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Project Proposal template

Neural representation of social information in prefrontal and somatosensory cortical areas of normal versus socially amnesic mice

Version 1

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1. EXCELLENCE (4 pages max)

1.1. Pre-proposal's context, positioning and objective(s)

Neural representation of social information in two cortical areas of normal versus socially amnesic mice

Context

Social interactions underlie various beneficial behaviors such as cooperation, group coordination, mating and protection. Most of these behaviors rely on the ability to form social memories, i.e., to remember familiar conspecifics, a process that can be affected in autistic syndromes.

Social interactions and social memory can be experimentally assessed in rodents using well-established behavioral paradigms (*1*). In the sociability task, a test mouse is placed in an arena containing two small cages—one housing a stimulus mouse and the other an inanimate object. Typically, socially normal mice spend significantly more time investigating the conspecific than the object. In the social memory task, both cages contain a mouse—one familiar, the other novel. Normal mice preferentially investigate the novel mouse, demonstrating social curiosity and the ability to distinguish familiar from unfamiliar individuals.

A central question in systems neuroscience, including in the study of social memory, concerns how individual neurons and neuronal assemblies encode specific behaviors in both time and space. Individual neurons can encode behavior by exhibiting selective activity during the given behavior (*1*, *2*). Others display mixed selectivity, firing during multiple behaviors (*3*, *4*), while some exhibit anti-correlated. At the population level, advanced computational techniques such as support vector machines (SVMs), Bayesian decoders, and linear classifiers allow the identification of cell assemblies involved in specific behaviors (*4*). These population analyses consider behavior-selective, mixed-selectivity, and non-selective neurons, all of which being able to contribute to the encoding of social interactions and memory at the cell population level (*3*, *4*, *6*).

Despite significant progress, several fundamental questions about social memory remain unanswered. While hippocampal subregions are clearly implicated (*1*, *7*), it remains unclear which downstream cortical areas might contribute to social memory formation, encoding, and retrieval, and how their neuronal subtypes participate in these processes (*8–11*). Among these regions, the medial prefrontal cortex (mPFC) has been the most extensively studied, yet its precise single-cell and population-level encoding mechanisms remain poorly characterized (*8–11*). Furthermore, the influence of familiarization on mPFC coding is still largely unexplored, leaving critical gaps in our understanding of the cortical regulation of social memory.

Other pressing questions include the stability of these cortical representations of conspecific identity across time and contexts. Do the same neurons and circuits consistently encode a given conspecific, or does this representation evolve with time and changing social contexts? Furthermore, how does social coding in cortical areas vary with factors such as familiarity and sex? Answering these questions is essential for uncovering fundamental principles of social cognition and its neural underpinnings. Finally, most cortical studies are constrained by limited neuronal recordings, making it difficult to achieve robust single-cell and population-level analyses.

Finally, other cortical regions beyond the mPFC may play a role in social memory, yet their contributions remain largely unknown. In particular, data in humans (*12*, *13*) and rodents (*14*, *15*) suggest that the somatosensory cortex may be involved in regulating and encoding social interactions and memory, an avenue that has yet to be fully explored.

Antoine de Chevigny, the principal investigator of this proposal, recently identified Neurod2 as a novel autism-associated gene expressed in cortical neurons (*16*) (Fig. 1). Mice lacking Neurod2, which serve as a model for the disorder, exhibit social amnesia (*16*), yet the underlying neural mechanisms remain unknown.

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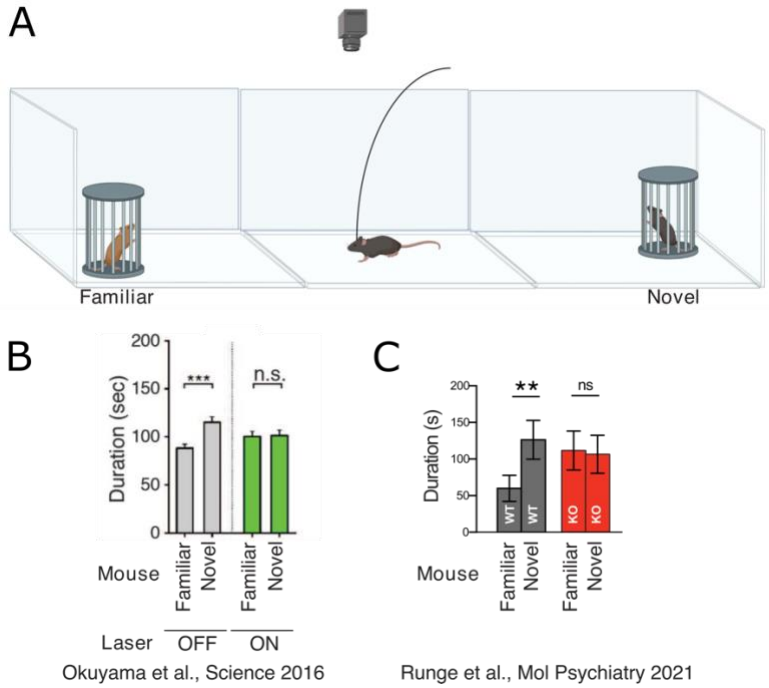

Figure 1: Neurod2 KO mice display social amnesia. (A) The subject mouse can choose to investigate familiar vs novel conspecific. (B) Okuyama et al. found that optogenetic inhibition of a forebrain region, i.e., the vCA1 inhibits the preference for the novel conspecific, a proxy for effective social memory *(1)*. (C) We confirmed that WT mice have social memory as they prefer investigating the novel partner. However, Neurod2 KO mice lose social preference, indicating social amnesia *(16)*.

Positioning and objective

In adult mice, our objectives are to:

1. Determine how excitatory neuron ensembles in the medial prefrontal cortex (mPFC) encode social interactions and social memory at both the single-cell and population levels.

2. Investigate whether, and to what extent, excitatory neurons in the somatosensory cortex encode social information.

3. Examine whether and how social coding in these two cortical areas is altered in Neurod2 KO mice, a model of social amnesia.

To rigorously address these questions, we require a dataset with a larger number of mice, a greater number of recorded neurons per mouse, and more recording sessions than previous studies (*1*). By "recording," we refer to the simultaneous collection of behavioral interaction data and neural activity measurements.

Over the past two years, we have developed and implemented the experimental framework, generating an extensive dataset by combining INSCOPIX microendoscopic calcium imaging with behavioral recordings in wild-type (WT) mice. This approach has enabled us to track neuronal activity in both the prefrontal and somatosensory cortices as mice engage in sociability and social memory tasks. Our dataset includes 7 WT mice per cortical area, with at least 200 recorded neurons per mouse and 18 trials conducted over an 8-day period, during which each mouse interacted with six different partners. We have successfully collected, processed, and integrated large-scale neuronal and behavioral data for WT mice.

We are applying to the SCHADOC program to recruit a PhD student who will:

1. collaborate with our in-house full-time research to replicate the WT experiment in Neurod2 KO mice, a model of social amnesia.

2. analyse datasets to determine how social recognition is encoded in the two cortical areas, how it is modulated by familiarization, and how it evolves over time and with repeated conspecific exposures. This analysis will involve a detailed investigation of both single-neuron contributions and population-level encoding, using supervised and unsupervised machine learning approaches.

Methodology

We will begin by providing a brief overview of the miniature microscope setup (Fig. 2) and the preprocessing pipelines (Fig. 4). While these components have already been implemented and utilized to record WT mice, they will be reused for the wet lab phase of the project (experiments on the Neurod2 KO mice).

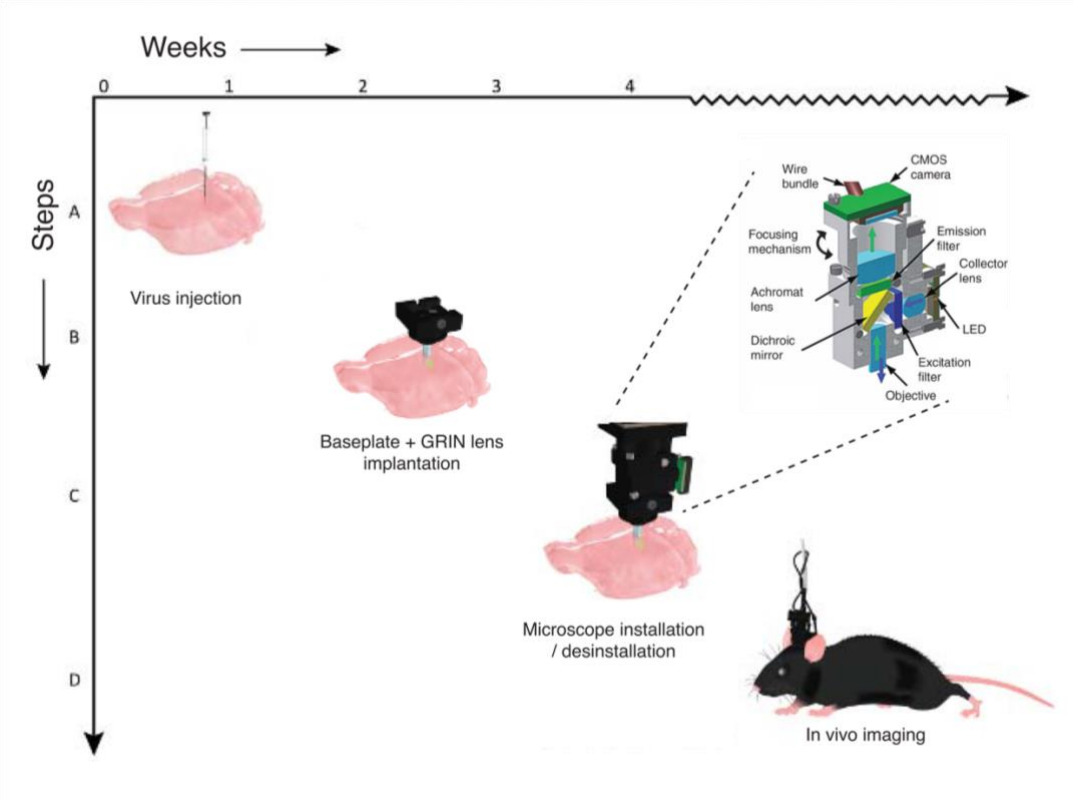

Figure 2: Miniature microscope set-up. The nVoke2 miniscope is a lightweight (2 g) one-photon microscope that can be easily clipped and unclipped from a baseplate affixed to the mouse's head. This baseplate is connected to a gradient refractive index (GRIN) lens, with its tip implanted 200 μm above the target imaging region—in this case, the somatosensory cortex. Imaging is achieved using a blue LED for excitation and a CMOS camera for detection. Below, we outline the key surgical steps involved in the implantation process.

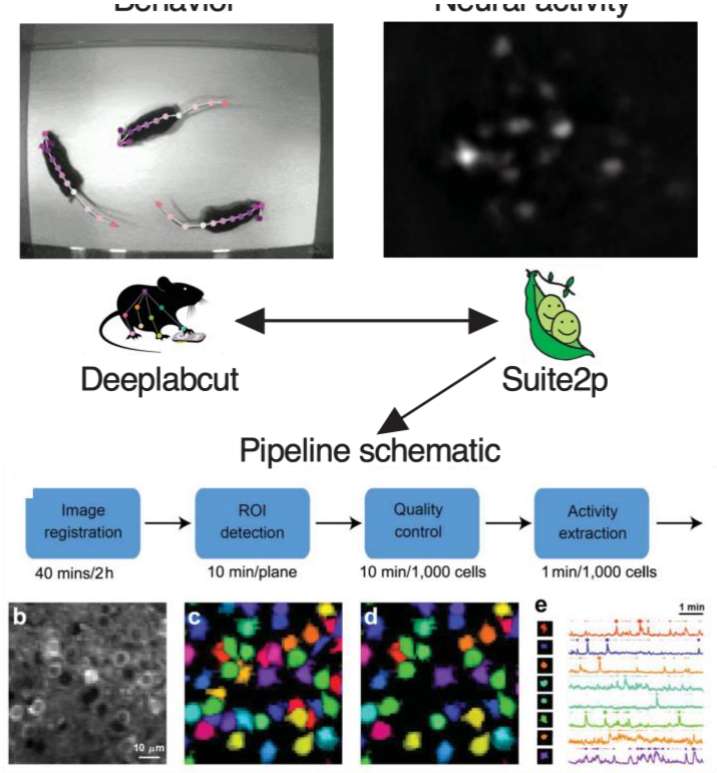

Figure 3: Preprocessing pipeline. Mouse behavior is recorded while mpfc or somatosensory cortex activity is imaged. Behavior annotation and calcium traces extraction feed an analysis pipeline that aims at correlating social interactions and cell assembly activity.

Description of the activity (see 3. Implementation for more details):

WP1: The student will assist our engineer in performing calcium indicator injections, miniscope implantations and recording both neural activity and social behavior during the social discrimination task of neurod2 ko mice (WT mice have already been recorded).

WP2: Encoding of social interaction with familiar vs novel conspecifics will be analyzed in greater detail than ever done before, at both individual cell (*1*) and population levels (*4*, *6*), in both WT and Neurod2 KO amnesic mice. We aim at designing computational models to predict the social partner based on neural activity recording in mPFC and/or somatosensory cortex. The models will help us to determine the relevant alterations in number and/or spatio-temporal dynamics of cells that encode social investigation of a given individual in Neurod2 KO mice.

Multiple computational and mathematical models will be developed for different tasks. Machine learning algorithms will be developed i) to analyze the datasets to cluster different behaviors in an automatic fashion and ii) to put in relationship the recording of the neurons and the recordings of the behaviors. These machine learning algorithms will be novel and will enable the modelling of mouse behavior in relation with neuronal activity.

1.2. Interdisciplanary and intersectoral dimension of the project

Complementarity of the partners

The expertise of both partners is highly complementary for the successful execution of this project. Antoine de Chevigny specializes in the wet-lab aspects, while Lorenzo Fontolan brings expertise in the dry-lab components. Both partners share a strong interest in social cognition, yet approach it from distinct but complementary disciplines.

Antoine de Chevigny is a neuroscientist specializing in neurodevelopment, synaptic plasticity, mouse behavior and neurodevelopmental disorders (*17–19*, *16*, *20*). His research focuses on the molecular and cellular mechanisms underlying neuronal circuit development, particularly in models of pathologies associated with cognitive and social deficits. He is known for his discovery that the loss of function of NeuroD2 in the cortex induces social amnesia (*16*), a finding he extended to identify autistic patients with similar NeuroD2 loss-of-function mutations, which were shown to be causative. This breakthrough sparked his interest in exploring the role of the cortex in the representation and encoding of social behaviors, particularly social memory, at the root of this proposal.

Lorenzo Fontolan is an expert in computational neuroscience, specializing in neural dynamics, network modeling, and data analysis of large-scale neural recordings (*21–23*). His research focuses on understanding circuit mechanisms underlying cognitive functions, particularly in sensory processing and decision-making. He applies advanced statistical and machine learning methods to analyze electrophysiological and imaging data, bridging theoretical models with experimental neuroscience.

Organisation of fellow's activities and hosting arrangements

The SCHADOC fellow will have two primary responsibilities:

1. Experimental Data Collection
The fellow will collaborate with Antoine de Chevigny and a full-time research engineer to simultaneously record social behavior and neural activity in the *Neurod2* conditional mouse model of social amnesia. This will involve working with approximately seven mice per cortical area (mPFC and somatosensory cortex). The fellow will receive training from the research engineer and will primarily focus on data acquisition, with minimal involvement in surgical procedures—unless they wish to develop this skill.

2. Data Analysis
Under the supervision of Lorenzo Fontolan, the fellow will analyze data from both wild-type (WT) mice (already available) and *Neurod2* KO mice (collected in point 1). Antoine de Chevigny will provide scientific guidance on the social neuroscience aspects of the project but will not be directly involved in coding.

The fellow's workload will be distributed approximately as follows: 30% wet lab work (data acquisition) and 70% dry lab tasks (data analysis).

Intersectoral dimension of the project

The non-academic partner for this project is Aquineuro, a Bordeaux-based company specializing in training, software development, and services in systems neuroscience. Aquineuro has extensive expertise in recording physiological data in freely moving animals, including electrophysiology, optogenetics, and photometry, as well as in behavioral data acquisition and analysis (*24*).

Through specialized training and collaborative exchanges, Aquineuro will provide valuable methodological support to the fellow, particularly in the analysis phase of the project. This partnership will ensure access to cutting-edge

techniques and foster a dynamic exchange of expertise, strengthening the project's analytical depth and translational potential. Additionally, it will expose the fellow to industry-oriented research, broadening career perspectives beyond academia.

2. IMPACT (2 pages max)

2.1. Expected impact of the project on the candidate's career

Before outlining how this project will benefit the Early Stage Researcher (ESR), it is important to clarify the profile we are seeking. We aim to recruit a highly motivated and talented ESR with a strong interest in machine learning, quantitative analyses, and the neural bases of cognition and neurodevelopmental disorders. While prior experience in Python programming is not mandatory, it would be a valuable asset.
Although the ESR will primarily focus on data analysis, we strongly encourage their involvement—at least partially—in wet lab experiments. This hands-on experience will provide a broader understanding of neuroscience, ensuring that their analytical work remains grounded in biological reality.

Why this project will have a strong impact on the ESR's career

1) The project's scientific ambition
This project offers an exceptional training opportunity due to its ambitious scope:

- Advancing statistical power and reliability in the analysis of neural activity and memory correlations by significantly increasing both the number of recorded neurons and the volume of behavioral data—pushing beyond current standards in the literature.
- Exploring a novel brain region—the somatosensory cortex—in the context of social coding, a largely uncharted territory in neuroscience.
- Investigating the neural basis of social amnesia in Neurod2-deficient mice, a model relevant to autism spectrum disorders, thereby contributing to our understanding of neurodevelopmental conditions.

2) A strong, interdisciplinary, and internationally recognized supervisory team

The ESR will benefit from the expertise, international recognition, and mentorship skills of leading scientists:

Expertise:

- Antoine de Chevigny's team brings strong expertise in neurodevelopment and disease mechanisms, along with proficiency in using INSCOPIX for simultaneous measurements of behavior and neural activity.
- Lorenzo Fontolan provides critical knowledge in complex neural data analysis, including Artificial Neural Networks and machine learning approaches.
- Léo Guignard's team, our AMU collaborator, will develop cutting-edge tools for automated quantitative analysis of calcium imaging data, leveraging computer vision techniques applied to neural and behavioral image analysis.

International Recognition:

- The co-PIs have published in top-tier journals, attesting to their leadership in neuroscience:
- Antoine de Chevigny: Nature Neuroscience (2x), Molecular Psychiatry (1x), etc.
- Lorenzo Fontolan: Nature (1x), Nature Neuroscience (2x), etc.
- Léo Guignard: Science (2x), Cell (1x), etc.

Mentorship:

- Antoine de Chevigny (46 years old) has successfully trained five PhD students, all of whom have pursued successful careers (industry, Allen Institute, Kenneth Harris's lab at UCL etc..) .
- Lorenzo Fontolan is a young and dynamic group leader, with an outstanding scientific track record and a promising research trajectory.
- Léo Guignard has a similar emerging leadership profile in the field of computational neuroscience.

Conclusion

With a highly ambitious project and strong mentorship in both experimental and computational neuroscience, the ESR will be exposed to state-of-the-art techniques, rigorous scientific training, and interdisciplinary collaboration. This combination will provide an exceptional foundation for a successful career in systems neuroscience, data science, or neurotechnology.

2.2. Expected impact for the thematic axis

The project has a clear expected impact on the health and well-being thematic axis of Schadoc. By deciphering the neural code underlying social interaction and memory representation in the mammalian cortex, it aims to provide new insights into human social behavior and potential treatments for disorders affecting social interactions. This could be achieved, for instance, by modulating the activity or connectivity of identified social neurons or neural assemblies.

Like most strong research endeavors, if successful, this project will generate more new questions than definitive answers—and that is precisely what we hope for. We envision that these findings will open exciting new avenues of research, which we at AMU are uniquely positioned to explore through our collaborations and expertise.

2.3. Dissemination, exploitation and communication activities planned

Our dissemination strategy follows our strong commitment to open science. We will ensure that both we and, as much as possible, the student involved in the project, actively share the results through multiple channels:

- Within the institute: Through data clubs and lab meetings.
- At conferences: By presenting findings at relevant scientific meetings.
- Preprints and publications: We will upload the manuscript to bioRxiv and submit it to an appropriate journal. We will ensure the paper is open access and actively promote it, for instance, through News & Views articles by other researchers.
- Public outreach: We will encourage the ESR to present the project at public science events focused on neuroscience communication, such as Pint of Science and La Semaine du Cerveau.
- General-audience article: We plan to write an article on social coding for The Conversation, an online media platform that has gained popularity in recent years for its ability to combine scientific rigor with a journalistic style. As an open-access platform with an international network, The Conversation provides excellent visibility and engagement with the general public.

3. IMPLEMENTATION (2 pages max)

3.1. Work plan

As mentioned in Part 1, the PhD student will undertake two primary work packages during the course of their thesis:
WP1: Collaborate with our in-house, full-time research engineer to replicate the wild-type (WT) experiment in NeuroD2 knockout (KO) mice, a model of social amnesia.
WP2: Analyze data to determine whether and how social recognition is encoded in the two cortical areas examined, in adult WT and NeuroD2 KO mice.

WP1: recording behavior and mPFC/somatosensory neural activity in Neurod2 KO mice
The student will work closely with Antoine de Chevigny and the team's engineer, Emmanuelle Buhler (INSERM), to carry out neural activity and behavior recordings in NeuroD2 KO mice. While the student will have the opportunity to assist in surgeries for GCaMP virus injections and Inscopix baseplate implantation (gaining valuable experience), these tasks will not be their primary responsibility in WP1. The main focus will be assisting with the recording sessions on days 1 and 8 of the experiment (Fig. 4).

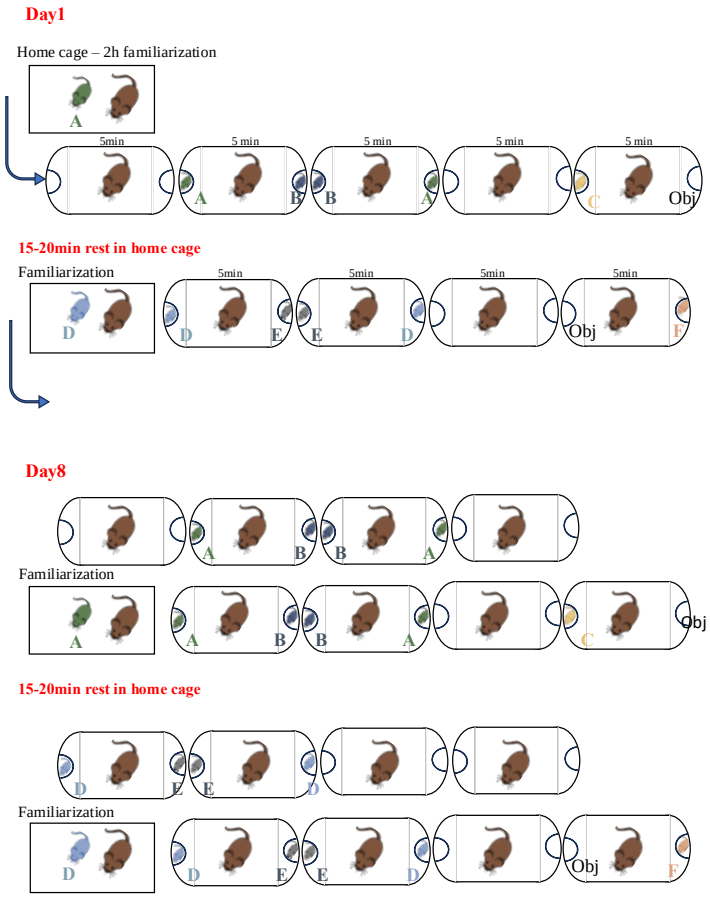

Figure 4: Protocol for behavioral recordings (previously conducted in WT mice; to be replicated in NeuroD2 KO mice by an engineer, with assistance from the PhD student). The swap between partners A and B will help distinguish place coding from social coding (i.e., a social A-coding cell fires preferentially when the test mouse is near A, regardless of A's location). The mouse vs. object sessions will assess sociability coding, while familiarization sessions in the home cage (rectangles) will measure familiarity coding. Finally, comparing Day 1 vs. Day 8 will allow us to evaluate the stability vs. flexibility of individual-specific coding over time.

WP2: Analyzing behavior and neural activity data to unravel social coding in mPFC and somatosensory cortex
See Fig. 5.
The ESR, under the supervision of Lorenzo Fontolan, will analyze behavioral and neural activity data to determine whether and how social interactions are represented in the mPFC and somatosensory cortex. Specifically, the student will investigate:
• How neuronal representations of social interactions differ between the two cortical areas., at both single cell and population levels.
• How these representations change with familiarization (i.e., repeated exposure to the same conspecific).

• How they evolve over time and across multiple social interactions, assessing the stability or flexibility of social coding.

The ESR will develop and apply advanced computational and mathematical models to investigate the correlations between neuronal activity and mouse social behavior. Initially, DeepLabCut will be used to track key anatomical features of the mice (e.g., nose, upper tail, limbs). However, we anticipate that improvements can be made in segmenting animal parts or refining cell contour detection. In this regard, the Kainmueller Lab, the international partner specializing in machine learning and combinatorial optimization for complex biological image analysis, will provide invaluable expertise. During the first year of the PhD, the student will temporarily join Dagmar Kainmueller's lab to gain hands-on experience with cutting-edge techniques for detecting, reconstructing, and tracking cells in fluorescence microscopy images. Beyond cellular image analysis, these advanced methods will also be applied to improve the precision and quality of behavioral tracking. By integrating sophisticated image analysis pipelines, the student will enhance the accuracy of tracking both neural structures and social interactions, leading to deeper insights into brain function and behavior. For instance, we believe that these techniques will enable, for the first time, the precise tracking of neuronal responses to key social events, such as social approach, physical contact, and the decision to disengage from a conspecific. This level of granularity will significantly refine our understanding of the neural dynamics underlying social interactions. Additionally, Léo Guignard (AMU) will facilitate knowledge transfer between the Kainmueller Lab and the PhD student, bringing his expertise in computer vision techniques to further optimize the computational tools used in the project. In summary, the integration of novel machine learning algorithms will greatly enhance our ability to model mouse behavior in relation to neuronal activity, bridging computational image analysis with neuroscience for a more scalable and precise approach to studying brain function in social contexts.

The ESR will analyze both single-cell and population-level coding of social information in the mPFC and somatosensory cortex, employing state-of-the-art analytical methods and improving them (*1*, *4*, *6*, *25*). This will be done under the guidance of both Lorenzo Fontolan and of the intersectoral partner, Aquineuro. Antoine de Chevigny will provide scientific guidance on the conceptual aspects of WP2 but will not be directly involved in coding. Our dataset, which is the largest and most comprehensive of its kind to our knowledge, will allow us to investigate whether familiarization alters the dimensionality of neuronal representations at the population level, as previously observed in the hippocampus (*6*). This should provide key insights into how social experience shapes neural coding in cortical circuits.

WP2 will also investigate a question raised by Okuyama et al. regarding ventral CA1, which may extend to cortical areas: Do "supernumerary mouse A cells," induced by familiarization, encode a specific individual (mouse A), or do they represent a broader social novelty signal? To test this, mice will undergo a social discrimination task with different partner pairs on consecutive days (e.g., A-B on day 1, C-D on day 2). By comparing the neural ensembles activated by familiarized mice (A vs. C), we aim to determine whether these cells encode a unique social identity or a general recognition of familiarity.

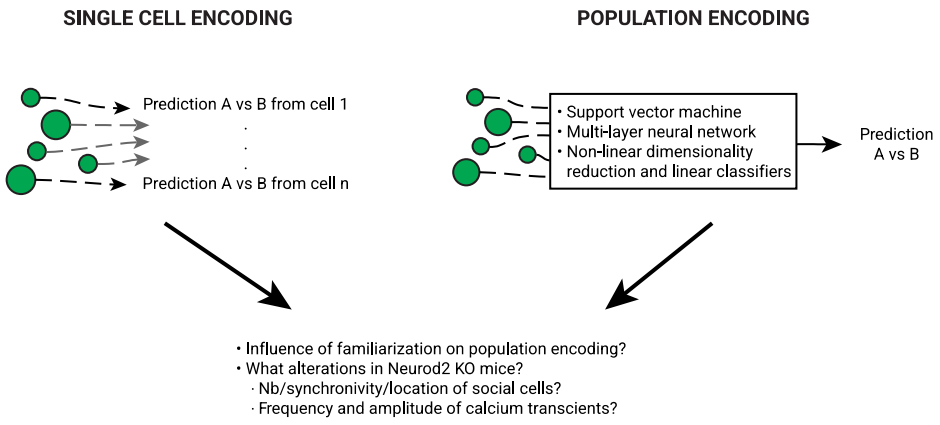

Figure 5: Principles of the planned computational pipeline. Single-cell and population coding schemes will be evaluated with respect to their behavior prediction power. Several classification algorithms based on population encoding will be tested and explored.

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5. ETHICS

1) Details on the numbers of animals to be used, nature of the experiments, procedures and techniques to be used.

For WP1, we will need 14 Neurod2 KO mice (7 for mPFC and 7 for somatosensory cortex). Given the success rate of ~70%, we will utilize in total 20 mice.

2) Details on species and rationale for their use.

We use Mus Musculus because that is the only rodent species for which the Neurod2 KO has been generated (*26*).

3) Details on procedures to ensure animal welfare.

Daily Monitoring: Animals are examined daily to detect any signs of pain or distress. Specific criteria such as weight loss, anorexia, respiratory difficulties, reduced activity, dull coat appearance, dehydration, and infected skin lesions

are monitored. Animals are euthanized if they exceed these limits.
Pain Management: Analgesics like buprenorphine and local anesthetics like lidocaine are administered pre- and post-operatively to manage pain. Anti-inflammatory drugs like carprofen are also used.
Housing Conditions: Animals are housed in enriched environments with access to food and water ad libitum. They are kept in temperature-controlled rooms with a 12-hour

4) Details on implementation of the 3Rs Principle.

Replacement

- **Use of Animal Models**: The study uses animal models (mice) because the complexity of the brain's cellular environment cannot be replicated in vitro. The research aims to understand the development of the cerebral cortex and associated neuropsychiatric disorders, which requires studying live animals.
- **Justification for Animal Use**: The project justifies the necessity of using animal models to study social coding, as no alternative methods can replicate the complexity of the brain's development and function.

Reduction

- **Statistical Power Analysis**: Previous experiments and literature reviews have helped define the minimum number of animals needed to generate statistically significant data. A statistical power analysis is conducted before each experiment to determine the exact number of animals required.
- **Efficient Use of Animals**: The study plans to use a total of 28 mice (14 for WT and 14 for Neurod2 KO). The number of animals per experiment is carefully calculated to ensure that only the necessary number of animals is used to achieve reliable results.

Refinement

- **Pain and Stress Management**: Animals receive analgesics and anesthetics to minimize pain and distress during and after procedures. Daily monitoring ensures early detection and management of any signs of pain or distress.
- **Humane Endpoints**: Specific criteria are established to determine when animals should be euthanized to prevent unnecessary suffering. These include significant weight loss, severe infection, and other signs of distress.
- **Enriched Housing Conditions**: Animals are housed in enriched environments with nesting materials, tunnels, and access to food and water ad libitum. This helps reduce stress and improve their overall well-being.
- **Post-Operative Care**: Animals are closely monitored after surgical procedures, and additional analgesics are provided as needed. Heating pads are used to keep animals warm until they fully recover from anesthesia.
- **Training and Habituation**: Animals are habituated to handling and experimental setups to reduce stress during procedures. For example, animals are acclimated to the treadmill used in imaging studies.

These measures ensure that the study adheres to the 3Rs Principle, prioritizing animal welfare while achieving scientific objectives.