus of the LHA causes social avoidance behavior[58]. Since both LHA/parvafox and PAG seem to suppress social interaction, we can suppose that GABAergic "familiarity" neurons in rLS projecting to these regions do not impinge directly on Foxb1^{+} glutamatergic neurons but rather onto local GABAergic ones.

We will express the terminal-specific silencing opsin eOPN3[63] in "familiarity" neurons and implant optical ferrules above AHA or LHA. We will silence each output during free social interactions with familiar mice, during the repetitive presentation test and during the 3-chamber test to determine which outputs suppress social interaction. In the future, we will focus on post-synaptic neurons and attempt to determine their role during social interactions. These experiments will indicate which downstream region is crucial for our circuit to suppress social interactions with familiar animals. We will also perform dual injections of the mono-synaptic Cre-dependent retrograde tracer Herpes simplex virus expressing mCherry or GFP after trapping the "familiarity" cells in order to determine whether fibers projecting to the AHA or LHA originate from the same familiarity neurons or distinct populations.

Aim 1d: What is the link between CRHR1^{+} and "familiarity" cells?

The simplest hypothesis is that CRHR1^{+} and "familiarity" cells consist of one and only population. However, measuring the overlap between CRHR1-TdT and c-fos neurons following familiar encounter show that only 3% of c-fos cells in rLS expressed CRHR1 (Fig. 2). Since CRHR1 neurons are GABAergic (Fig. 5) and CRHR1^{+} cell excitability is likely increased by CRH[59], the most parsimonious way CRH release can lead to disinhibition of "familiarity" cells calls for a third population of "relay" neurons (which would also be GABAergic as every LS cell[50]). CRH release would activate CRHR1^{+} cells which would increase their inhibition of the "relay" cells. Increased inhibition of the "relay" cells would lead to a decrease of the inhibition they provide onto the "familiarity" cells (Fig. 6). Accessing the "relay" neuron population is challenging but we can express channelrhodopsin in CRHR1^{+} neurons and patch post-synaptic neurons in voltage-clamp configuration, looking for neurons responding with monosynaptic inhibitory post-synaptic current (IPSC).

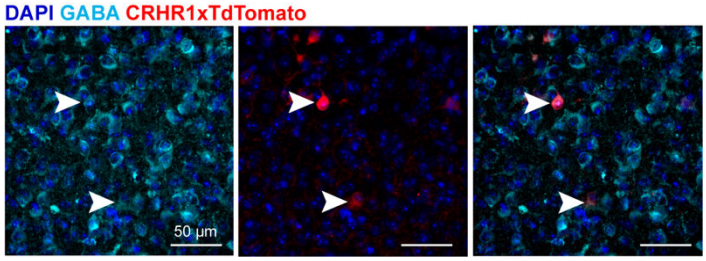

Fig. 5. CRHR1^{+} cells in LS are GABAergic. Immunohistochemical labelling of CRHR1^{+} neurons expressing TdTomato.

Then, we will apply the rabies retrograde monosynaptic tracing technique starting from trapped "familiarity" cells. We will inject a Cre-dependent helper AAV expressing TVA, the G protein and GFP before injecting a pseudotyped rabies virus expressing mCherry. "Familiarity" starter cells will be labelled with GFP and mCherry while pre-synaptic relay neurons will only express mCherry. We will observe the location of these neurons within LS, co-label them for known molecular markers of LS populations (PV, SST, CCK, etc.). Finally, we will use the FACS technique in collaboration with the lab of Dr. Jose-Lopez Atalaya at the institute of Neurosciences (IN) to sort them and sequence their RNA in order to look for the expression of a particular marker.

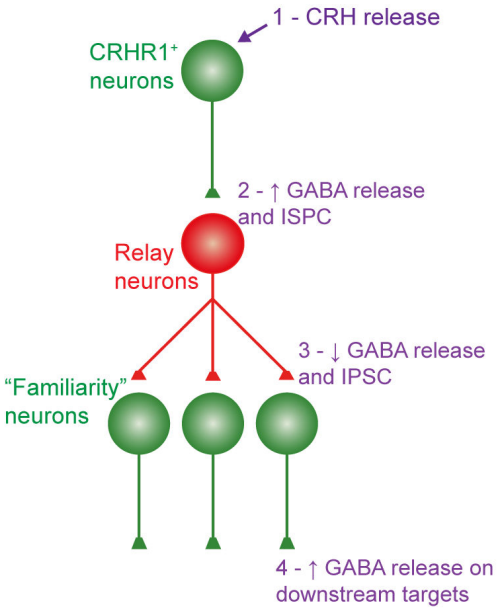

Fig. 6. Putative local LS circuit linking CRHR1⁺ cells to "familiarity" neurons.

Aim 1e: Can CRH release in rLS support other social preferences?

Social preference, the decision to interact with one member of the same species over another, is a key feature of optimizing social interactions. In rodents, social preference relies on both extrinsic factors, such as sex, strain and kinship, and intrinsic ones such as social memory. Here, was ask whether CRH release in rLS from ILA^{CRH} neurons suppress social interactions with other non-preferred conspecifics in order to support other social preference. Rodent's preference for mice from the opposite sex is well-known[7] but the mechanisms responsible for it are unknown. We will perform a sex preference test in mice following knock-down of Crh in ILA. Briefly, male and female C57Bl6 littermates will be placed under pencil cups located at opposite corners of a large area. A male test mice will be introduced in the center and given the choice to interact with both mice during 10 min. Our preliminary results indicate a strong preference for the female. It has also been described that mice prefer to interact with other mice from the same strain so we will repeat the social preference test by placing mice a C57Bl6 mouse under one pencil cup and a Balb/c mouse under the other one.

Anticipated outcomes. We will be able to understand how CRH release in rdLS can suppress social interaction with familiar mice. We will link CRHR1⁺ cells activation to disinhibition of "familiarity" neurons and explain how activation of familiarity neurons suppress social interaction through projection to identified downstream areas. This will conclude our investigation of the PFC to LS to hypothalamus circuit supporting SNP.

Aim 2: Synergistic LS circuits suppressing interaction with familiar mouse and promoting interaction with novel mouse.

Previous hypotheses about the mechanisms underlying social novelty preference supposed the existence of circuits promoting social interactions with novel mice, perhaps under control of the rewarding properties of social novelty. This would be sufficient to explain the SNP and familiarization since the novelty factor decrease upon repetitive presentations. Very little is known however about the mechanisms supporting the rewarding properties of social cues. The lateral habenula, nucleus accumbens, dorsal raphe nucleus and ventral tegmental area modulate social reward[56,64–67], some of them under control of oxytocin[56,64,65].

In addition, the kin preference displayed by young mice toward mothers or siblings[18,57] when CRH expression is not yet mature[47] supposes the existence of other circuits controlling social preference. Numerous studies have shown that kin preference in pups and SNP in adult depends on LS[3,19]. The lateral septum, which is heavily modulated by dopamine, vasopressin and oxytocin[19], may act as a hub and also integrate inputs promoting interaction with novel mice in order to regulate social preferences. Interestingly, Liu et al. (2022) showed that silencing dorsal LS neurons (located posterior to rLS) suppresses social approach and facilitates avoidance with novel but not familiar mice. This confirms the importance of LS to modulate social interactions and suggests that different LS regions can regulate different types of social interactions and may work together to promote SNP.

Aim 2a: Identification of the LS neurons responding to social novelty and promoting social interactions.

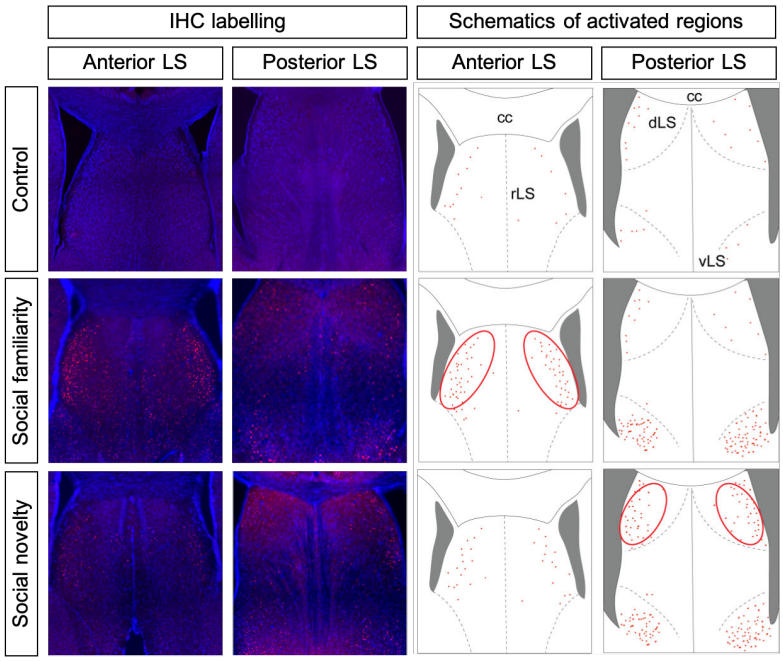

Fig. 8. Familiar and novel social encounters activate different LS regions. c-Fos labelling following no social interaction, novel social interaction and familiar social interaction (left). Schematics on the right depicts activated regions. Red ellipses denote non-overlapping activated regions.

Liu et al. (2022) showed that pyramidal neurons located in the dorsal CA3 region of the hippocampus responds to social interactions and send glutamatergic projections to dLS. This is in keeping with my own results showing that dCA2 pyramidal neurons which respond to social interaction in general[52] and novel social interactions in particular projects[68] to dLS as well. In addition, optogenetic silencing of dLS neurons (located posterior to rLS) suppresses social approach and facilitates avoidance with novel but not familiar mice (Liu et al 2021) which suggests that this region contains a population of neurons facilitating interaction with novel mice but not familiar ones. We plan to identify LS neurons responding to social novelty and facilitating interactions with novel mice ("sociability" neurons). How do dLS neurons respond to social interactions? We will express GCaMP6f in dLS to perform calcium imaging in freely moving mice (Fig. 7). We will focus on the social tuning of each cell. We expect to find non-overlapping populations responding to one or the other social stimulus. We will also present novel and familiar object to the mice as controls.

C-fos labeling in LS following social interaction with novel but not familiar mice demonstrate the existence of a dLS population responding to social novelty (Fig. 8). This contrasts with the population of neurons responding to social familiarity found in rLS and described above (Fig. 8). In order to obtain the molecular profiling of the "sociability" and "familiarity" neurons, we will use an unbiased approach named phosphoTRAP. The goal is to come up molecular markers specific for each population. This method relies on the fact that neural activation results in phosphorylation of ribosomal protein S6 (pS6), which is a structural component of the ribosome. Phosphorylated ribosomes can be captured from mouse brain homogenates, thereby enriching for the mRNA selectively expressed in neurons activated by a stimulus. Once captured the mRNA can then be sequenced to reveal the molecular identity of the activated cells[69]. Following social isolation overnight, mice will be challenged with a novel mouse before labelling LS slices for pS6. As a control, we will use mice presented with a novel object. Our c-fos data suggests specific activation of no-overlapping population within LS, we expect to

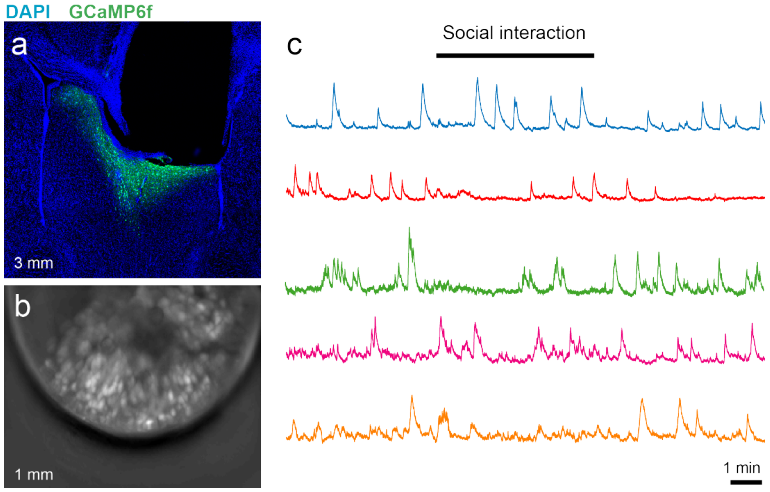

Fig. 7. LS calcium imaging. a. AAV1 syn-GCaMP6f-WPRE-SV40 injection in LS. Note the hole where the Grin lens was implanted. **b.** Field of view through the lens with multiple cells recorded. **c.** Individual cell calcium responses during social interaction.

see similar separated patterns of expression in dLS and rLS respectively. We will then repeat the social presentations on other mice and immunoprecipitate pS6 ribosomes from homogenates of the microdissected LS. We will then sequence this RNA to determine the fold-enrichment for each gene. We will repeat the same approach following presentation of a familiar animal to identify markers of the "familiarity" neurons. As a control, we will use mice presented with a familiar object. Once we have identified specific markers of the novelty and familiarity neurons, we will look for Cre- or FLIP-expressing mouse lines that could be used to target "familiarity" and "sociability" neurons within LS. The goal of this aim to confirm the existence of "sociability" neurons and find some molecular markers to target "familiarity" and "sociability" neurons to avoid using the TRAP technique.

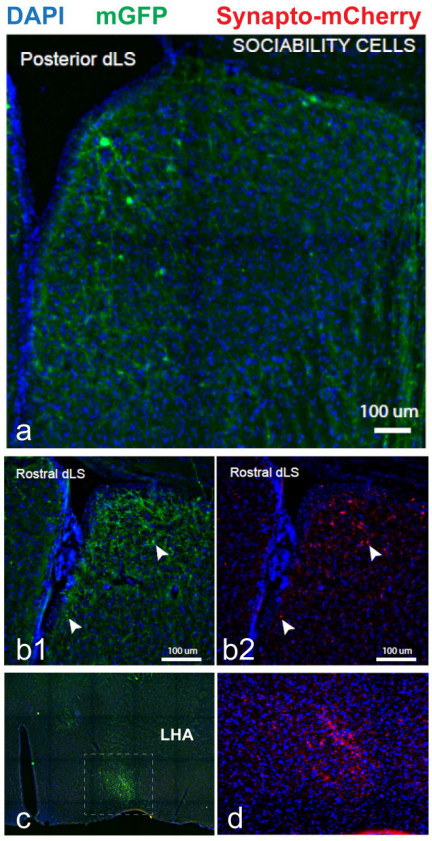

Fig. 9. Trapping of "sociability" cells. a. Sociability cells trapped in posterior dLS**. b-d.** Axon terminals in anterior dLS (b) and parvafox nucleus of the LHA (c-d).

Aim 2b: Are social novelty neurons regulated by dopamine, vasopressin and oxytocin?

LS is known to be heavily neuromodulated[19]. Indeed, one important feature of rodent LS is that it receives extensive peptidergic inputs such as vasopressin (AVP), oxytocin[70–72], corticotropin-releasing factor (CRF)[73,74] and enkephalin[75] as well as cholinergic and monoaminergic inputs such as dopamine and serotonin[76,77]. LS neurons themselves express neuromodulatory peptides such as enkephalin[78,79]. These substances act through membrane receptors expressed by discrete LS neuronal populations[80,81]. Interestingly, these neuromodulatory receptors such as *Drd3*, *Galr1*, *Crhr2* and *Tac1r* are scattered across discrete gene expression domains within LS and their effects on LS function remain mostly unknown. One attractive hypothesis is that the combination of neuromodulatory substances - reflecting the animal's internal states and interoception[82] - may orchestrate the recruitment of specific intra-septal sub-circuits due to the heterogenous expression of their membrane receptors. The role of AVP receptor 1a (AVPR1a) in SNP has been particularly studied. AVP injection in LS[83] of male rats enhances SNP and the deficit of SNP exhibited by male mice lacking the AVP receptor 1a can be rescued using a viral vector injected into LS[84]. This suggests that AVP is released in LS of male rodents following an encounter with a novel conspecific to regulate social recognition[85]. Ex vivo studies in acute slices have proposed a potential mechanism: AVP application on acute septal slices from rats induces long-term potentiation[86,87] and application of a AVPR1a agonist excites LS^{AVPR1a} neurons which, in turn, inhibit LS neurons that do not express this receptor and are otherwise tonically active[88]. This example of LS neurons assembled in series is similar to the internal LS circuit modulated by CRH proposed in Aim 1. In addition, early-life stress applied to mice was shown to reduce the ratio of excitatory to inhibitory inputs (E/I ratio) in a population of $rvLS^{DRD3}$ neurons[81]. These effects were accompanied with long-lasting deficits in social behavior which could be normalized by restoring $rvLS^{DRD3}$ neuron activity [81]. Although the source of inhibitory inputs to $rvLS^{DRD3}$ neurons remains to be established, an intriguing hypothesis is that changes in the E/I ratio could stem from increased lateral inhibition proceeding from neighboring LS neurons. Although the authors targeted the LS population based on its expression of a dopaminergic receptor, they left out the crucial question of DA modulation of these cells.

We will label "sociability" neurons in LS using the $Fos^{2A\text{-}iCreERT2}$ mouse line (Fig. 9) or another line targeting "sociability" neurons (cf. Aim 2a) and co-label for peptide receptors known to be expressed in LS (*Drd3*, *Galr1*, *Crhr1, Crhr2, Avpr1a, Oxtr,* and *Tac1r*) using in situ hybridization. If we observe a high level of co-labelling (>50%), we will perform targeted patch-clamp recordings and apply specific agonist to confirm whether the receptors can modulate the activity of "sociability" neurons. We will

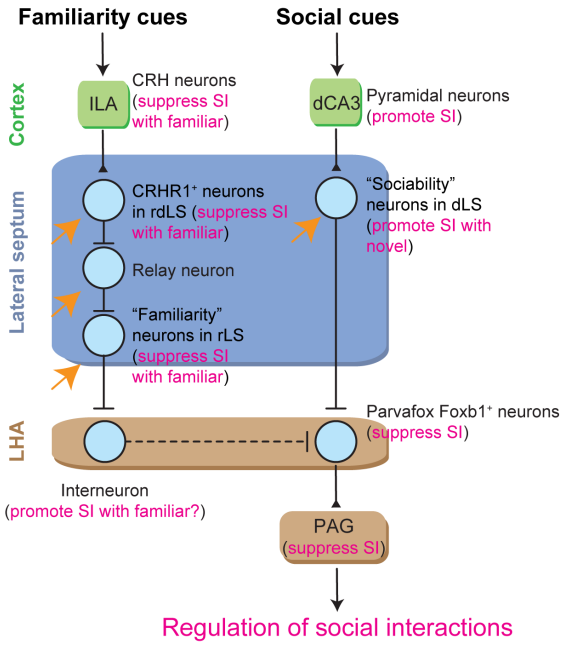

Fig. 10. Putative "familiarity" and "sociability" circuits in LS. Familiarity and sociability neurons receive different social cues to suppress social interactions with familiar or promote interaction with novel mice respectively through regulation of the parvafox nucleus. The orange arrow represents possible neuromodulation from dopamine (DA), vasopressin (AVP) and oxytocin (OXT).

perform the same approach with "familiarity" neurons. We hope this characterization of each population neuromodulation will shed light on how monoamine and peptide release in LS can influence defined LS circuits. This is different to previous approaches starting from the cells expressing specific receptors which lead to confusing results since the same neuromodulator is likely to influence several motivated behaviors[19,73].

Aim 2c: How do familiarity and sociability neurons regulate social interactions and cooperate to support SNP?

Our preliminary results indicate that silencing "familiarity "neurons decrease social interactions with familiar but not novel mice. Is the opposite true when silencing "sociability" neurons? We will express inhibitory DREADD in "sociability" neurons in LS using the *Fos*$^{2A\text{-}iCreERT2}$ mouse and test the mice for direct social interaction with novel and familiar mice. We expect that chemogenetic silencing of novelty neurons will decrease social interaction with novel but not familiar mice, in keeping with Liu et al. (2022). We will also perform the repetitive presentation and 3-chamber tests while silencing "sociability" neurons. We expect familiarization to proceed from a shorter interaction time during the first presentation (first interaction with the novel mouse) and SNP to be impaired. Conversely, we will try to stimulate sociability neurons during social interactions using channelrhodopsin. We expect an increase in social interaction during presentation of novel mice. Liu et al.[58] showed that the activity of dCA3$^{CaMKII\alpha}$ → dLS^{GABA} projection produced signals to suppress PFN^{Foxb1} neuronal activity and enhanced social approach behavior. Conversely, silencing the circuit disinhibited PFN^{Foxb1} neurons and caused social avoidance behavior. This suggests that "sociability" neurons project directly to the parvafox nucleus of the LHA as well as other possible targets (Fig. 10, sociability circuit a). Indeed, our preliminary experiment expressing the anterograde virus synaptotag in "sociability" neurons shows a direct projection to the parvafox nucleus of the LHA (Fig. 9c-d) but also projections to the anterior part of LS where familiarity neurons can be found (Fig. 9b). Similar to our investigation of "familiarity" cells, we will also use and retrograde tracing technique (CtB and herpes simplex virus) to investigate the downstream projections of "sociability" neurons.

We will perform terminal-specific silencing of each output using the eOPN3 opsin in order to determine which ones facilitate social interactions. Overall, we expect "sociability" neurons to target specifically fox1b^{+} neurons in the parvafox nucleus (PFN) meanwhile "familiarity" neurons would target LHA interneurons impinging on PFN^{fox1b} neurons (Fig. 10) given their opposing effect regulating social interactions (Fig. 3d and Luo et al. 2011). Another possibility is that "sociability" neurons synapse onto relay GABAergic vLS neurons which in turn projects to the same neurons than "familiarity" neurons. Such disynaptic LS circuit has been shown previously in the dCA2-dLS-vLS-VMH circuit regulating aggression[52]. Conversely, Luo et al. (2011) described a dCA3 to dLS to VTA circuit where LS fibers project on VTA^{GABA} neurons, inhibiting dopaminergic neurons in the VTA[53]. Whether located in the downstream hypothalamic region or within LS, the addition of an extra "sign-changing" GABAergic neuron would reconcile the opposite effect "sociability" and "familiarity" neuron populations exert on social interactions.

Anticipated outcomes. This second aim will identify the LS circuit responding to social novelty and facilitating social interactions with novel mice ("sociability" neurons). It will also provide some insight into the neuromodulation of "sociability" neurons and their counterpart, the "familiarity" neurons. Finally, it will demonstrate how both circuits work together in order to promote social novelty preference. In the future, we will test whether the "sociability" neurons regulate other social preferences. We will also continue our investigation of circuits regulating social preferences. Specifically, we will ask how defined hypothalamic nuclei combine LS inputs to control social interactions and whether the hypothalamus feedbacks onto the cortical areas projecting to LS.

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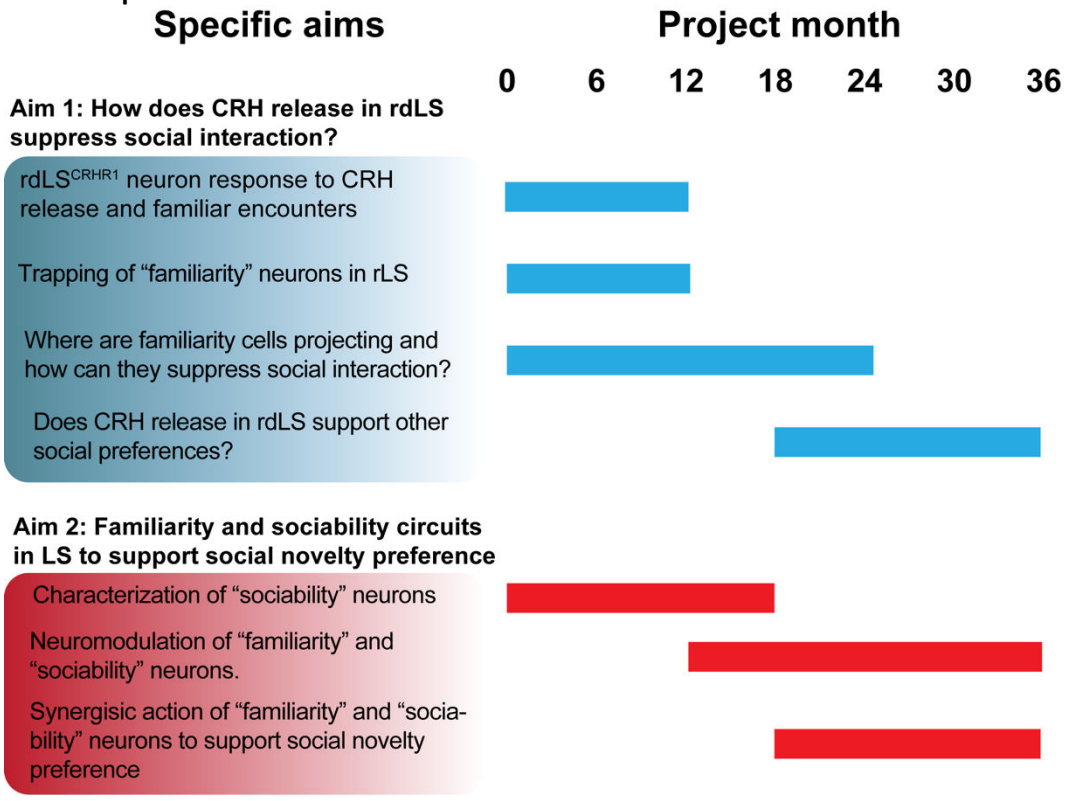
3.3 Work plan and schedule.



3.4 Identification of critical points and contingency plan.

All techniques are routinely used in our lab and we expect no particular difficulties. In this application, we demonstrated that the TRAPPING method worked to capture "sociability" neurons (Fig. 9). We also showed that we can perform calcium imaging in LS successfully (Fig. 7). In addition, we will benefit from my interaction with Denise Cai and Tristan Shuman that pioneered the miniscope as well as with the lab of Amar Sahay which performed the first miniscope imaging of LS. We recently began collaborating with the lab of Raoul Andero in Barcelona which has extensive experience acquiring and

analyzing calcium imaging films. As an alternative to trapping neurons, we could leverage a high-titer AAV1 (>10^{13} vg/mL). At this titer, AAV1 expressing Cre has been reported to "jump" synapses in an anterograde fashion. To test this possibility, we injected a high-titer AAV1 expressing Cre in dCA3 of the Ai9 TdTomato Cre-reporter mouse. we found dLS neurons receiving CA3 inputs expressed TdTomato after Cre expression jumped the dCA3 to dLS synapse. We could use this technique to label "sociability" neurons in LS after double-checking using rabies tracing that familiarity neurons do not receive dCA3 inputs.

Recordings: This project relies on challenging techniques investigating the function of neural circuits. Our previous work demonstrates our experience using such techniques. We have extensive experience using patch-clamp and immunohistochemistry to investigate neural circuits. In addition, we have published extensively using behavioral assay coupled to chemogenetic or optogenetic and already validated the behavioral paradigms we plan on using for this project.

Neuron targeting: Many experiments described here rely on the use of viral injection, which can become a burden and introduce variability. Our group has already tested most injections and verified that they properly expressed the proteins of interest in the desired neuronal cell types. Normalization to the signal at the injection site will help quantify and compare results from different viral injections. The brains of all animals expressing a virus will be collected post-mortem in order to assess the spread of viral expression and whether it correctly targets the region of interest. Following opto- or chemogenetic manipulation of animal's behavior, I will correlate the behavioral change with the spread of viral injection. If necessary, I will resort to multiple injections to cover a larger region. AAVs are prepared by Dr. Cristina Fregola who runs IN vector core and can package any plasmid into various AAV serotypes. Our group also has an ongoing collaboration with Dr. Rachel Neve who runs the Massachusetts General hospital viral vector core. In addition, our group has an ongoing collaboration with Susan Morton at Columbia University who is proficient in making of new antibody.

3.5 Previous results of the team in the theme of the proposal.

During my post-doctorate I published a study in the journal *Nature* as first and corresponding author where I described a new CA2 to LS to hypothalamus circuit regulating social aggression. Using this study as a blueprint, my laboratory began exploring the role of PFC^{CRH} cells projecting to LS. We found that these cells suppress social interaction with familiar but not novel mice in order to support social novelty preference. A detailed description of these results is presented in section 2.2 Current status. A manuscript presenting these results have been uploaded to BiorXiv in March 2022[47] and the manuscript is currently invited for resubmission at the journal *Cell*.

3.6 Human, material and equipment resources available for the execution of the Project.

The Neuroscience institute (IN) in Alicante is a state-of-the-art research center selected as a "SEVERO OCHOA" CENTRE OF EXCELLENCE in 2014 (renewed in 2018 and 2022). The IN has core facilities freely accessible to researchers, which include live cell imaging, cell sorting, cell culture rooms. Importantly, a SPF mouse facility is run directly by the IN, which optimized the maintenance costs and set up a service for in-house embryo cryopreservation and transgenic mouse generation. In addition, the IN has incorporated a functional Magnetic Resonance Imaging (fMRI) core facility (equipped with a 7-Tesla machine for small animals) and it has the most modern modalities of microscopy (super-resolution, light-sheet, etc.), which in combination with in vivo electrophysiology, state-of-the art molecular biology and equipment for animal behavior assessment, provides new tools to further strengthen the research performed at the IN. My own laboratory is supported by several grants, including an ERC starting grant and is well-equipped to conduct the experiment outlined above. We have two vibratomes, two rigs for in vitro patching, 3 miniscopes, 1 fiberphotometry setup, our own Thunder epifluorescent microscope, 1 stereotax with two arms and 2 nano-injector for viral injections and several behavioral setups (elevated plus maze, thermal camera, IR cameras, 2 Any-Maze licenses, blue and green lasers). Besides myself, my laboratory employs 1 post-doc, 3 PhD students, 2 technicians and one lab manager. I am planning to hire a second post-doc to lead this project.

4. EXPECTED IMPACT OF THE RESULTS.

4.1. Expected impact on the generation of scientific-technical knowledge in the thematic area of the proposal.

Social preference, the decision to interact with one member of the same species over another, is a key feature of optimizing social interactions. In rodents, social preference relies on both extrinsic factors, such as sex, strain and kinship, and intrinsic ones, such as the memory of previous encounters, which favors interactions with novel compared to familiar animals (social novelty preference). At present, it is unclear which neuronal circuits guide social preferences and whether such circuits promote social interactions with the preferred individuals or suppress interactions with the non-preferred ones. We recently identified a population of inhibitory neurons in ILA that express the neuropeptide corticotropin-releasing hormone (CRH) and project to the rostro-dorsal region of LS (rdLS). Release of CRH from ILA in rdLS during interactions with familiar mice disinhibits rdLS neurons, thereby suppressing interactions with familiar mice and contributing to social novelty preference. The goal of this project is to understand how CRH release disinhibits the rostral lateral septum and more generally how distinct LS circuits process various cues in order to regulate social preferences. Alteration of the lateral have been described in schizophrenia and bipolar disorder; two psychiatric diseases associated with impaired social interactions. Understanding how the lateral septum regulate social preferences in healthy condition will open new avenues to investigate LS dysregulation in mouse models of psychiatric diseases and leads to the development of new drugs designed to help patients suffering from impaired social cognition.

4.2. Social and economic impact of the expected results.

Most patients afflicted with psychiatric diseases suffer not only from positive symptoms such as hallucinations but also from negative ones such as impaired social cognition are difficulties in setting up priorities. Therefore, understanding how the brain regulates and orchestrate motivated behaviors is crucial to understanding impaired and maladaptive behaviors in disease contexts. The project presented here will investigate how male and female mice regulate social preferences, a key behavior in gregarious species such as the mouse and human. It will also provide some insights into how distinct motivated behaviors such as feeding and social interactions can be orchestrated in order to allow us to select the appropriate behavior. Overall, our research aims to unravel and rescue potential changes occurring in neuropsychiatric disorders associated with altered social interactions.

4.3. Plan for scientific communication and internationalization of the results

The results obtained here will be presented at broad national and international scientific neuroscience conferences such as such as the Spanish Society of Neurosciences meeting (SENC) or the Federation of European Neuroscience Societies meeting (FENS) as well as more specific conference such as the Hippocampus meeting in Taormina or the neural circuits meeting in Cold Spring Harbor. We plan to publish the results in top-tier journals and expect to produce 2 publications from this project. Manuscript will be uploaded on BiorXiv before submission to important journals in our field such as those from *Cell Press* (*Neuron, Cell Reports*), Nature publishing group and societies' journals (*J. Neurosci*). I am reviewing for all these journals. We will also aim at publishing in the top multidisciplinary journals. We will follow Open Access policy, which favors public and quick dissemination of scientific data. Specifically, we will follow the "green path" (making available pre-print formats or depositing the files in public repositories) when the "golden path" (paying to publish Open Access in hybrid journals) is not available. We will diffuse our scientific results through social networks. Internal seminars within our institute also will serve as progress evaluation and dissemination strategy at the host institution. I am usually invited to present my work at 3-4 universities per year which will give me a chance to share our results. The results obtained during this project will also help building collaborative research projects and open new of research lines such as translational research aiming at understanding psychiatric diseases. Finally, this project will contribute to the development of Spanish I+D+i policy through the formation of young investigators at PhD and Postdoc levels.

4.4. Plan for dissemination of the results to the most relevant groups for the theme of the project and to society in general.

We will use the following communication strategies. All press release and communication strategies will be controlled from the institute in order to respect embargo and allow dissemination to wider audiences. The IN maintains various social media outlets and communicates scientific findings to the general public through major news sources. Also, through the IN social networking (Twitter, Facebook and Instagram) helping to keep the general, young public and disease associations informed about news and publications carried out by the IN.

4.5. Transfer plan and valorization of results

It is likely that some data may be susceptible of technology transfer and IPR (intellectual property rights). In particular, identified targets for disease treatment and new animal model of psychiatric diseases. If this is the case, we will immediately inform our tech transfer officers based at CSIC and UMH, both with proven experience of successful transfers to industry and protection of IP. Furthermore, within the context of the Severo Ochoa Excellence award, and supported by the Generalitat Valenciana, the Institute has recently established a Unidad Científica de Innovación Empresarial (UCIE), to facilitate and help in the identification of results susceptible to be transferred and/or protected.

4.6. Summary´s management plan of the planned data

The data collected will be kept in the lab for at least 10 years and shared upon request to the principal investigator. In addition, we will consider uploading the data in an online repository that meets the criteria for digital longevity, implementation of FAIR standards and community support.

4.7. Effects of gender inclusion in the content of the proposal

Male and female mice exhibit social recognition and social novelty preference. We will therefore use mice for both sex during this study and pool the data together if they appear to be similar. However, since social recognition in rodents can be dimorphic (particularly its LS neuromodulation[19]), we will be particularly looking for differences in aim 2e "Are social novelty neurons regulated by dopamine, vasopressin and oxytocin?". We already demonstrated that CRH release from the PFC to LS suppress social interaction in both sexes.

5. JUSTIFICATION OF THE REQUESTED BUDGET: The requested grant total is **300.000€**

Staff recruitment costs (142.948,2€)

Personnel costs will cover the salary of a post-doc who will work full-time for 3 years on this project (47.649,40€/year). They will design and conduct most of the experiments described above. They will be responsible for injecting viruses, perfuse the mice and perform immunohistochemistry and in situ hybridization as well as microscopy imaging. They will also perform chemogenetic, optogenetic and calcium recording experiment in freely moving mice. The PI will supervise the project and conduct patch-clamping experiments. Other members of the team will provide sporadic support. We have also applied for a FPI fellowship. This will allow the post-doc to get help from a PhD student. The PhD student will learn to perform some experiments and will work in tandem with the postdoc in order to complete this project.

Implementation and operating costs (133.200€)

-Equipment (27.500€): The experiments proposed in the project require the purchase of some specific laboratory equipment. We will purchase 1 high-end computer workstations to process calcium recordings (aim 2a, 5.000€). We will also need to purchase a 532 nm laser to optimally stimulate the new eOPN3 opsin (aim 1c and 2c, 8.000€). For patching we will need a new camera able to perform epifluorescence imaging (pco.panda 4.2, aim 1a, 1b, 2c, 9.000€).

-Consumables, materials and animals (106.552€): We will need to cover the costs of mouse line maintenance and genotyping (30.000 €). We will also require to purchase reagents and kits, antibodies and probes to perform immunohistochemistry, in situ hybridization (25.000€). We will also need

purchasing various receptor agonists and antagonists (aim 1a and 2b, 6.552€) as well as several AAVs (10.000€). For miniscope experiments we will need to purchase Grin lenses and baseplates (aim 2a, 15.000€) and a care package for our N'Vista miniscope (aim 2a, 15.000 €). Finally, we will need to cover the cost of FACS sorting in our institute (aim 1d, 5.000€) and sequencing in external facilities which can be quite high (aim 1d, 20.000€).

-Travel cost (10.000€): The recent pandemic cruelly reminded us of the importance to attend scientific meetings. I am planning to attend to one national (SENC, SEDB) and one international (SfN, FENS) meetings with the postdoc and PhD Student so they can present their results, obtain valuable feedback and build their network around the end of the project.

-Publication cost (10.000€): We will follow the "Open Access" policy, which favors public and quick dissemination of scientific data. We expect to publish 2 articles describing the results of this project which will be published in high-impact factor journals. Top-tier hybrid journals currently request extremely high article processing charges (up to 12.000€ for Nature Neuroscience) which is regrettable and a clear abuse of the "Golden path" publication policy. However, given the importance of publishing in these journals for a young researcher we have no other choice but to budget a significant amount for publications.

-Certificate of Financial Statement (3.000€): According to the guidelines we have allocated 1% of the project costs for external audit of our project.

6. TRAINING CAPACITY

6.1 Training program in the context of the proposed project

Our institute (IN) enrolls PhD student within the neuroscience PhD program of the Miguel Hernandez University. Indeed, the IN is a joint research center of the Consejo Superior de Investigaciones Científicas (CSIC) and the Universidad Miguel Hernández of Elche (UMH). In addition, the IN has been accredited "*Center of Excellence Severo Ochoa*" in 2014 which was renewed in 2018. It offers the perfect environment for young scientists to be trained. Through the UMH, the IN offers training at master and PhD level. Students selected from all over the world join the master program and perform their master thesis in the laboratory they select. I typically choose my PhD student this way since the year-long internship allows them to learn the skills they will need during their PhD. It also gives ample time to make sure the laboratory is a good fit for them. Within the IN, students work closely with PI and post-docs. This program is complemented by a wide range of weekly seminars given by leading international researchers, different courses about scientific writing and workshops for career development. Additionally, students in the lab typically take 1 extra-curricular course every year such as the Cajal courses. For this project, I will enroll the student to learn more about calcium imaging through dedicated courses (Cajal course organized by Inscopix in Bordeaux) or through a short stay in the lab of our collaborator, Raul Andero Galli at The Universidad Autonoma de Barcelona. Dr. Andero is an expert in acquiring and analyzing films of calcium activities. I will also suggest to the student to follow our in-house python class since this language is commonly used in neuroscience. For example, we are currently using code written in Python in order to perform unbiased analysis of the mice' behavior after tracking them suing DeepLabCut. Through my direct mentoring and the one of the post-doc which will lead this project (Noelia de Reyes Leon), our PhD student will quickly become independent and able to contribute significantly to this project.

6.2 Thesis completed or ongoing

6.2.1 Ongoing PhD thesis

· Helena Bortolozzo (2020-2024): AVP release in LS regulates social aggression.

· Paula Sierra Diaz (2021-2025): Dysregulation of LS in a mouse model of schizophrenia.

Publication: Corticotropin-releasing hormone signaling from prefrontal cortex to lateral septum supports social novelty preference. Noelia Sofia de León Reyes, Paula Sierra Díaz, Ramon Nogueira, Antonia Ruiz-Pino, Yuki Nomura, Christopher de Solis, Jay Schulkin, Arun Asok, Félix Leroy. *BioRxiv* 2022.03.15.484224; doi: 10.1101/2022.03.15.484224

· Lucia Illescas (2022-2026): TRMP8 neurons in LS control thermoregulation (FPI from the Severo Ochoa: PRE2021-100185).

6.2.2 Former TFM students mentored
2022: Feimeng Wu, master student, Institut National d'Agronomie, Paris.
2022: Sidonie Bourgoin, master student, Ecole Normale Supérieure, Saclay.
2021 – 2022: Auriane Gerbelot-Barillon, master student, Ecole Normale Supérieure, Saclay.
2021 – 2022: Paula Sierra Diaz, master student UMH (Impaired calretinin and parvalbumin-expressing neurons in the septum of a mouse model of schizophrenia).

Prior to 2020, I supervised the following rotation and PhD students during my postdoctoral stay in the laboratory of Steve Siegelbaum.
2018 – 2021: Olivia Lofaro, rotation student, Columbia University.
2018 – 2019: Shivani Bigler, rotation student, Columbia University.
2017 – 2019: Lara Boyle, PhD student, Columbia University.
2016 – 2018: Jung Park, rotation student, Columbia University.
2015 – 2018: Torcato Meira, PhD student, Columbia University.
2014 – 2016: David Brann, undergraduate, Columbia University.

Publications with my mentees (underlined):
· Corticotropin-releasing hormone signaling from prefrontal cortex to lateral septum supports social novelty preference. Noelia Sofia de León Reyes, Paula Sierra Díaz, Ramon Nogueira, Antonia Ruiz-Pino, Yuki Nomura, Christopher de Solis, Jay Schulkin, Arun Asok, Félix Leroy. *BioRxiv* 2022.03.15.484224; doi: 10.1101/2022.03.15.484224
· Enkephalin release from VIP interneurons in the hippocampal CA2/3a region mediates heterosynaptic plasticity and social memory. Leroy F, de Solis CA, Boyle LM, Bock Tobias, Lofaro OM, Buss EW, Asok A, Kandel ER, Siegelbaum SA. *Molecular Psychiatry* 2022 Jun;27(6):2879-2900.
· A circuit from hippocampal CA2 to lateral septum disinhibits social aggression. Leroy F, Park J, Asok A, Brann DH, Meira T, Boyle LM, Buss EW, Kandel ER and Siegelbaum SA. *Nature* 2018 Dec 5; 564(7735):213-218.
· A hippocampal circuit linking dorsal CA2 to ventral CA1 critical for social memory dynamics. Meira T, Leroy F, Buss EW, Park J and Siegelbaum SA. *Nature Communication* 2018 Oct 9; 9(1):4163. PMID: 30301899

6.3 Description of the scientific development of former trainees.
· David Brann graduated from Harvard University. While in the lab of Robert Datta, he published several articles in prestigious journals (Cell, Nature etc.). He is currently doing his post-doc.
· Jung Park completed his PhD at Columbia University in the lab of Dr. Randy Bruno. He is currently performing his post-doc.
· Lara Boyle completed her PhD at Columbia University in the lab of Dr. Steve Siegelbaum. As a MD-PhD student she is currently completing her medical training.
· Torcato Meira went back to Portugal where he finished his medical residency. He's quickly becoming a rising star MD-PhD and recently published as a first author in the New England Journal of Medicine.
· Olivia Lofaro and Shivani Bigler are currently completing their PhDs in the lab of Steven Siegelbaum at Columbia University.

6.4 Scientific context of the group
In the Fall of 2020, I joined the Instituto de Neurociencias de Alicante (the largest Spanish neuroscience institute managed by the Consejo Superior de Investigaciones Cientificas and the Universidad Miguel Hernández) as Principal Investigator. There, I am leading the cognition and social interactions laboratory where we investigate how social cognition (past experiences and decisions) prioritize, determine and calibrate innate social behaviors. My group consists of 1 lab manager, 1 personal assistant, 1 technician, 3 PhD Students and 1 postdoc. My research is supported by the European Research Council, the Generalitat Valenciana and the Brain and Behavior Foundation.

The PhD student selected for this project will have access to important financial and technical resources within the lab (ERC strating grant to the PI) and through the numerous viral cores of the IN (SPF mouse facility, behavioral facility, multiple stereotaxes for viral injection and implants). We hold weekly lab meeting during which the student will be able to present its results, receives feedback on his project but also learn about the other projects ongoing in the lab. Our in-house PhD program co-organized with the UMH offers several classes throughout the year. Each PhD student selects a mentor amongst the other PI of the institute which they can refer to in case of conflict with their supervisor. We also have several PIs directly involved in the PhD program that can help students with any issues. In addition, the student will interact with other laboratories expert in the acquisition and analysis of calcium imaging data (laboratory of Raoul Andero Garcia in Barcelona, Center for computational neuroscience at Columbia University with Larry Abbot, Stefano Fusi). Should the FPI grant conceded, we will announce the position on social media, on our website (felix-leroy.com) and diffuse it through my personal network in EU and US universities as well as institutional social media outlets (IN, CSIC and UMH). Finally, we will post the call on some of the most visited websites for post neuroscience jobs (https://www.fens.org/careers/job-market and https://www.senc.es/empleo/).

7. SPECIFIC CONDITIONS FOR THE EXECUTION OF THE PROJECT

6.1 Ethical considerations

Investigating the role of LS in regulating motivated behaviors requires the use of live animals with a septum and all experiments proposed in this grant will be performed in WT and transgenic mice which will enable precise targeting of neuronal populations. This animal model is adequate for the aims of the current proposal and is regularly used to study cognitive processes such as decision-making or learning on one hand and motivated behavior on the other hand. The proposal therefore involves research on vertebrate animals. It also involves genetically modified laboratory animals and viruses. Experiments will be performed in accordance with the European Communities Council Directive of 24 November 1986 and the European Directive 2010/63/EU on the Protection of Animals used for Scientific Purposes. In addition, experimental procedures will follow the recommendations of the UMH and CSIC ethics committee. The number of mice bred and then used will be minimized through tight management of breeding and use of the same mouse for multiple procedures when possible. We used G-power to perform a power analysis and calculated the number of mice needed for each experiment. Overall, we calculated that this project would require using 368 mice over 2 years. In addition, every effort will be taken to reduce animal suffering and distress (use of analgesic and anesthetic whenever painful procedure cannot be avoided). Through frequent discussions with our in-house veterinarians, we will keep informed of new techniques refining our methods. For all these reasons, the project proposed here is in accordance with the three Rs: Replacement, Reduction and Refinement.

6.2 Facilities

The IN holds a SPF mouse facility with optimized maintenance costs and services for in-house embryo cryopreservation and transgenic mouse generation. Several measures are in place to ensure compliance with required ethical principles. Both, the CSIC and the IN are supporting the European Directive 2010/63/UE on the protection of animals used for scientific purposes and have joined to the agreement on Openness on Animal Research, promoted from the Federation of Scientific Societies in Spain (COSCE), with the collaboration of the European Association of Research Animals (EARA). Animals will be housed under specific pathogen-free (SPF) conditions at the RMG (genetically modified mice) animal house (REA Code: SEA-RMG 03 119 000 1001, A/ES/13/I-26). The AAV viral vectors injected in the mice are considered biosafety level 1.

6.3 Authorizations

I hold an animal research certificate for proceedings a, b, c and d. As of now, 5 members of the laboratory also holds certificates a, b and c. Procedures similar to the ones described in this project have already been reviewed positively by the IN internal ethics committee, the UMH office for responsible investigation and the CSIC ethics and biosafety committees. We expect to receive the authorization to perform the experiments described in this proposal before its start.