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**Ensemble encoding of self- and other-related behaviors in the medial  
prefrontal cortex**

PhD thesis

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# List of abbreviations

**mPFC - medial prefrontal cortex**

**ACC - anterior cingulate cortex**

**PL - prelimbic cortex**

**IL - infralimbic cortex**

**CS - conditioned stimulus**

**US - unconditioned stimulus**

**M2 - secondary motor cortex**

**AON - anterior olfactory nucleus**

# Abstract

Social transfer of information (also called observational learning) is fundamental to establishing a functional social structure in which individuals can benefit from being part of a group. Key advantages of social groups - such as increased safety, improved food security, and enhanced offspring survival - rely heavily on the transfer of social information. While there is evidence in support of social information transfer in rodents, exactly where and how those observed behaviors are encoded in the brain remains unclear.

The current mainstream approach to this problem centers on mirror neurons. This framework is based on the fundamental assumption that recognizing states or behaviors in others requires the activation of at least a subset of the same neurons that would be engaged during the observer's execution of similar behaviors. However, as of now, the research on mirror neurons has mainly focused on the motor and proprioceptive areas of the brain, with only recent findings reporting the existence of 'emotional mirror neurons' in the anterior cingulate cortex of rats. Given the role of the medial prefrontal cortex in observational learning and other social tasks, we decided to investigate further if and how behaviors of others can be encoded within this brain structure.

This study aimed to elucidate the role of the medial prefrontal cortex in interaction between individuals by combining a well-established behavioral protocol with modern approaches to behavioral and electrophysiological data analysis. We show that rats - at least when under threat - pay attention to the behavior of their social partners. Furthermore, using extracellular electrophysiological recordings with silicon probes in freely moving animals, we show that the information about the partner's behavior is encoded at the population level in the medial prefrontal cortex along with encoding of self behavior. Importantly, the same information could not be reliably extracted at the level of single neurons.

We propose that studying the medial prefrontal cortex's activity at the populational level, rather than focusing on the mirror activity of single cells, is better suited to capturing the associative role of this brain region.

# Streszczenie

Spółeczny transfer informacji (zwany również uczeniem się przez obserwację) odgrywa kluczową rolę w tworzeniu więzi społecznych. Jednostki czerpią szereg korzyści z przynależności do grupy, takich jak zwiększone bezpieczeństwo, lepsza dostępność pożywienia i zwiększona przeżywalność potomstwa, w dużej mierze dzięki przekazywaniu informacji społecznych.

Chociaż istnieją dowody potwierdzające, że gryzonie przekazują sobie informacje, wciąż nie jest jasne, gdzie i w jaki sposób obserwowane zachowania innych są kodowane w mózgu. Obecne, dominujące podejście do tego problemu koncentruje się na neuronach lustrzanych. Zakłada ono, że rozpoznanie stanów emocjonalnych lub zachowań u innych wymaga aktywacji przynajmniej podzbioru tych samych neuronów, które są zaangażowane podczas wykonywania przez obserwatora podobnych zachowań lub przeżywania podobnych stanów emocjonalnych. Dotychczasowe badania nad neuronami lustrzanymi skupiały się głównie na obszarach mózgu związanych z motoryką i propriocepcją. Dopiero niedawno pojawiły się doniesienia o istnieniu „emocjonalnych neuronów lustrzanych” w przedniej korze zakrętu obręczy u szczurów. Biorąc pod uwagę rolę przyśrodkowej kory przedczołowej w uczeniu się przez obserwację i innych zadaniach społecznych, postanowiono zbadać, czy i w jaki sposób zachowania innych mogą być kodowane w tej strukturze mózgu.

Celem przeprowadzonych badań była analiza aktywności neuronów w przyśrodkowej korze przedczołowej podczas obserwacji nacechowanych emocjonalnie zachowań innego osobnika i przejawiania takich zachowań. W tym celu wykorzystano rozwinięty wcześniej protokół behawioralny, w którym szczury odpowiadają na zachowania partnera świadczące o wyższym bądź niższym poziomie strachu. Protokół ten połączono z nowoczesnymi metodami analizy danych behawioralnych i elektrofizjologicznych.

Otrzymane wyniki wskazują na to, że szczury – przynajmniej w sytuacji zagrożenia – zwracają uwagę na zachowanie swoich partnerów. Wykorzystując zewnątrzkomórkowe rejestracje elektrofizjologiczne przeprowadzone za pomocą sond krzemowych u swobodnie poruszających się zwierząt, wykazano, że informacje o zachowaniu partnera są kodowane na

poziomie populacyjnym w przyśrodkowej korze przedczołowej, podobnie jak zachowania własne, które są kodowane przez odrębne populacje neuronów w tej samej części mózgu. Co istotne, tych samych informacji nie można wiarygodnie wyodrębnić na poziomie pojedynczych neuronów.

Na podstawie otrzymanych wyników zaproponowano, że aktywność przyśrodkowej kory przedczołowej na poziomie populacyjnym lepiej odzwierciedla rolę tego obszaru mózgu w procesach asocjacyjnych niż aktywność lustrzana pojedynczych neuronów.

# 1. Introduction

## 1.1. Social behaviors of rats

### 1.1.1. Rats live in social groups

Rats (*Rattus norvegicus*) are highly social animals that live in complex communities and exhibit a wide range of behaviors (Barnett, 2017). Their social structure and behaviors make them an ideal model for studying the neural basis of social dynamics and communication (Wöhr & Schwarting, 2013; Kondrakiewicz et al., 2019; Gorkiewicz et al., 2023). Understanding the social behaviors of rats provides valuable insights into how social information is processed and encoded in the mammalian brain (Knapska et al., 2010; Song et al., 2021).

Rats naturally form large colonies, typically up to 150 individuals (Schweinfurth, 2020). These groups are often found in underground burrow systems, which provide shelter and a space for cooperative living. Multiple generations coexist within a colony, creating a dynamic social environment.

Living in groups offers numerous advantages, such as increased protection from predators (Endres et al., 2007; Brudzynski, 2009), efficient foraging through shared information (Laland & Plotkin, 1990; Galef et al., 1996) or foraging in groups (Weiss et al., 2017), and cooperative care for offspring – for instance through adoption (Cramer et al., 1990). Nevertheless, living in a group also comes with challenges, including competition for resources and establishing social hierarchies (Bobrov et al., 2014; Wesson, 2013), which play an important role in resource management (Hoshaw et al., 2006) and reducing within-group aggression. Despite these challenges, social organization in rat colonies promotes overall group survival, for instance, by increasing reproduction efficiency (McClintock et al., 1982) and the chances of offspring survival (Meyza & Knapska, 2017). This social structure relies on a wide range of behaviors that help rats interact with each other and adjust to their environment. Additionally, many individual behaviors can be influenced by this intricate social organization, a diverse range of which has been extensively studied and categorized to understand their adaptive properties to environmental challenges.

### 1.1.2. The behavioral repertoire of rats

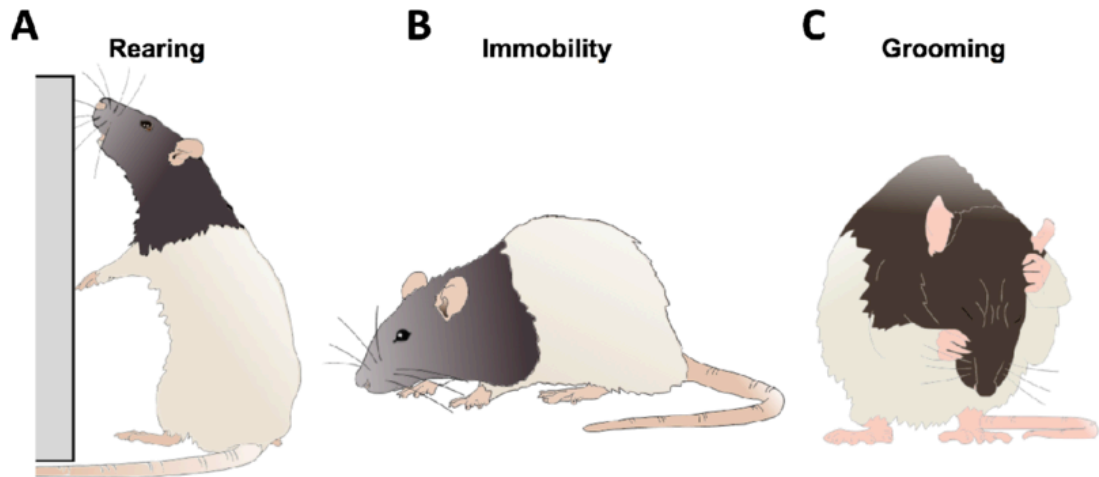
Rats present a wide range of behaviors, which in literature were characterized several times with ethograms (Grant, 1963; Casarrubea et al., 2009; Ivanov & Krupina, 2017), sometimes created using automated approaches (van Dam et al., 2013; Wang et al., 2018). Two of the commonly studied behaviors in rats are freezing and rearing (McGregor et al., 2004; Brandão et al., 2008; Han et al., 2019; Andraka et al., 2021)[Fig. 1]. **Freezing** is defined as a cessation of movement that reduces the likelihood of detection by a predator in situations where escape is not possible. In rats, this behavior typically occurs when the distance between the animal and the threat exceeds 1 meter. If the threat approaches closer, the response shifts to defensive threat behavior and escalates to a defensive attack when the predator is within 0.5 meters (Eilam, 2005; Blanchard et al., 2011).

Freezing can also occur as a conditioned response to specific stimuli, such as a tone previously paired with an unpleasant stimulus, such as a foot shock. Additionally, it can be triggered by social cues - for example, observing freezing behavior in others can prompt rats to exhibit freezing themselves (Cruz et al., 2020). Freezing can also be observed when an animal briefly stops all movement while exploring a novel or threatening environment (LeDoux, 1994).

**Rearing**, in contrast, serves two primary functions: facilitating environmental exploration and responding to novelty (McCall et al., 1969; Lever et al., 2006). It is commonly observed during the habituation phases of experiments when animals are acclimating to a new environment (Poltyrev et al., 1996). Additionally, rearing can aid in risk assessment, with vigilant rearing directed toward a threatening stimulus, such as cat odor (Dielenberg et al., 2001).

Other behaviors are also studied extensively in rats – some of them are key behavioral measures in multiple laboratory tests. For example, **grooming** (and being well groomed) is usually a sign that a rat is not overly stressed – animals under chronic stress and exposed to poor living conditions can present hunched posture, ungroomed coat, and nasal and lacrimal secretions (Rinaman & Schwartz, 2004; Kaliste & Mering, 2007). However, under experimental conditions, excessive grooming can be a sign of stress, and stereotyped chains of rostral grooming often occur as a stress response in fear extinction experiments (Velooso et al., 2016; Reimer et al., 2018; Rojas-Carvajal et al., 2018) or upon exposure to novelty (van Erp et al., 1994). Taken together, the same body posture in rats can convey different

meanings depending on the context, underscoring the nuanced nature of their social behaviors. To understand this complexity and its origins, it is important to explain how these behaviors develop and what is their role in shaping group dynamics.



**Figure 1.** Visual representation of rearing, freezing, and grooming which are the most commonly studied behaviors of rats in a laboratory setting (Leanoardis et al., 2022).

### **1.1.3. Role of social behaviors and their development**

For rats, the ability to display normal social behaviors is crucial for the establishment of stable social structures (Scott & Fredericson, 1951; Lehner et al., 2011), conflict avoidance (Rychlik & Zwolak, 2005), information sharing, and cooperation (Wöhr & Schwarting, 2018). These behaviors are shaped during adolescence, with social play and play fighting serving as critical mechanisms for developing social skills and influencing future behavioral phenotypes (Vanderschuren & Trezza, 2014). Preventing adolescent rats from play fighting (through social isolation) leads to decreased social approach, increased self-grooming, and lack of social behavior-induced conditioned place preference in adulthood (Van den Berg et al., 1999). The latter is mediated by increased opioid receptor expression, especially in the basolateral amygdala (Van den Berg et al., 1999). Thus, social play and interactions with others during adolescence are essential for healthy social and neural development in rats.

Interactions with peers and across generations (i.e., with adults) not only facilitate the development of social skills but also enable animals to exhibit adaptive behavioral responses even without direct experience. For example, animals can adaptively respond to a stimulus by observing a conspecific's behavior or a parent's reaction (Debiec & Sullivan, 2014; Olsson et

al., 2020). Notably, it has been shown that through interactions between different generations, specific traits can be transmitted, akin to the cultural passage of information between different generations. For instance, food preference is retained in colonies across generations even if new sources of food, potentially more palatable, become available (Terkel, 1996). It is also important that learning between generations depends entirely on observing behavior of older conspecifics, starting from a very young age. Rat pups born by dams that could efficiently strip pine cones for food but reared by foster mothers did not develop the efficient technique themselves (Aisner & Terkel, 1992). In sum, adolescence is a critical period for rats, during which social play and interactions with peers and adults shape socio-cognitive development, establish adaptive behavioral responses, and facilitate the transmission of behaviors and preferences across generations. Notably, observational learning remains adaptive and effective into adulthood.

#### **1.1.4. Observational learning**

Animals, including rats, often learn about threats and opportunities in the environment by observing the behavior of others, the phenomenon called observational learning. For example, it has been shown that rats that observe others freezing often start to freeze themselves (Keysers et al., 2022). This phenomenon of response to perceived distress of a conspecific is what we call emotional contagion.

A common way of studying emotional contagion is observational fear learning, where animals are separated by a perforated wall, and the demonstrator is fear-conditioned using a paired footshock and sound cue (Atsak et al., 2011). The observer's response is then measured and correlated to the activity of the demonstrator. An increase in the observer's freezing is considered a defensive emotional response. Learning in this task can be measured by testing animals in a similar experimental context, but without a stressed partner, on the following day. Although behavioral mimicry, where an animal copies the behavior of the demonstrator (Nakahashi & Ohtsuki, 2015), could be considered a source of this phenomenon, a lack of temporal synchrony and differing length of behavioral responses seem to point against it (Andraka et al., 2021). Similarly, increased negative arousal in others, perceived outside of fear-conditioning context, causes an increase in exploration of the environment, including rearing (Andraka et al., 2021). Thus, observing the behavior of others provides important information about the environment, and animals are attentive to these

cues. Animals can use such socially acquired information in a flexible and context-dependent manner, which indicates that observational learning cannot be fully explained by simple behavioral mimicry.

Social behavior, including observational learning, is largely dependent on the medial prefrontal cortex (mPFC), which plays a critical role in understanding social situations and regulating emotional responses. While other brain regions, such as the amygdala and insula, also contribute to social interactions, the mPFC is essential for coordinating these processes effectively (Jurado-Parras et al., 2012; Hill et al., 2016).

## **1.2. Role of medial prefrontal cortex**

The medial prefrontal cortex (mPFC) is a brain region located in the forebrain. It is an aggregate term used to describe multiple prefrontal cortex regions, mainly the anterior cingulate cortex, prelimbic cortex, and infralimbic cortex. It is widely considered a hub for high-order information processing and association, with bidirectional connections to many other brain areas, such as the thalamus, amygdala, hippocampus, or other cortical regions (Dixsuat & Graeff, 2021). This associative role is vital in behavioral planning and can be described by four main functions (Euston et al., 2012).

1. *Retrieval of sensory information.*

Sensory information is primarily processed in the thalamus, which relays and modulates signals from sensory areas. These sensory cues, integrated within the thalamus, are then conveyed to the mPFC (Rainer & Miller, 2000). This includes sensory cues of different modalities derived from observing others, which are processed to assess potential threats or safety cues.

2. *Executive function over the retrieval of long-term memory.*

The mPFC plays a crucial role in guiding behavior toward specific goals. To achieve this, it retrieves long-term memories, primarily through top-down projections to the parietal cortex. The mPFC integrates sensory information, including inputs from other individuals, with information derived from memory, e.g., past instances of observed behavior (Tomita et al., 1999).

3. *Integration of sensory information and retrieved memories with context.*

The integration of sensory information and retrieved memories is heavily influenced by context, which plays a vital role in how the mPFC guides downstream responses (Prabhakaran et al., 2000). Context includes not only the physical environment, such as light conditions, visual cues, and familiarity but also emotional and social factors. The hippocampus is crucial for encoding and retrieving contextual memories, allowing the brain to assess the relevance of past experiences to current situations. The amygdala, on the other hand, is involved in processing emotional aspects of stimuli, particularly those related to negative and positive stimuli. These contextual elements shape the perception of physical stimuli, as demonstrated by their influence on sensory processing (Prabhakaran et al., 2000).

4. *Maintenance of short-term memory and ordinality.*

Working memory is defined as the short-term retention of information that is relevant to current goals. It allows for the integration of multiple sources of information, such as sensory inputs, memory-retrieved and contextual information, without losing the meaning of those different stimuli (Reeders et al., 2021). The mPFC plays a crucial role in guiding behavior by processing both anticipated and actual outcomes, including reward expectancy (Pratt & Mizumori, 2001). Its activity is notably modulated by adverse outcomes, highlighting its sensitivity to deviations from expected results. Within the mPFC, the anterior cingulate cortex (ACC) is particularly important, as it has been implicated in the subjective experience of pain (Johansen et al., 2001) and the anticipation of aversive events (Baeg et al., 2001). This intricate interplay between sensory processing, memory, and expectation underscores the critical role of the mPFC in maintaining the sequential structure and order of information critical for goal-directed behavior.

Notably, the mPFC is central to interpreting social cues (Huang et al., 2020) and directing social approach behaviors (Lee et al., 2016). In particular, studies have shown a significant role of the ACC in observational learning. The ACC has been implicated in the transfer of information about danger during observational fear learning (Han et al., 2019). Furthermore, the PL has been shown to play a role in cooperative learning of a task

(Conde-Moro et al., 2019). This highlights that the mPFC plays a crucial role in evaluating and responding to the actions of others within a social framework. Furthermore, alterations in the mPFC circuitry have been implicated in models of autism spectrum disorder, demonstrating its significance in social cognition and behavior (Mediane et al., 2024).

Collectively, it creates a picture of the mPFC that, most of all, guides behavior towards a specific goal, where the sensory and memory-derived information (including information provided by the conspecifics) is integrated towards achieving a goal of proper, for a given context, behavioral response. Past experiences and clues present in the environment provide information that can be weighed to predict the best possible outcome, guide decision-making, and exert control over motor areas. How does the mPFC achieve this integration and guidance within a social context?

This broad problem can be broken down into two separate questions. The first one regards mirroring as a possible mechanism of perceiving social cues. *Mirror neurons* are cells that respond to both the presentation and execution of a motor function. It was hypothesized that understanding what others are doing and being able to learn from it is driven by activation of such cells. Similarly, emotional mirror neurons, whose existence has been proposed recently (Carillo et al., 2019), would be necessary to understand the emotional state of others. Alternatively, observed and first-hand executed behaviors could be encoded by separate, specialized groups of cells.

The second question regards single-neuron versus population coding. Historically, many brain functions have been explained by response properties of single cells. For example, the primary auditory cortex is able to discriminate between different sounds because individual neurons located there are typically responsive only to one frequency. In a similar manner, one could expect to find in mPFC neurons specialized in detecting a particular behavior of conspecifics. Another possible scenario is *population-based coding*, where individual neurons are not tuned strongly enough to discriminate between different stimuli, but activity of the population as a whole still robustly represents them. In this approach, the tuning of individual cells is not discarded but is treated as part of a larger whole; the state of this ensemble and its dynamic changes contain the information rather than the activity of single cells.

To sum up, mPFC could potentially integrate social information using different coding schemes: either on single-neuron or population level, and either through mirroring or

without it. The following sections will discuss the historical and theoretical background behind each of these options.

### 1.3. Single-neuron vs. population coding

When examining the representation of behavior in the brain, two primary frameworks emerge. As outlined in *Two Views on the Cognitive Brain* (Barrack & Krakauer, 2021), these approaches offer distinct perspectives on the study of cognition. The first, referred to as the **Sherringtonian view**, emphasizes node-to-node connections, where neural connections and single-cell computations take center stage in explaining cognition. Within this paradigm, the focus lies on the connections between neurons, their synaptic weights, and the mechanisms by which single cells interact. Information representation is understood as a series of processing steps involving intracellular ion flows, signaling cascades, and the transmission of transformed signals from one node to the next. This approach has traditionally been prevalent in studies of sensorimotor phenomena. One of the examples of a Sherringtonian view is Konorski's idea of gnostic units, cell assemblies or single cells that are at the tip of the information processing and that combine all the inputs related to recognition of particular objects and people (John, 1975). Such highly specialized neurons indeed can be found in the brain - for example, it was reported that some cells in medial temporal lobe are activated only in response to photos of a single person (Quiroga et al., 2005).

The second framework, known as the **Hopfieldian view**, highlights the distributed nature of neural computation. Here, cognition arises from the collective activity of neuronal populations, creating a neural space where single neurons serve secondary roles. In this paradigm, individual cell activity is often disregarded during analysis, with the emphasis instead placed on the ensemble's representation of information. Features such as geometry and trajectory within this neural space become central to understanding cognitive processes. This approach is more commonly applied to regions involved in higher-order functions, where the explanatory power of single neurons diminishes.

While these two perspectives may seem distinct, they are not mutually exclusive. Population activity, after all, is derived from the combined activity of single cells that comprise the population. To study the medial prefrontal cortex (mPFC) and potential mirror activity, it is essential to consider both levels of analysis. If mirror activity exists at the

single-cell level, the same neurons should respond both to the experimental animal's behavior and the partner's behavior. On a population level, this would manifest as similar trajectories representing the population's activity. Conversely, if the partner's behavior is encoded differently, the trajectories should diverge.

An additional challenge arises when considering the mixed selectivity of neurons in the prefrontal cortex. Single neurons in this region often respond to multiple stimuli, complicating their classification. This mixed selectivity suggests that population-level representations are more informative, capturing relationships between neurons and their states in response to specific stimuli or actions. In such cases, a single neuron might respond to one event in one trial and a different event in another. Fortunately, these dynamics are well-suited to linear classifiers, though, as noted by Rigotti et al. (2013), achieving consistency across trials requires non-linear mixing.

Ultimately, the choice of a theoretical framework depends on the brain region under study and the type and quantity of data available. While connection-based approaches may be suitable for highly specialized and conserved regions - though these can also be studied using a Hopfieldian approach (Mountoufaris et al., 2024) - studying the mPFC often necessitates a more dynamic framework. This approach must account for the region's responsiveness to a constantly changing environment and the need to distinguish signal from noise.

However, most studies focusing on the encoding of observed behavior have concentrated on more specialized structures, such as the premotor cortex, primarily relying on single-cell classification. Therefore, it is important to examine both historical and contemporary ideas regarding mirror neurons and their role in the coding of observed behavior.

## **1.4. Mirror neurons**

### **1.4.1. Motor mirror neurons**

Mirror neurons have first been reported in the ventral premotor region of the macaque (Di Pellegrino et al., 1992) and are described as neurons that are activated when the subject executes a specific behavior or motor action and when the subject observes the same act being performed by another individual. In the first study reporting the discovery of mirror neurons, they were a small part of the recorded population, divided into 3 categories:

- Neurons that specifically encoded the same behavior when executed and when observed (6.5%)
- Neurons that encoded the executed behavior and visually similar behaviors that were observed (3.2%)
- Neurons that encoded both the executed behavior and observed behaviors that were logically similar - such as those that are visually distinct but produce the same effect (6%)

Later studies have usually shown a higher percentage of mirror neurons in the recorded populations, up to to 70% (Tkach et al., 2007). Most of the studies have focused on non-human primates, namely macaques. The recordings were originally performed almost exclusively in two regions: the F5 region of the ventral premotor cortex and the posterior parietal cortex. Typically, only hand movement or reaching behavior was studied. The discrepancy in the number of reported mirror neurons can be partially explained by the change in methodology - in more recent studies, neurons have been chosen for their specificity in response to very precisely described motor actions and then investigated further if they were also responsive during the complete behavior that the motor action is a part of. This narrowed down the pool of all reported neurons to only previously filtered out as responsive cells. As a consequence, the stricter the inclusion criteria for neurons were, the higher the reported percentage of mirror neurons (Kilner & Lemon, 2013). This limitation was partially offset by the development of higher-throughput recording techniques, such as silicon probes. More recently, a number of studies have shown mirror neurons in other brain areas: ventral premotor cortex, dorsal premotor cortex, primary motor cortex, and inferior parietal lobule – all related to specific motor actions (Ishida et al., 2010; Yoshida et al., 2011; Dushanova & Donoghue, 2010). These findings have broadened our understanding of the mirror neuron system and its potential implications for motor action perception. Furthermore, the investigation of mirror neurons has recently extended beyond motor areas, prompting researchers to explore their role in emotional processing.

#### **1.4.2. Mirror neurons in mPFC - emotional mirror neurons?**

Do mirror neurons exist beyond motor areas? Recently, we have seen an increasing body of research related to social mirror neurons and their presence in the prefrontal cortical areas. For example, in a study by Ninomiya and colleagues (Ninomiya et al., 2020) about the

coordination of social monitoring, macaques were exposed to 3 different experimental conditions. In the first one, a real partner (another monkey or a human experimenter) performed a reaching action – allowing for social interaction during the trial. In the second, a filmed partner performed the action, so social interactions were not enabled, and finally, in the third condition, a video of a stick reaching for the object was presented. Those conditions were scaled as most to least biological actions – from performed by live, available partners to performed by an inanimate object on a video. The authors have shown that the mPFC increases its responsiveness if actions are more biological. They reported around 22% of neurons recorded in the mPFC to be preferentially responsive to behavior of self, 32% of neurons to be mirror-type, but also a large population of neurons (47%) to be responsive specifically to actions of the partner. The last group was also significantly more activated by partners' incorrect actions than the correct actions.

After years of research involving humans and monkeys, a substantial body of evidence supports the existence of mirror neurons across multiple brain regions, including the prefrontal areas. As these parts of the cortex are essential for processing emotions, the question arises whether these mirror neurons encode more than just simple motor activity. Do they also represent emotional responses? A relatively recent study discovered emotional mirror neurons in the rat ACC (Carillo et al., 2019), encoding pain and fear of both self and others. In this study, the authors pre-exposed experimental animals to footshocks by performing classical fear conditioning (pairing footshocks with a tone). Then, 3 recordings were performed: one with the exposure of an experimental animal to the other animal being shocked, second with pain triggered by laser, and third with exposure to the conditioned tone. The authors reported 11 (15% of recorded neurons) fear mirror neurons, so responsive both to the footshock of the partner and exposure to the CS, and 25 (34%) pain mirror neurons, so responsive to the partner's footshock and laser-induced pain. Three (4%) of neurons responded in all conditions. Interestingly, the response to the partners' shock seemed to be guided by its squeaks and jumping, as maximal ACC response occurred in the same window of the post-shock delivery as the partner's behavioral response to it. The methodological downside of this study is a lack of control for the motion of self. The authors have not measured the motion of the experimental animal during the observational condition, which could be a source of the ACC activity. If the experimental animal exhibits a behavioral

response to the partner's shock, this may activate the ACC in relation to its own behavior rather than encoding the partner's actions.

To summarize, the existence of emotional mirror neurons in mammals is not well established, as the research data remains inconclusive. Further studies are needed to distinguish between different possible coding schemes within mPFC . Recognizing the need for further investigation into this issue, we aim to enhance our understanding of emotional mirror neurons and population-based coding through the application of modern behavior analysis techniques.

## **1.5. Modern approaches to behavior analysis**

For a long time, the analysis of behavior has been a time-intensive process, fraught with subjectivity and inconsistencies. While manual scoring based on established ethograms offers a degree of replicability and is generally interpretable, it often results in the loss of information when repetitive behavioral patterns fall outside the predefined ethogram. However, advancements in high-resolution, high-speed cameras and modern data compression methods have enabled the collection of unprecedentedly detailed recordings, creating new opportunities to apply computational tools to behavior analysis.

In a modern framework for behavior analysis, manual scoring serves as an initial step, providing a broad, high-level understanding of behavior. On a finer scale, analysis can focus on kinematics and motion sequencing - repetitive motion patterns associated with specific behavioral phenotypes. These two levels of abstraction introduce methodological considerations. Behaviors, particularly social ones, typically unfold over relatively long timescales, measured in hundreds of milliseconds or seconds, and can often be captured adequately at a standard frame rate of 30 Hz or lower. In contrast, kinematic analysis benefits from higher temporal resolution, which improves data quality and supports robust data correction methods, such as signal filtering and interpolation.

One of the most widely used methods in behavioral neuroscience for acquiring movement data is markerless pose estimation. This technique enables researchers to identify specific body parts on an animal and track their movement across a recording, effectively generating a downsampled representation of the animal's motion. This data can then be used

to extract kinematic features, such as speed, velocity, acceleration, angles between body parts, or body tortuosity, while also tracking the animal's location within the experimental space. Such data can be applied in supervised learning approaches - training models to identify user-defined behaviors based on annotated data - or unsupervised approaches, such as clustering poses to identify common postures or using autoregressive models to detect patterns in motion sequences. For behaviors occurring on shorter timescales, a recording frame rate of 50 Hz strikes a balance between data quality and storage requirements. Certain behaviors, however, demand higher frame rates for accurate capture - for instance, grooming in fruit flies or whisker movements in rodents, which typically require a minimum of 100 Hz.

High-quality machine vision cameras also allow seamless synchronization with other tools, such as electrophysiology probes or two-photon microscopes, minimizing the need for post-hoc alignment methods like dynamic time warping.

Combining these approaches offers the dual benefit of explainability (analyzing behaviors within a defined ethogram) and flexibility (enabling the discovery of new behavioral patterns). These patterns may only become interpretable when considered in a specific context and integrated with additional data, such as electrophysiological activity, thereby enriching our understanding of complex behaviors. By leveraging this combined approach, we can establish a robust behavioral paradigm that not only captures complex interactions but also facilitates the exploration of socially significant information through the behaviors of observing rats.

## **1.6. Using Social Buffering as a Paradigm for Coding Behavior of Conspecifics**

To effectively study coding of both one's own and observed behavior, a robust behavioral paradigm is necessary. The experimental design should incorporate both multimodal information transmission and the ability to convey socially significant information, as both factors were demonstrated to enhance efficiency of communication between rodents (Rao et al., 2014). An observing rat must be motivated to focus on the behaviors of its partner; thus, the situation should be ambiguous enough to encourage the rat

to seek information from others. Simultaneously, the stimuli (behaviors) must be clearly defined and emotionally significant. To account for the effects of locomotor activity, it would be ideal to incorporate behaviors that elicit both passive and active responses - that is, responses that differ in their levels of physical activity and the nature of the information being conveyed.

Already in the mid-20th century, a phenomenon later called ‘social buffering’ was discovered in rats (Davitz & Mason, 1955). Research demonstrated that the presence of a non-fearful conspecific significantly reduced fear response to the conditioned stimulus, compared to when the animals were tested in isolation. Since then, extensive studies implicated the mPFC in this phenomenon (Mikami et al., 2016; Jung et al., 2021; Gorkiewicz et al., 2023).

We have developed a behavioral paradigm in rats that utilizes the social buffering phenomenon. The paradigm measures threat-related responses in rats previously subjected to fear conditioning during the fear extinction process, accompanied by a less fearful partner. When presented with a calmer conspecific, the animals modulate their behavioral responses, showing fewer instances of freezing and more rearings (Gorkiewicz et al., 2023). In this paradigm, freezing signifies higher levels of fear, while rearing indicates a reduced response to threat. Importantly, freezing and rearing are passive and active responses, respectively, which involve different levels of activity. The tested animals show significant interest in the conspecific and seek social contact by approaching the mesh wall separating the animals. This creates ideal conditions for testing the coding of observed behavior, as the observers have a documented interest in their conspecifics and process the information received, which alters their behavioral responses to stimuli.

## 2. The aim of the dissertation

As discussed in the previous sections, the study of self- and other-related behaviors and their neural representations has thus far focused primarily on humans and non-human primates. These studies are often limited to simple tasks, such as reaching or other constrained activities, and predominantly record activity from motor areas of the brain. In contrast, much less is known about how own and observed behaviors are processed in emotional contexts.

A key question remains: Does observing another's behavior recruit some specialized cells, or does it influence mPFC activity predominantly on the population level? In each case, does execution and observation of a behavior engage the same or different neuronal ensembles?

Rats provide a good model for addressing these questions. They exhibit a rich social repertoire, allowing the study of complex social behaviors. They also offer opportunities for precise manipulation of neuronal activity to test its functional relevance.

Numerous studies have demonstrated the well-established role of the medial prefrontal cortex in integrating social, emotional, and cognitive information. Thus, it is plausible that the mPFC encodes not only self-generated behaviors but also the behaviors of others.

To address these gaps, we posed the following questions:

- 1. Can the behaviors of self and others be decoded based on the activity of the mPFC?**
- 2. Are the behaviors of self and others encoded by specialized individual cells or on population level?**
- 3. Is there evidence for mirroring in rat mPFC, either on single-cell or population level?**

## 3. Methods

### 3.1. Animals

All research was conducted on experimentally naïve Wistar rats. Adult animals (250-300g) were provided by the Center of Experimental Medicine in Białystok, Poland (total N = 12). They were housed in pairs under a 12h light/dark cycle in standard cages of 43 x 25 x 18.5 cm. All experimental procedures corresponded to the light phase and were carried out during the day. The water and food were provided *ad libitum*. One animal from each pair was randomly chosen to be chronically implanted with a Neuropixels probe. This animal will be referred to as an experimental animal whose cage mate is the partner. All experimental procedures were approved by the Local Ethical Committee (No. 126/2016).



**Figure 2.** Experimental cage layout. The scene is lit with IR light, providing a dimly lit environment for the animals. Experimental animal on the right with a headstage cover during recording.

### 3.2. Behavioral testing

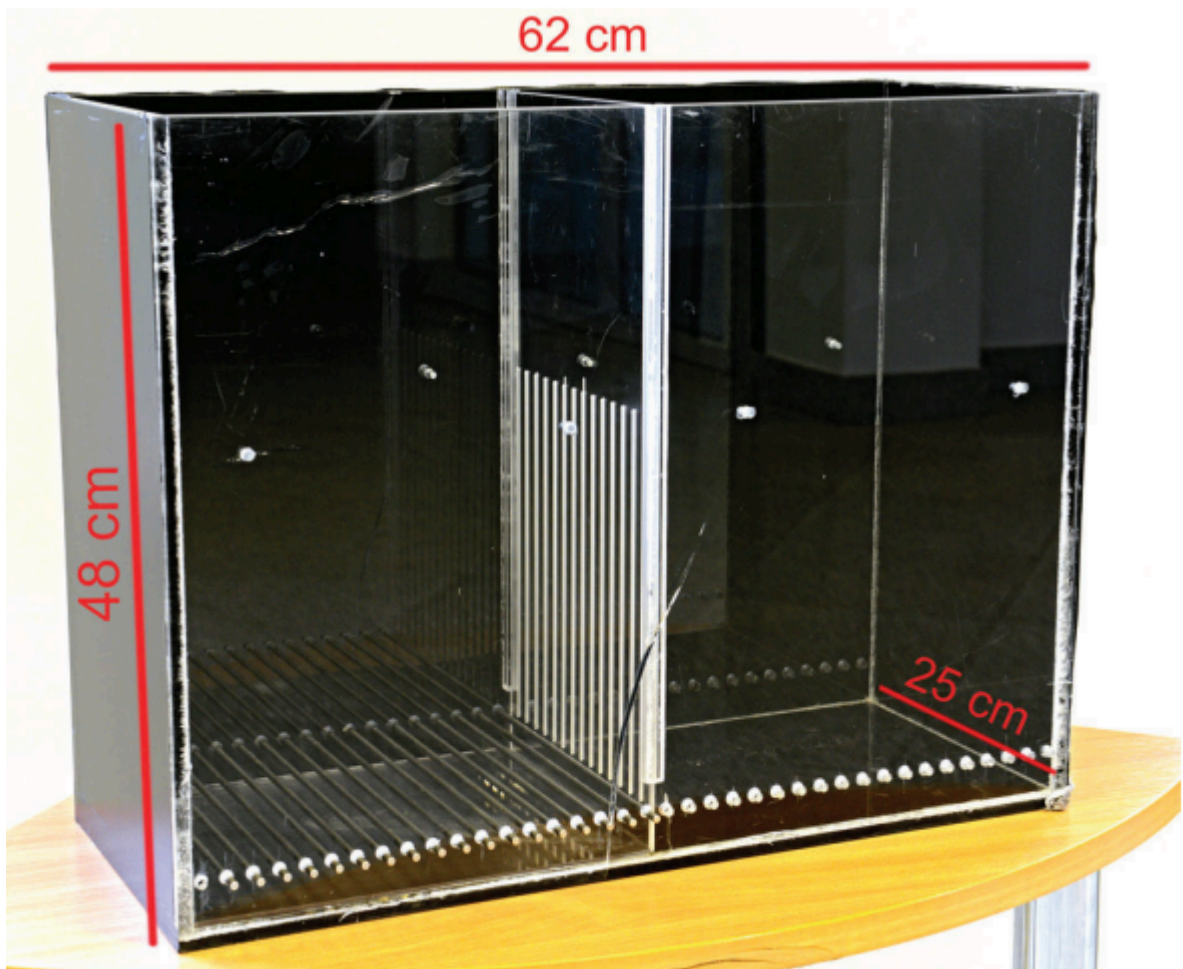
#### 3.2.1. Habituation

All the animals were handled two weeks before the surgeries and experiment to get used to the experimenter. The procedure involved lifting them, placing them on the forearm, and petting them for about 5 minutes. Additionally, before the behavioral experiment, the

animals were habituated to the experimental room for three consecutive days by being placed there for 30 minutes.

### 3.2.2. Behavioral paradigm

The behavioral procedure was performed similarly as described in Gorkiewicz et al., 2023 (see below). The experiment was conducted in a custom-made plexiglass cage split into two compartments with a perforated wall, separating from each other the compartments with paired rats – allowing for the transfer of visual, olfactory, and tactile information. The cage has been placed in a Faraday cage and lit with 12 V DC-powered infrared LED lights (850 nm). Additionally, fear conditioning has been done with bright white light to provide a different context.



**Figure 3.** Experimental cage picture with dimensions (Kondrakiewicz et al., 2019).

During the first day, fear conditioning was performed on both animals separately. For this purpose, one of the compartments has been equipped with a metal grid floor, which

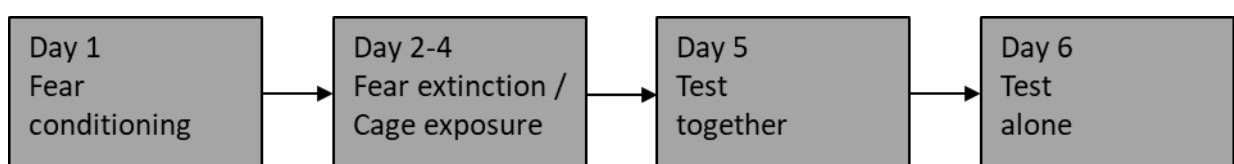
allows the delivery of a foot shock using an electrical stimulator (ENV-414SA, Med Associates). After 2 minute habituation period, the animals received five auditory CS (pure tone, 20s duration, 85dB, 2000 Hz) co-terminated with an unconditioned stimulus (US) (foot-shock, 1 s duration, 0.85 mA) with subsequent stimuli being delivered at a 60-second inter-trial interval (ITI). The animals were fear-conditioned in the compartment assigned to them for the rest of the experiment.

During the next three days, the partners underwent a fear extinction protocol involving exposure to 30 CS presentations with the same 60-second ITI following a 2-minute habituation period. The experimental animals were not subjected to the fear extinction procedure but instead exposed to the experimental cage for a time equivalent to the partners' fear extinction - 42 minutes.

On the fifth day, both animals were placed in their respective compartments in the experimental cages. Following a 2-minute habituation period, they were exposed to 30 presentations of the CS with the 60-second ITI. The number of CS presentations was extended compared to the original protocol to collect more electrophysiological data (Gorkiewicz et al., 2023).

Only the experimental animal was tested in its compartment on the sixth day. Following a 2-minute habituation period, it was exposed to 30 CS presentations with the 60-second ITI.

This experimental design has been chosen for two main reasons. Firstly, it provides an external stimulus (CS) confirmed in the literature to be encoded by the medial prefrontal cortex (mPFC). The CS was evenly spaced in time and relevant to the behavior presented by the animals. Secondly, the experimental design puts animals into a situation where meaningful information is shared between individuals due to similar experiences, i.e., the experimental animal has a reason to pay attention to its partner for information about the threat related to the CS. Additionally, the design and size of the cage, combined with the presence of a conspecific, allow us to study both freezing and rearing.



**Figure 4.** Schematic of the experimental design.

### **3.3. Experiment recording**

The procedure was recorded with a camera (BFS-U3-16S2M-CS) placed above the testing apparatus controlled via a hardware trigger with a microcontroller (Teensy 3.5). The foot-shock delivery was controlled by a microcontroller (Arduino Uno), triggered from Bonsai software (<https://bonsai-rx.org/>) using a custom-written script. The electrophysiological recordings were done using the Neuropixels 1.0 (n=3) or Neuropixels 2.0 (n=3) probes developed for high-density extracellular recordings (Interuniversity Microelectronics Centre - IMEC) with SpikeGLX software. The probes were connected to the PXIE\_1000 (Interuniversity Microelectronics Centre - IMEC) acquisition module mounted in the NI PXIe-1083 (National Instruments). Recording was performed at 30kHz, 500x amplification with a common average reference filter.

### **3.4. Stereotactic surgeries**

The experimental animals were implanted with either Neuropixels 1.0 (1 shank, n=3) or Neuropixels 2.0 (4 shanks, n=3) silicon probes into the mPFC. Rats underwent stereotactic surgery two weeks before the recordings were conducted. All the surgical instruments were sterilized before the procedure. For anesthesia, isoflurane was used (5% induction, 1% maintenance), and an additional injection of analgesic butorphanol (1mg/kg subcutaneously). Anesthetized animal was placed in a stereotactic apparatus (David Kopf Instruments) on a heating pad (Stoelting). The scalp was shaved, and the eyes were lubricated with an ocular gel Vidisic (Dr. Mann Pharma). After being disinfected with 70% ethanol, the skin on the skull was incised, moved to the sides, and temporally fixed in position using surgical clamps (Fisher Scientific). The skull was cleared, and two holes were drilled, one at the following stereotactic coordinates: anteroposterior (AP) – 1.2mm, mediolateral (ML) – 0.8mm, dorsoventral (DV) – 4mm relative to bregma (Paxinos & Watson, 2007), and one for the reference screw above the cerebellum.

In the case of Neuropixels 1.0, the procedure involved two separate stereotactic surgeries. During the first surgery, a protective 3D-printed case was fixed to the skull, and a ground screw was implanted. The second surgery – performed a few hours after the fear

conditioning – involved opening the protective case, performing a craniotomy, and implanting the probe. Before implantation, the probe was submerged in the fluorescent carbocyanine dye – DiI (Invitrogen, Thermo Fisher Scientific) to mark the trace of the probe shank. Insertion was performed at the rate of 0.1mm per minute. The exposed part of the probe was covered with a mixture of bone wax and mineral oil (50:50) and cemented to the skull using C&B Metabond® (Parkell). The probe fixture was then covered with an additional custom 3D printed case covered in Duracryl™ Plus (SpofaDental) to ensure its safety, as the rats were housed in pairs the whole time. Because the second surgery was much less invasive than the first one (and did not result in damaging any tissue with pain receptors), the rats showed signs of complete recovery a few hours after the procedure.

In the case of Neuropixels 2.0, firstly, the probe was glued inside a plastic protective fixture (as described in van Daal et al., Nat. Protoc. 2021). Following this, the surgery protocol was identical, except for all the steps being performed during one surgery instead of two.

## **3.5. Behavioral data analysis**

### **3.5.1. Manual scoring**

The video acquired during the experiments has been manually scored using BehaView software (<http://www.pmbogusz.net/?a=behaview>, P. Boguszewski) for three behaviors, two of which could be presented independently by either the experimental animal or the partner. Annotated behaviors were freezing, rearing, and social interaction. Freezing has been defined as the cessation of all movement and tense posture presented by the animal for a prolonged period – above 2 seconds. Rearing has been defined as an erection of the head followed by standing on hind paws; supported and unsupported rearing were not distinguished. Social interaction has been defined as a nose-to-nose interaction by the separation wall. Differences in behavioral phenotype were tested with a two-way ANOVA using GraphPad software (<https://www.graphpad.com/>).

### **3.5.2. Pose estimation**

Furthermore, a custom neural network based on a resnet-50 architecture has been trained using the DeepLabCut framework (Lauer et al., 2022) to track 12 body parts per

animal. Raw detections were assembled and then tracked using an ellipse-based tracker. Data was later smoothed and cleaned with a custom Python script. Pose estimation-based data analysis was done using a custom Python script to calculate repetitive poses, distance and angles between animals, distance traveled, velocity, and acceleration of each animal. Repetitive poses were retrieved using a K-Means clustering method. Animals' coordinates were first transformed such that the body part coordinates corresponded to their location in relation to the animal's centroid. After that, the animal's position was fixed to face a specific direction so that the long axis of the animal (a line between the nose and the centroid) was fixed in their angle to ensure that the clusters reflected actual pose changes and not just the movement direction.



**Figure 5.** Visualization of the body part placement for the pose estimation model.

## **3.6. Spike sorting and electrophysiological data analysis**

### **3.6.1. Spike sorting**

Raw data has been preprocessed using the ecephys spike sorting module ([https://github.com/AllenInstitute/ecephys\\_spike\\_sorting](https://github.com/AllenInstitute/ecephys_spike_sorting)), which extracts the binary data recorded with the Neuropixels and retrieves the timestamps of the camera and CS presentation for data synchronization purposes. Automatic spike sorting was done using Kilosort. 2.5 with the default parameters (<https://github.com/cortex-lab/KiloSort>), after which manual curation of the sorted clusters was done with the use of Phy (<https://github.com/cortex-lab/phy>) to remove possible noise or multi-unit clusters.

### **3.6.2. Analysis of electrophysiological data**

Preprocessed electrophysiological data was analyzed using a custom Python package for exploratory time-series data analysis. Simple Ephys-Behavior Analysis – SEBA

(<https://github.com/KonradDanielewski/SEBA>). Peri-stimulus time histograms (PSTH) were created for a window of 2 seconds before and 4 seconds after the onset of the analyzed events. The firing rate was calculated using a rolling Gaussian window (100 ms - 3000 samples), followed by the calculation of the z-score for the same PSTH windows. Responsive neurons were selected with a Wilcoxon rank test between the pre-and post-onset firing rate, followed by a correction for multiple comparisons (FDR) with a  $p > 0.01$ . Neurons with inconsistent responses (no spikes in more than 50% of the trials) were discarded even if identified as responsive by the statistical test.

### **3.7. Dimensionality reduction and population trajectory analysis**

To check whether the dimensionality of the population activity could be significantly reduced, principal component analysis was performed with the default values provided by the scikit-learn package, reducing neuronal activity to 20 dimensions (number of dimensions used in the Procrustes analysis). The data was first z-scored and 225 (4.5 seconds) samples per trial of an event were taken - 25 from the baseline and 200 after the event. Two different PCA models were fit - one for performed and observed freezings and another one for performed and observed rearings. This was done on a per animal basis to capture the dynamics specific to each animal. Mean trajectory per trial has been calculated and the first three dimensions have been plotted for visual inspection of the trajectory. To further test the difference between trajectories of the population activity between performed and observed behaviors, a Procrustes analysis was performed, which resulted in a disparity measure. Procrustes analysis is used to test the difference in shape between two objects. Given 2 matrices of 20 columns (one for performed behavior and one for the observed behavior) the matrices are standardized - centered around the origin - and a most optimal transformation is applied to both matrices to minimize the sum of the squares of the pointwise differences between the two input datasets. This sum is our disparity measure - essentially a leftover difference between initial matrices after transformation to make them as similar as possible.

### 3.8. Population activity-based decoding

To decode the behavioral classes and the CS from neural activity, all the recordings from different animals were combined to increase the number of features that code specific events. Due to a different number of event occurrences between animals, a permutation method was used to extract from each animal the maximum possible number of events equal to the minimum number of occurrences of a specific event for any animal, choosing random trials from each animal. An 80/20 split was done, with 80% of the data placed in the training set and 20% of the data placed in the test set. One hundred splits were created to train a 100-gradient boosted linear model with parameters chosen via a grid search, focusing mainly on selecting the proper number of estimators and learning rate to lower the likelihood of the model overfitting. Because the number of trials for each event was variable, to ensure no preference in the model samples, weights were adjusted inversely proportional to their frequency in the data. For each trained model, a confusion matrix was calculated for recall, precision, and f1-score, the mean of which was calculated and used as a final result.

To further test the coding of behaviors of the experimental animal and its partner, binary classifiers were trained using the same approach with a binary *logistic* target instead of a multiclass *softmax* target.

Probability estimations were plotted to confirm the f1-score and provide more information on the classification on a per-sample basis.

Additionally, to test whether this coding is led by a few neurons or it is instead coded by the state of the whole population, we trained 30 additional runs of 50 models per run where at each consecutive run, we removed the 10 most important features (neurons) from the previous run. This way, we can ensure that any potentially “leading” neurons with very specific, strong responses do not skew the decoding performance.

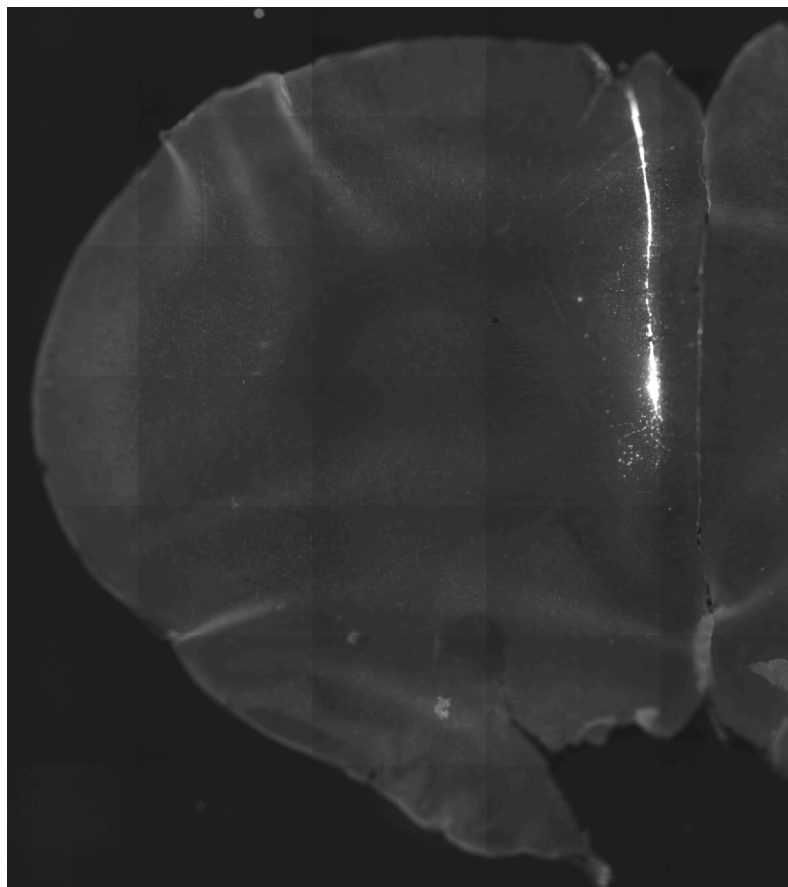
Pose clusters were decoded with a similar approach to verify whether simple kinematic features – considering the pose as a down-sampled representation of the animal’s behavior – can also be decoded.

### 3.9. Image collection and analysis

Two days after the last behavioral test (test without the partner), rats received a lethal dose of morbital (133.3 mg/ml sodium pentobarbital, 26.7 mg/ml pentobarbital). They were

transcardially perfused with ice-cold 0.1M PBS (pH 7.4, Sigma) and 4% (wt/vol) paraformaldehyde (POCh) in PBS (pH 7.4). The brains were removed and stored in the same fixative for 24 h at 4°C and immersed in 30% sucrose (wt/vol) at 4°C. The brains were then slowly frozen and sectioned at 40 µm on a cryostat. Coronal brain sections containing the traces left by the silicon probes were collected for analysis.

Coronal brain sections were photographed with a Nikon Eclipse Ni-U fluorescent microscope to analyze the location of recorded neurons. It was equipped with a color camera (QImaging QICAM Fast 1394) and a laser, emitting a wavelength of 568 nm (Kr laser). Images were acquired using the 10x objective and tiled using the Image-Pro Plus software (v. 7.0.1.658, Media Cybernetics) to include the whole area of interest in a single image. They were then saved as 16-bit .tiff files. Histological E-data Registration in Brain Space – HERBS software (<https://github.com/Whitlock-Group/HERBS>) has been used to align the slices to the Waxholm Rat atlas (Papp et al., 2014) and retrieve the location of each channel based on the shanks' trace. SEBA has been used to retrieve the neurons' location based on the channels' position according to the atlas coordinates.

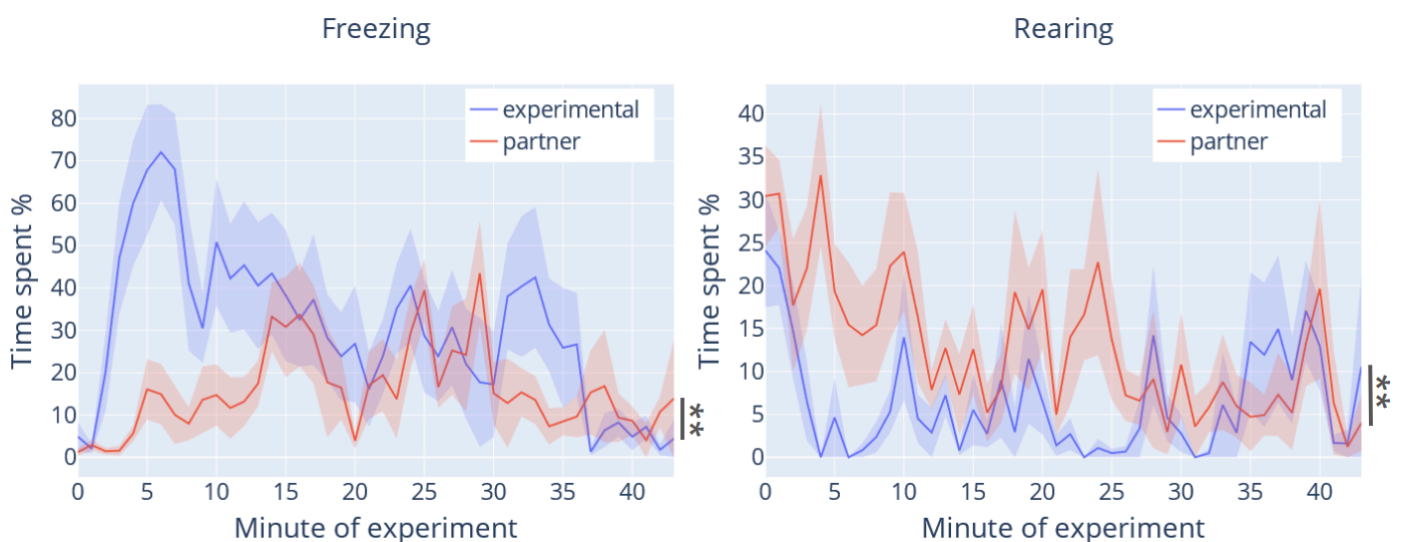


**Figure 6.** Example of the DiI staining marking of one of the shank's locations.

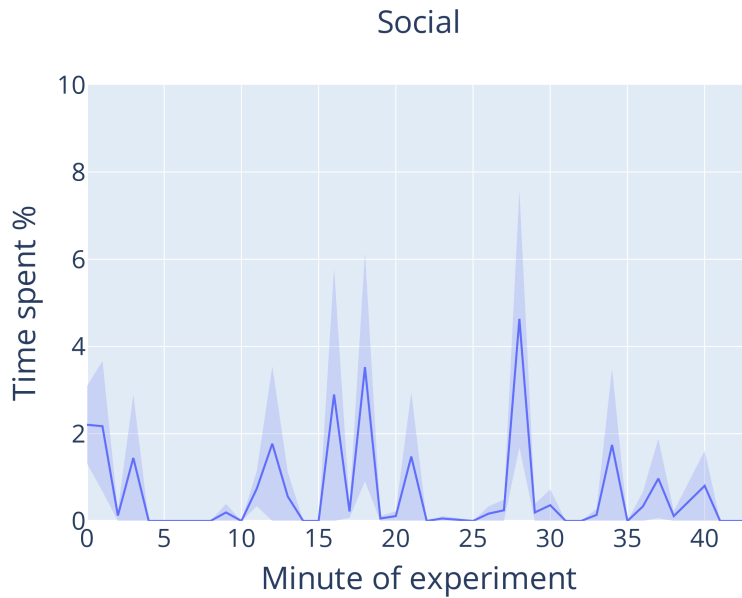
## 4. Results

### 4.1. Testing with a partner: behavioral demonstration over time

We calculated the percentage of time spent performing specific behaviors in each minute of the experiment to assess potential differences in the behavioral expressions of the experimental animal over time and compared to the partner. The experimental animal and the partner presented a different behavioral phenotype. A two-way ANOVA showed significant differences between the experimental animals and partners in both freezing [ $F(1, 10) = 16.55$ ,  $p = 0.0084$ , Fig. 7] and rearing [ $F(1, 10) = 13.24$ ,  $p = 0.0045$ , Fig. 7] with the experimental animals freezing more and rearing less. Furthermore, there was also a difference between the groups in how the behavior evolved over time regarding both freezing [ $F(42, 420) = 1.748$ ,  $p = 0.0035$ , Fig. 7] and rearing [ $F(42, 420) = 1.415$ ,  $p = 0.0495$ , Fig. 7] behaviors. Moreover, time spent on social interaction was also calculated (Fig. 8).

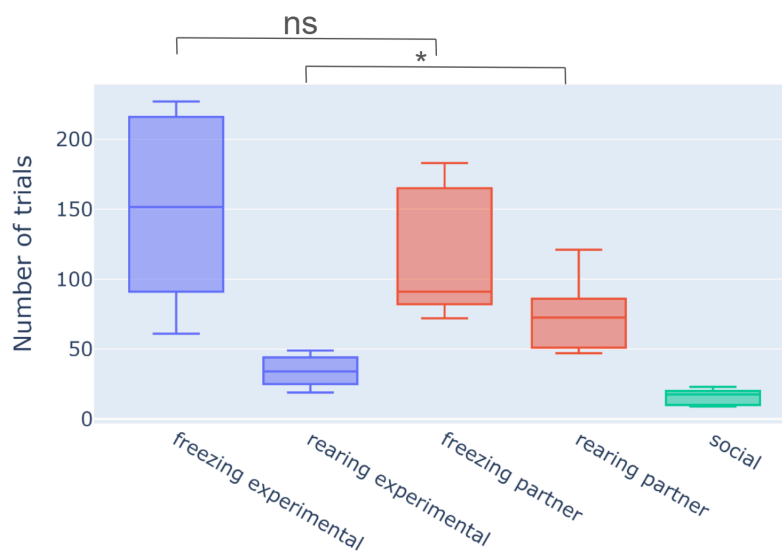


**Figure 7.** Overview of the time spent performing freezing and rearing behavior.



**Figure 8.** Time spent on social interaction.

The animals displayed varying numbers of behaviors due to the free-moving nature of the experiment. On average, the experimental animals froze more than their partners at  $149.9 \pm 68.9$  times vs  $114 \pm 47.39$  times. However, the difference was not statistically significant [ $t=1.044$ ,  $df=8.858$ ,  $p=0.3242$ , Fig. 9]. Experimental animals also performed significantly fewer rearings than their partners, with  $75 \pm 27.84$  rearings compared to  $34.12 \pm 11.25$  [ $t=3.331$ ,  $df=6.591$ ,  $p=0.0137$ , Fig. 9]. Social interactions occurred less frequently than freezing or rearing, with an average of  $16.2 \pm 5.8$  instances.



**Figure 9.** Number of specific behavior trials.

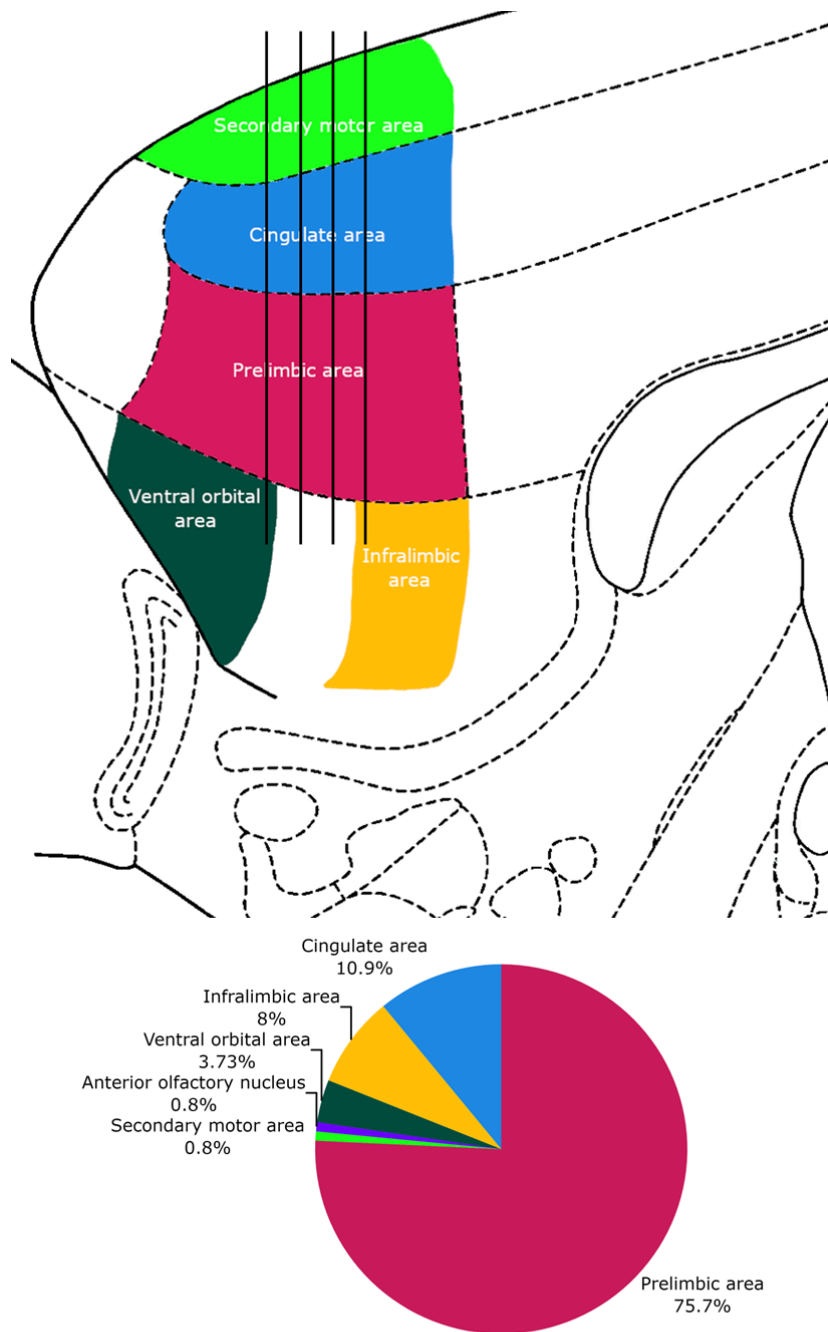
Taken together, the analysis of the percentage of time spent on specific behaviors revealed distinct behavioral phenotypes between the experimental animals and their partners, as well as variations in behavioral evolution over time. Importantly, both freezing and rearing behaviors were recorded in the experimental rats and their partners throughout the entire recording session.

## 4.2. Recorded neurons location and response characteristics

To determine the location of recorded cells, we used HERBS data obtained from histology-based probe alignment and selected channels that recorded neurons identified as valid single cells through spike sorting. Overall, 375 well-separated single units were recorded ( $62.5 \pm 16.87$  neurons per animal). The number of neurons per structure has been extracted from HERBS by assigning a neuron to a structure based on the location of the channel that recorded it. Most neurons (N=284) were recorded from the prelimbic area (PL), and the fewest (N=3) were recorded from the anterior olfactory nucleus (AON) and secondary motor area (M2).

Structure	Number of cells
Prelimbic area	284
Cingulate area	41
Infralimbic area	30
Ventral orbital area	14
Anterior olfactory nucleus	3
Secondary motor area	3

**Figure 10.** Number of recorded neurons per structure.



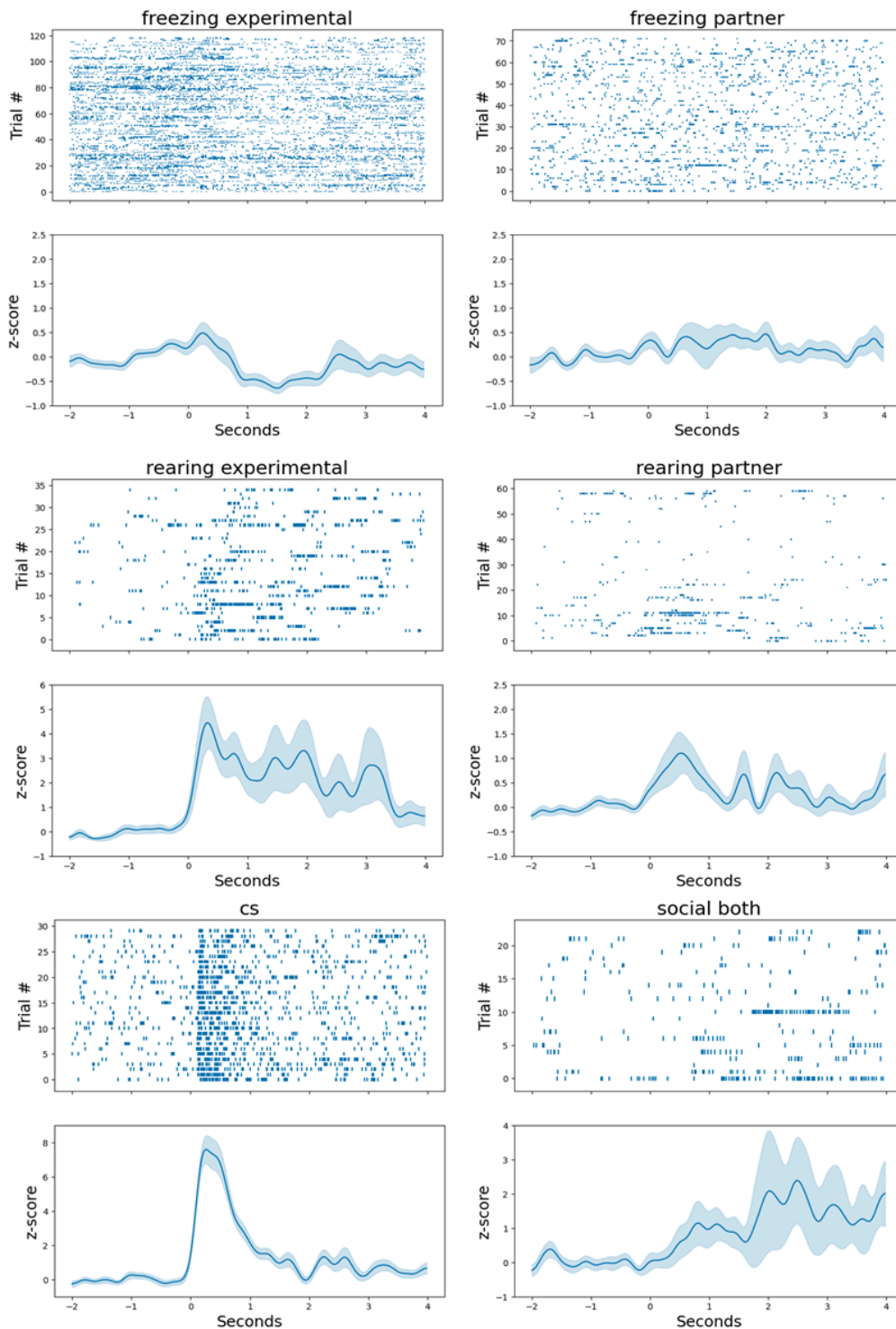
**Figure 11.** Structure location and proportion of the number of recorded neurons per location.

In summary, we identified 375 well-separated single units recorded from different parts of the prefrontal cortex, with the majority of neurons located in the prelimbic area (PL) and fewer in the anterior cingulate cortex (ACC) and infralimbic area (IL).

### 4.3. Single-cell responses

We first focused on single-cell-based statistical analysis. Of the 375 recorded cells, 147 were deemed responsive based on the Wilcoxon rank test (see Methods). Figure 12

shows representative peri-stimulus time histograms of the responses of individual cells to specific events.

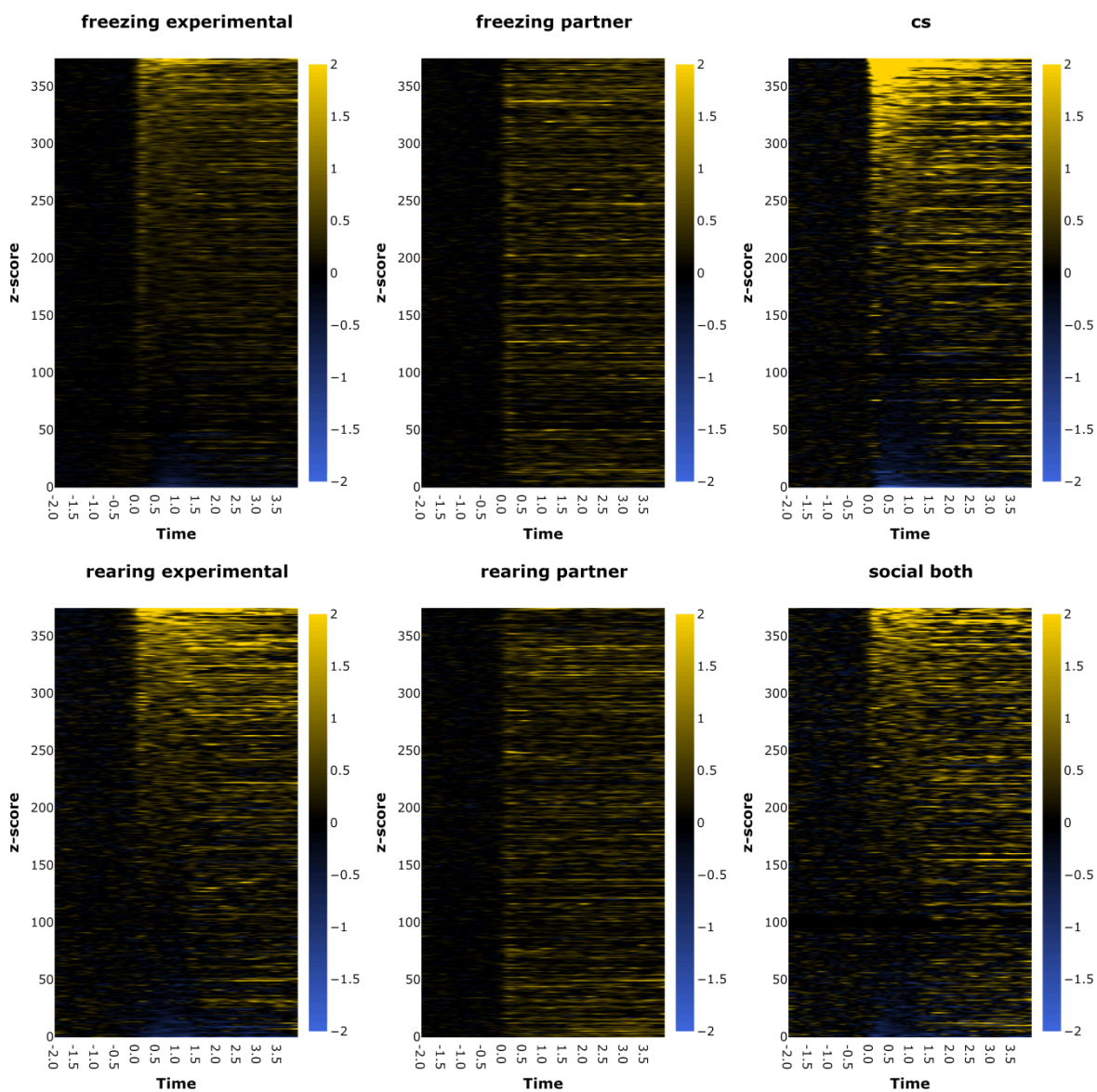


**Figure 12.** Peri-stimulus time histograms of the z-scored firing rate of representative cells.

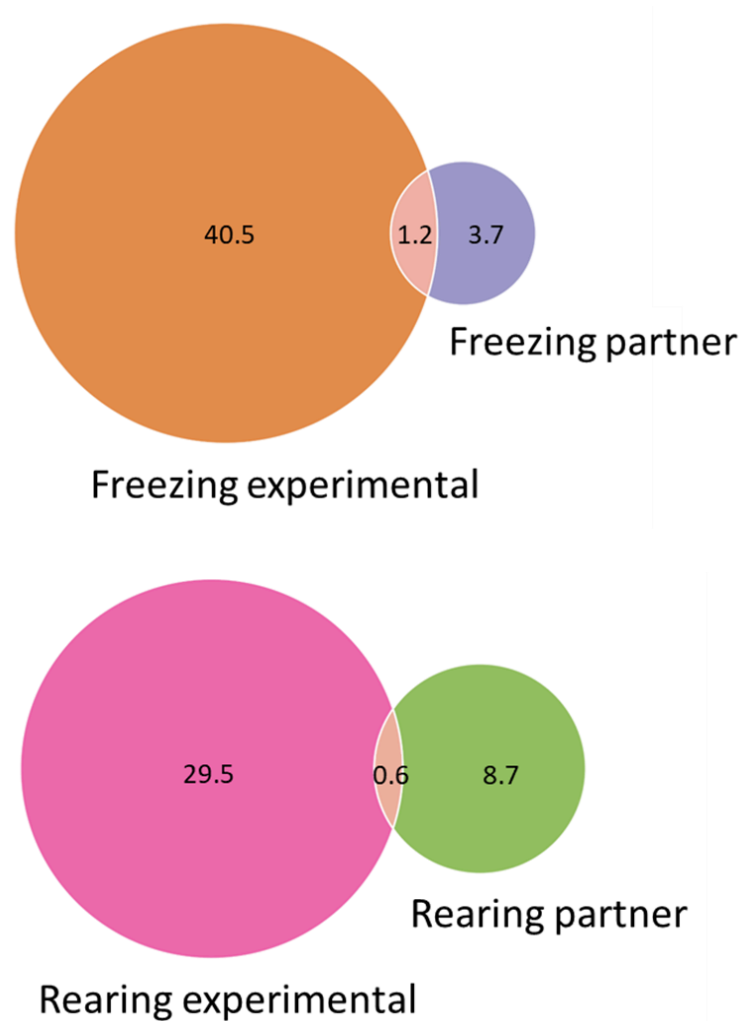
Of the responsive cells, 65 (44.2%) responded to the CS, 66 (44.8%) to the freezing of self, 6 (4.1%) to the freezing of the partner, 48 (32.6%) to the rearing of self, 14 (9.5%) to

the rearing of the partner, and 16 (10.8%) to the social interaction. Regarding mirroring, only three neurons of the recorded 147 responded to the same behavior performed and observed, 2 for the freezing and 1 for the rearing behaviors, respectively.

Mean z-scored responses of the neurons are presented on the heatmaps. The neurons are sorted based on their average response. For behaviors occurring in both animals, responses to the partner's behaviors are sorted by the response z-score of the experimental animal (Fig. 13).



**Figure 13.** Heatmaps of z-scored neuronal responses to event onsets. Mean responses to specific events are sorted by the response z-score. In the case of the events that were performed and observed, both are sorted based on the activity of neurons during behavior of self.



**Figure 14.** Overlap of coding based on Wilcoxon rank test. The graphs show the percentage of the whole responsive population per event.

Taken together, among the recorded neurons, a significant proportion responded to freezing behaviors (44.8% for self-freezing and 4.1% for partner-freezing) and rearing behaviors (32.6% for self-rearing and 9.5% for partner-rearing), indicating distinct neural responsiveness to these social behaviors.

#### **4.4. Principal component analysis - feature colinearity testing**

To test how our cell populations code behaviors of the experimental rat and its partner, we performed a principal component analysis (PCA) on a per recording basis. The PCA was performed on rescaled data - namely the z-scores of neural activity. 225 samples

were used per event, consisting of 25 baseline samples before each event and 200 samples of post-onset signal - overall 4.5 seconds of data per event trial was used. This provided us with two types of information:

1. Is the neural activity linearly correlated?
2. Is the population activity similar for the own and observed behaviors?

The explained variance is presented in Figure 15. The first observation is that the neural activity does not appear to be highly correlated. In all cases, it was impossible to reach the commonly used threshold of 95% variance explanation, even when using the first 20 components. This suggests that the neural activity within the mPFC does not exhibit a high linear relationship between cells.

A.	Animal	First 3 PCs variance	N components @ 95% variance / N neurons recorded
	1	25.9%	51 / 62
	2	31.6%	28 / 37
	3	22.3%	68 / 93
	4	24.9%	43 / 52
	5	21.4%	51 / 66
	6	32.2%	47 / 65

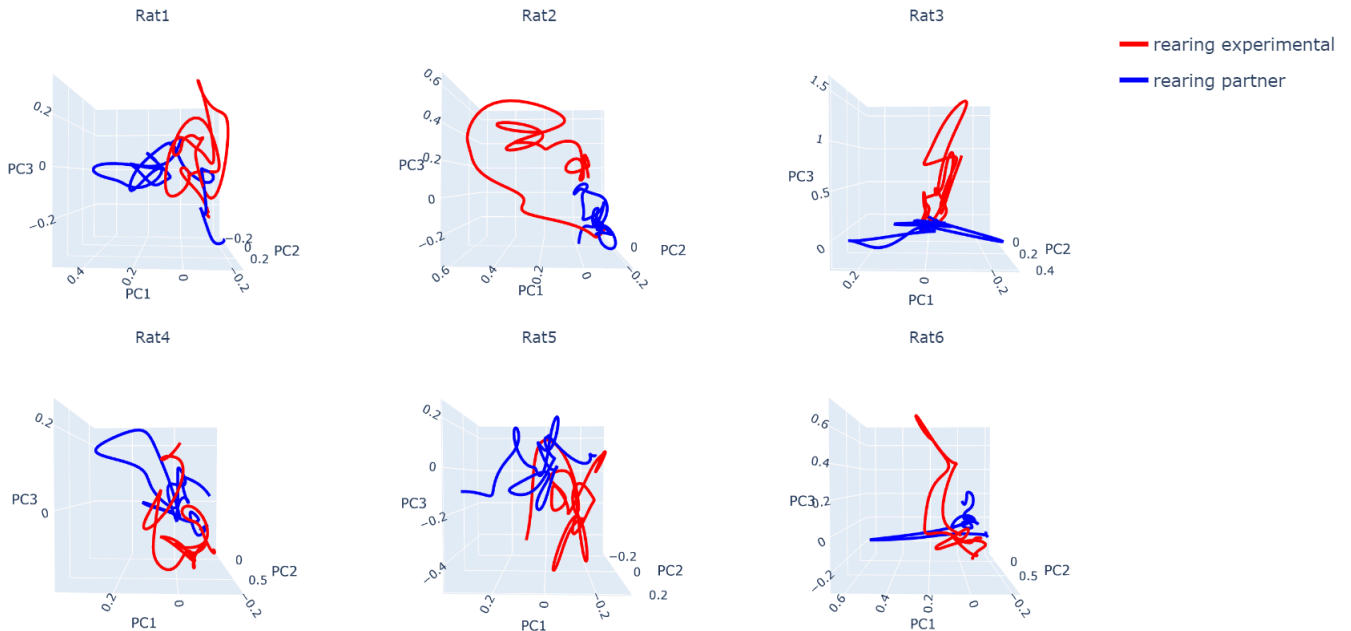
B.	Animal	First 3 PCs variance	N components @ 95% variance / N neurons recorded
	1	25.8%	51 / 62
	2	30.8%	29 / 37
	3	22.9%	68 / 93
	4	21.5%	42 / 52
	5	18.3%	55 / 66
	6	30.5%	50 / 65

**Figure 15.** A. Breakdown of dimensionality reduction results in the PCA fit on the rearing samples of both animals. B. Similar breakdown for the freezing behavior samples.

Mean trajectories of the first two components were plotted against time to compare them between the neural activity representing behavior of the experimental animal and its partner, with the results shown in Figures 16 and 17. These figures reveal a clear difference between the trajectories. A Procrustes analysis was performed to assess the shape disparity

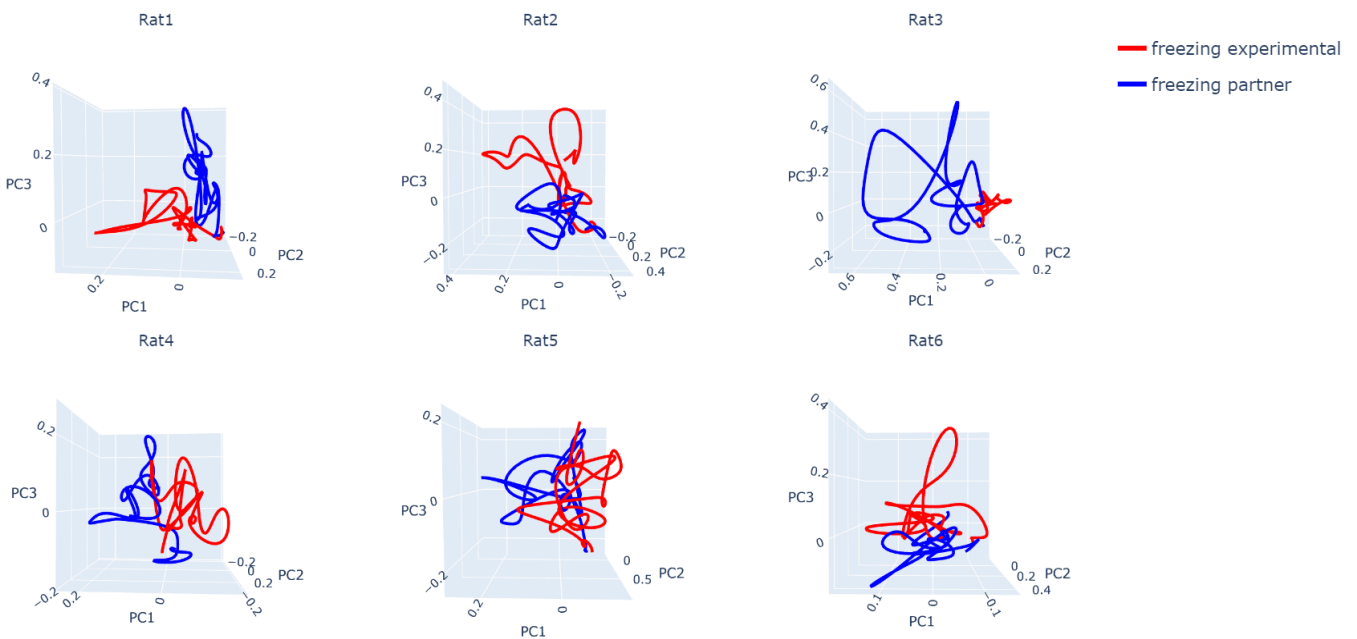
between the trajectories of the experimental animal and its partner, resulting in high disparities for both behaviors representations, with a mean disparity of 0.373 (SD = 0.05) for rearing and 0.39 (SD = 0.04) for freezing. Figure 18 displays a simplified trajectory, stretched over time, using only the first two components.

Components over time - Rearing



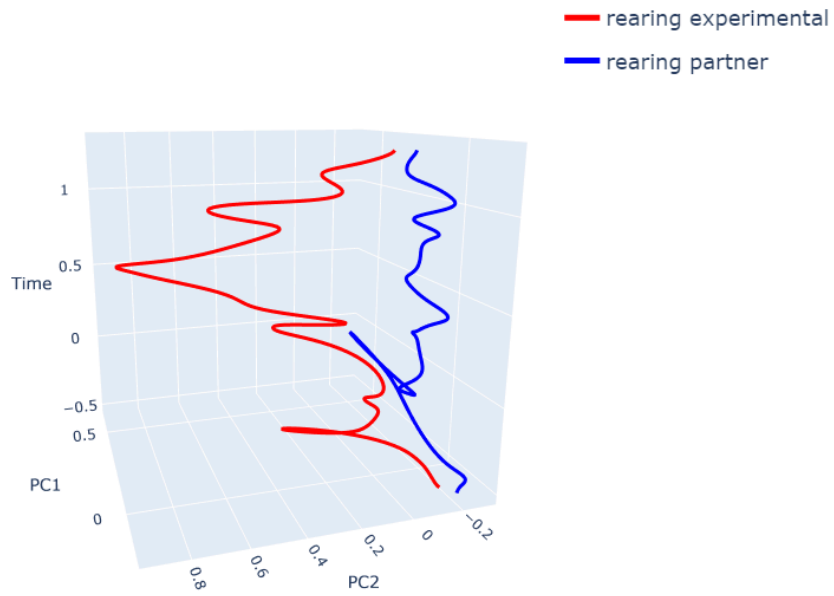
**Figure 16.** 3D visualization of the trajectories of the first three components for the rearing behavior of both animals.

Components over time - Freezing

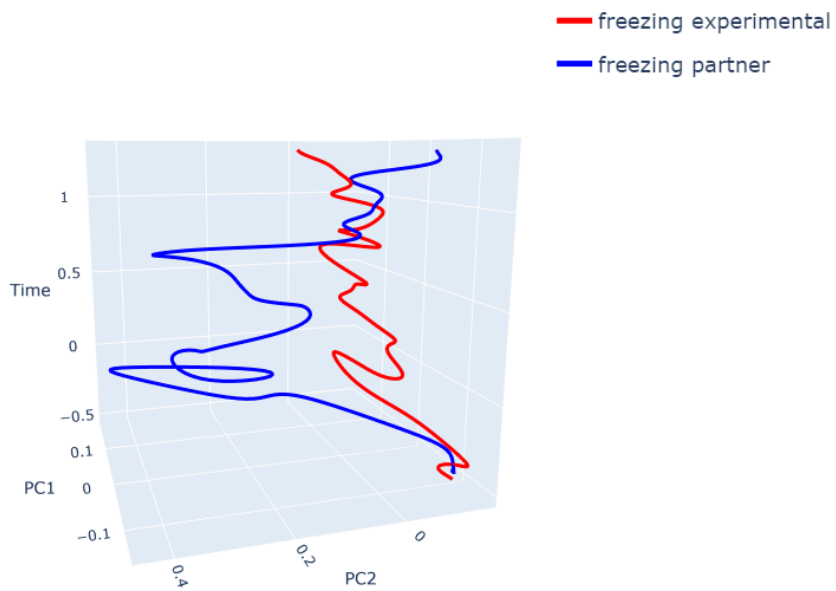


**Figure 17.** 3D visualization of trajectories of the first three components for the freezing behavior of both animals. The plots present a mean trajectory. A set of points corresponding to the samples of the event.

### Components over time - Rearing



### Components over time - Freezing



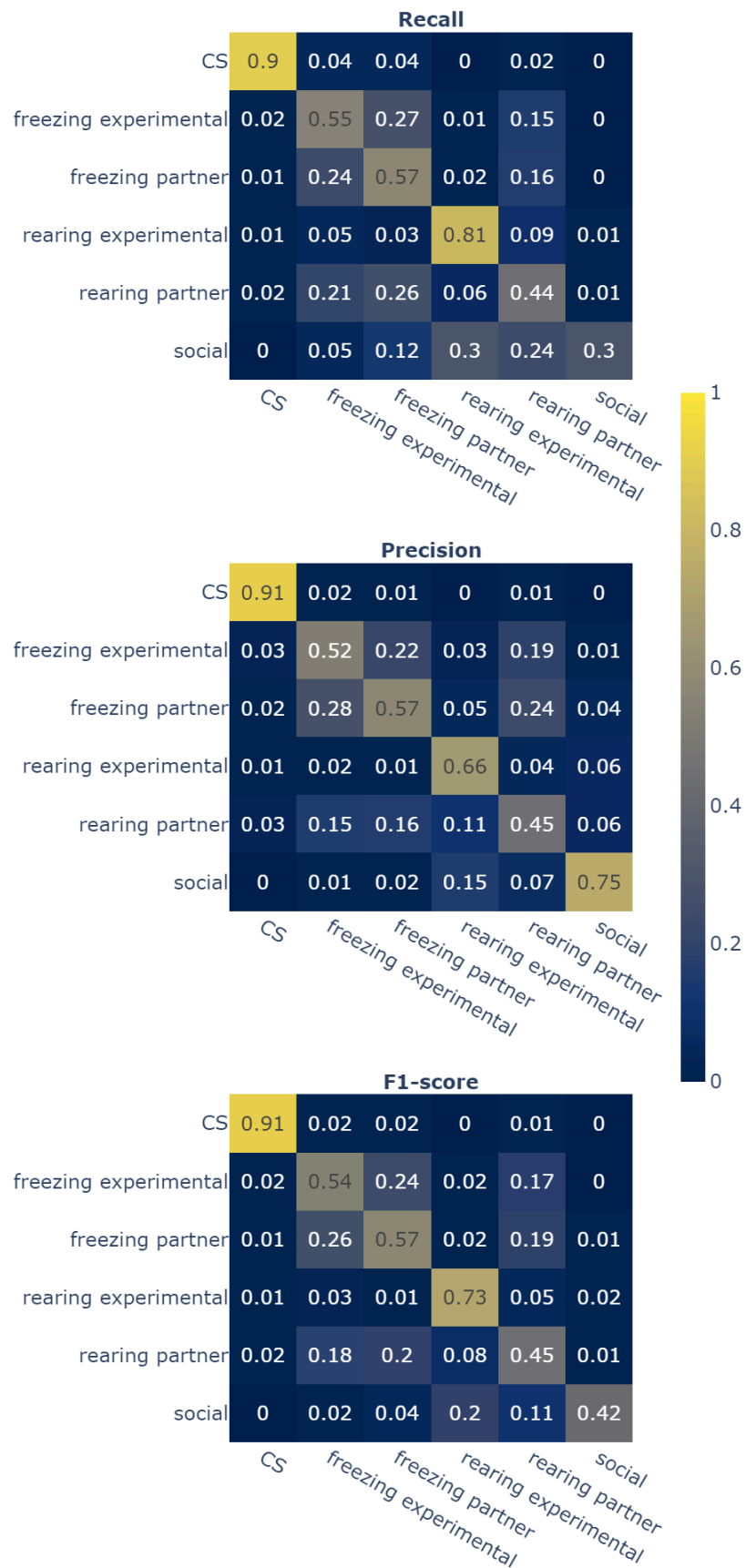
**Figure 18.** Example of the trajectory of the first two components stretched over time. Using data from Rat 6.

In summary, the analysis of neural activity in the mPFC indicates a lack of strong correlation and linear relationships between cells, as well as high-dimensionality of the data. Procrustes analysis revealed significant shape disparities in the mean trajectories of neural activity representing the behaviors of the experimental animal and its partner.

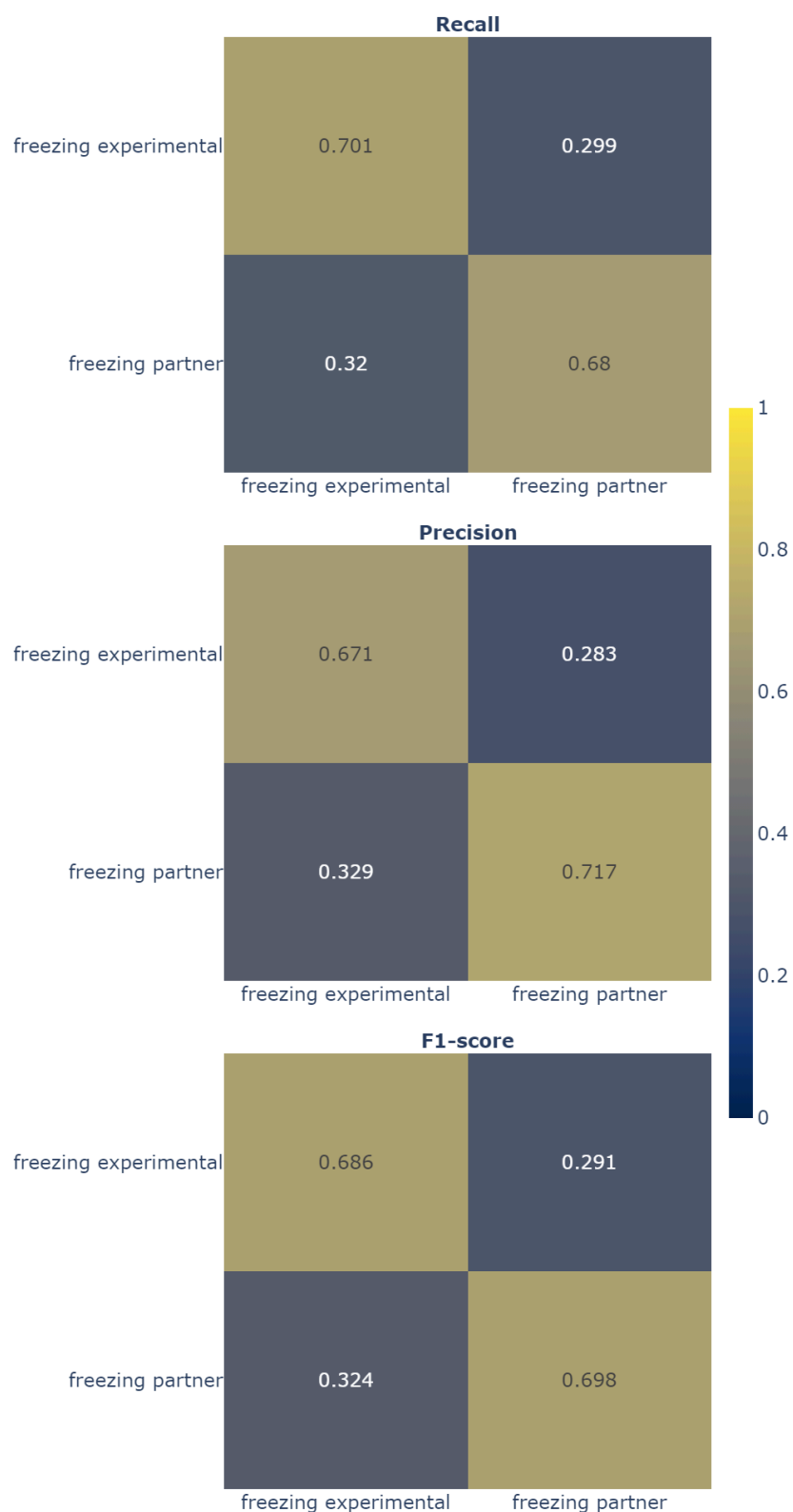
## **4.5. Decoding of the behavior based on population activity**

Given the limitations of single-cell-based statistical analysis in capturing the broader scope of neural activity, we next moved to a machine-learning decoding approach. Six events overall were taken into account. Onsets and offsets of the CS, freezing of the experimental animal and the partner, rearing of the experimental animal and the partner, and social interaction. Onsets were used as they indicate a transition in a behavioral state and potentially evoke the strongest response. A similar approach was used to decode offsets of the listed events to further prove the robustness of this transitive activity.

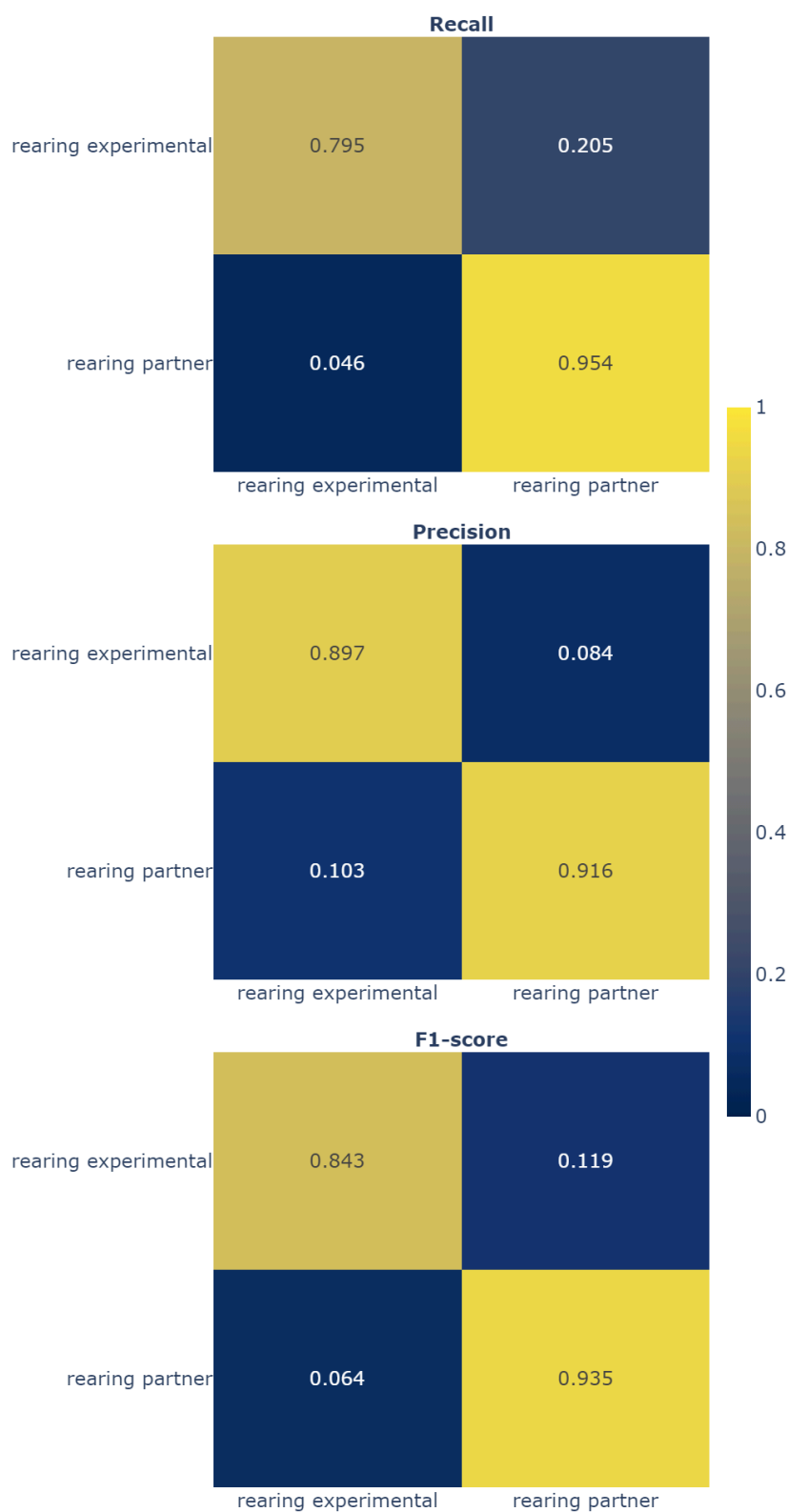
With both multiclass and binary classifiers we were able to successfully decode all of the analyzed behaviors and differentiate between the experimental animal's and partner's event behavior. For the onsets of the events, F1-score confusion matrices presenting the results are shown on Figure 19 (for all classified events) and Figures 20 and 21 (for the binary classifiers). For the offsets of the events, F1-score confusion matrices presenting the results are shown in Figure 22. For the multiclass classifier, the chance level was **0.166** for each class - due to sample weighing - and for the binary classifiers, it was **0.5**. To fully present the model's performance, we also show the recall and precision matrices, where recall represents the fraction of retrieved relevant instances, precision represents the fraction of relevant instances among the retrieved instances, and the F1-score is their harmonic mean [Figures 19, 20, 21, 22]. Both the onsets and offsets of behaviors for both the self and the partner have been decoded with greater accuracy than chance levels. Notably, CS was no longer encoded at the offset, which indicates a difference in its processing compared to social stimuli.



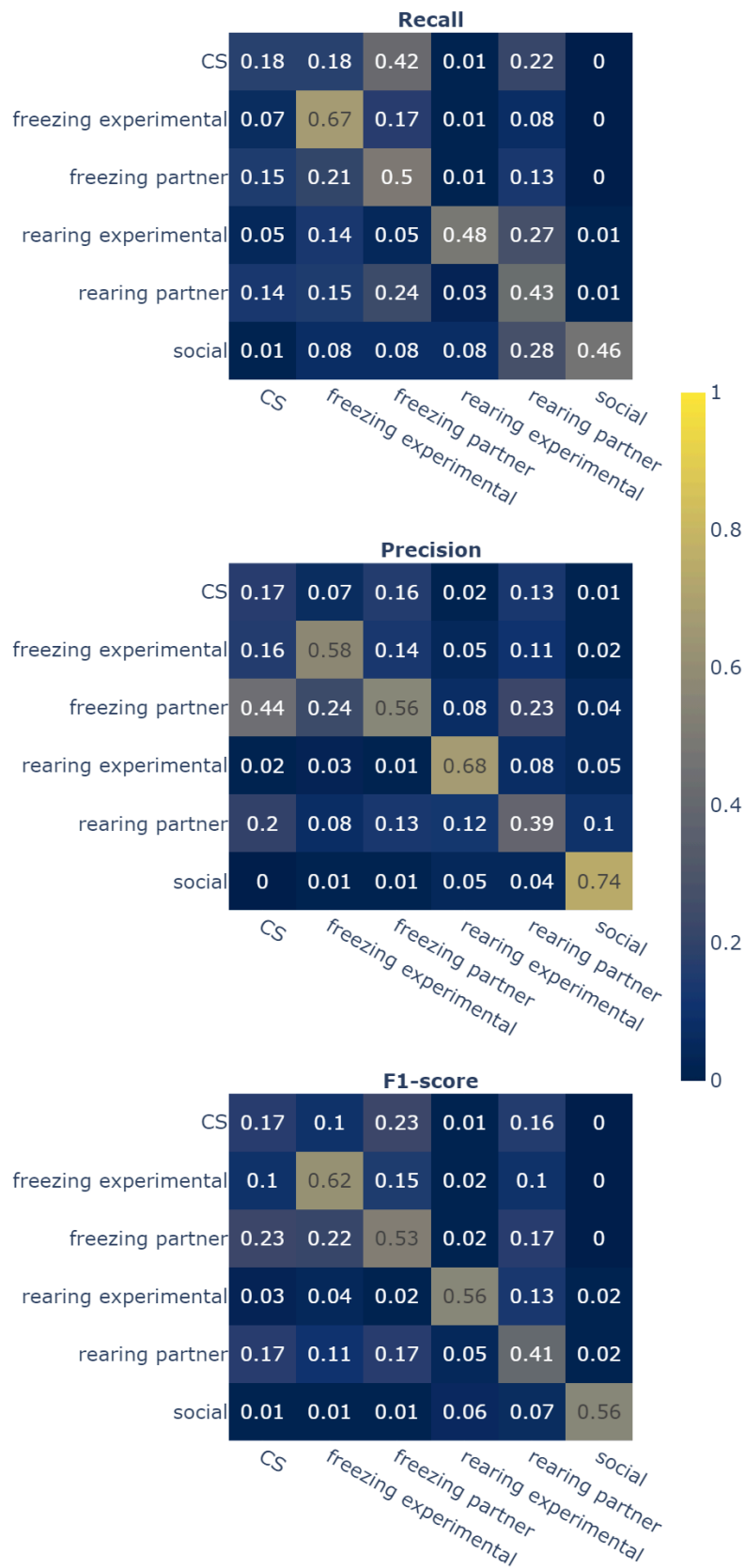
**Figure 19.** Multiclass classification confusion matrices for all classified events.



**Figure 20.** Confusion matrices of the binary classification for the performed and witnessed freezing.



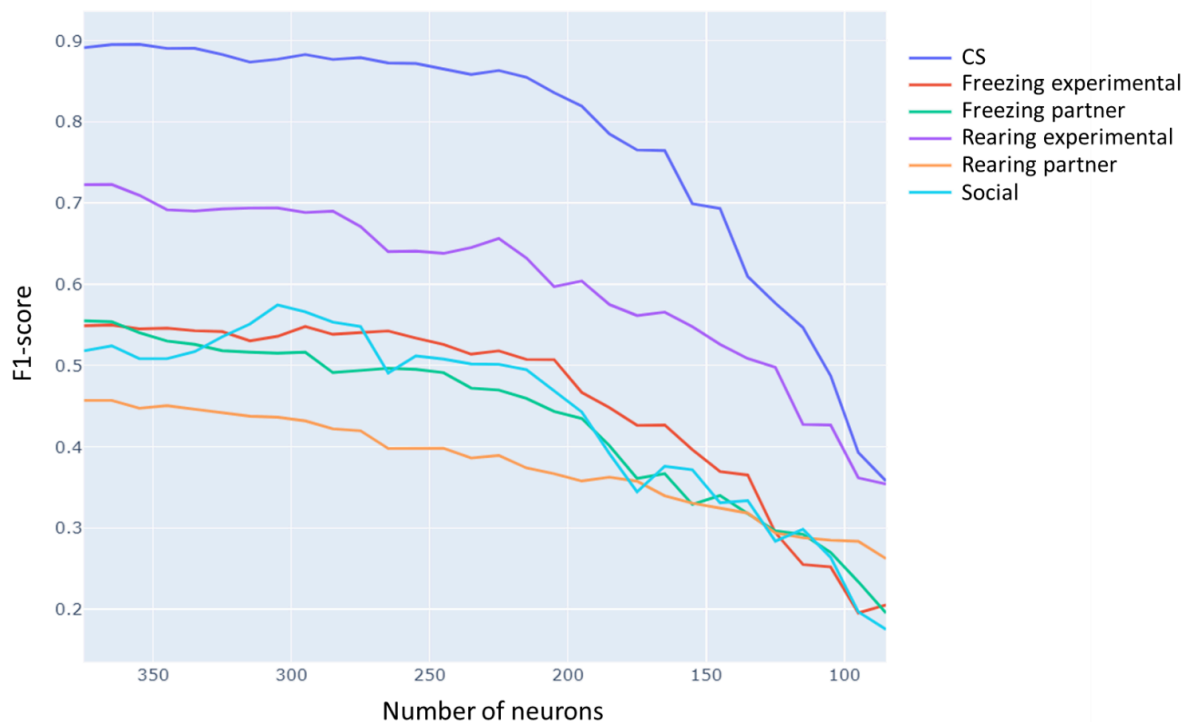
**Figure 21.** Confusion matrices of the binary classification for the performed and witnessed rearing.



**Figure 22.** Decoding performance on offsets of the classified events.

In sum, to address the limitations of single-cell analysis, we employed a machine-learning approach to decode the onsets and offsets of six behavioral events. We successfully differentiated between the experimental animal's and partner's behaviors with greater accuracy than chance level. Additionally, the analysis that the conditioned stimulus was not encoded at the offset, highlighting its distinct processing compared to social stimuli.

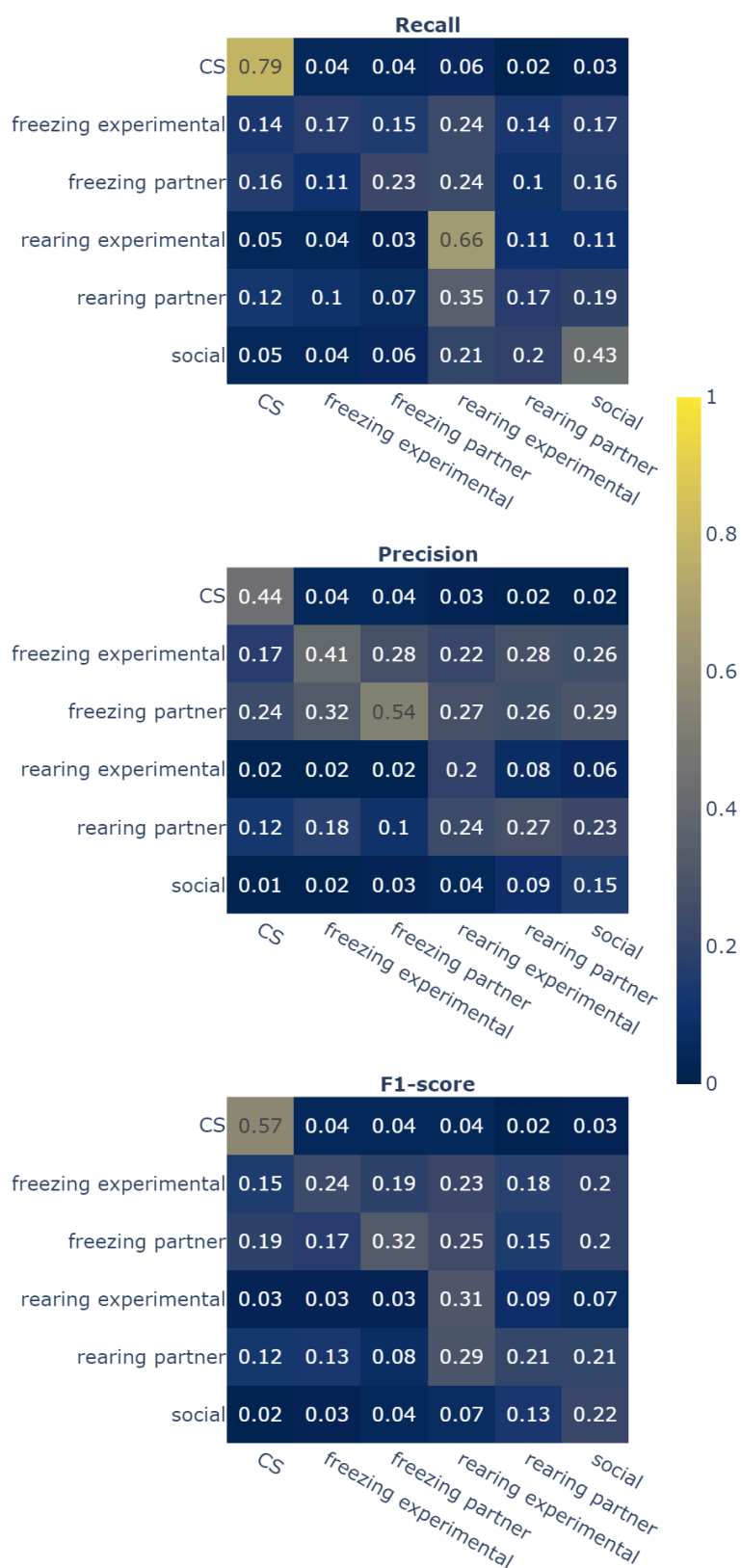
To further test whether this coding is led by a few neurons or is coded by the state of the whole population, we trained 30 additional runs of 50 models per run, removing the 10 most important features (neurons) at each run. Removing the most important features slowly decreased the accuracy, with a sharp decline at around 50% of the population size. This indicates that even when the most important, most responsive cells are discarded, the behaviors can still be decoded from the leftover population.



**Figure 23.** Change in the decoding performance with removing the most important features per run (10 neurons per run).

To assess how the model would perform using only the neurons with the most prominent responses to decode the events, we ran the training in a manner similar to the previous runs of 100 shuffles. We chose the 10 most responsive neurons per classified event.

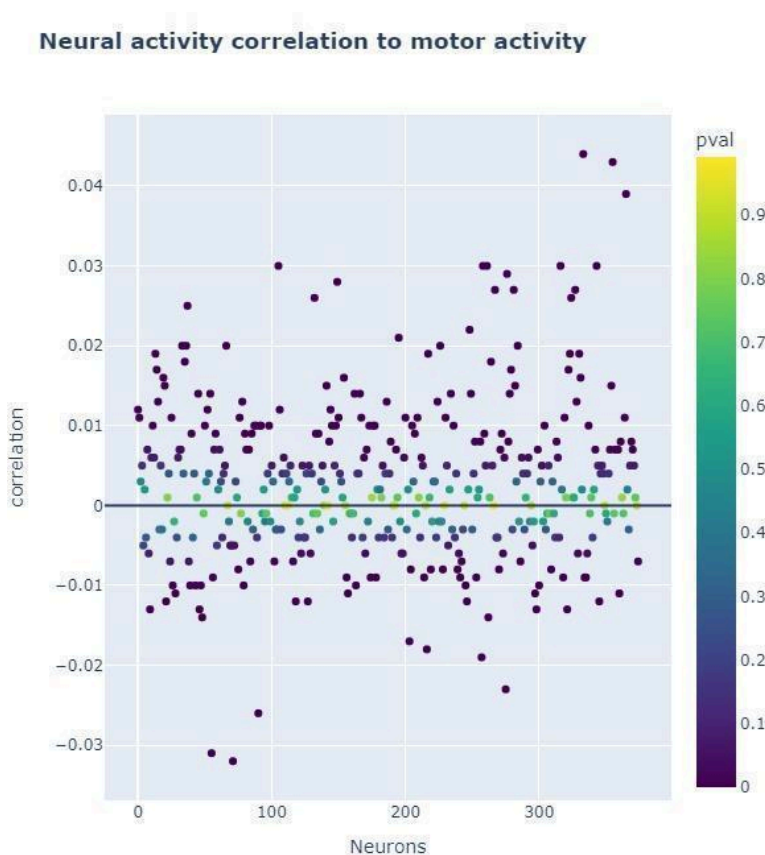
As expected based on the previous results, the model performed poorly, with low decoding performance and high confusion between classes. The results are presented in Figure 24. This overall low performance in the behavioral classes compared to CS decoding indicates a more population-based coding scheme.



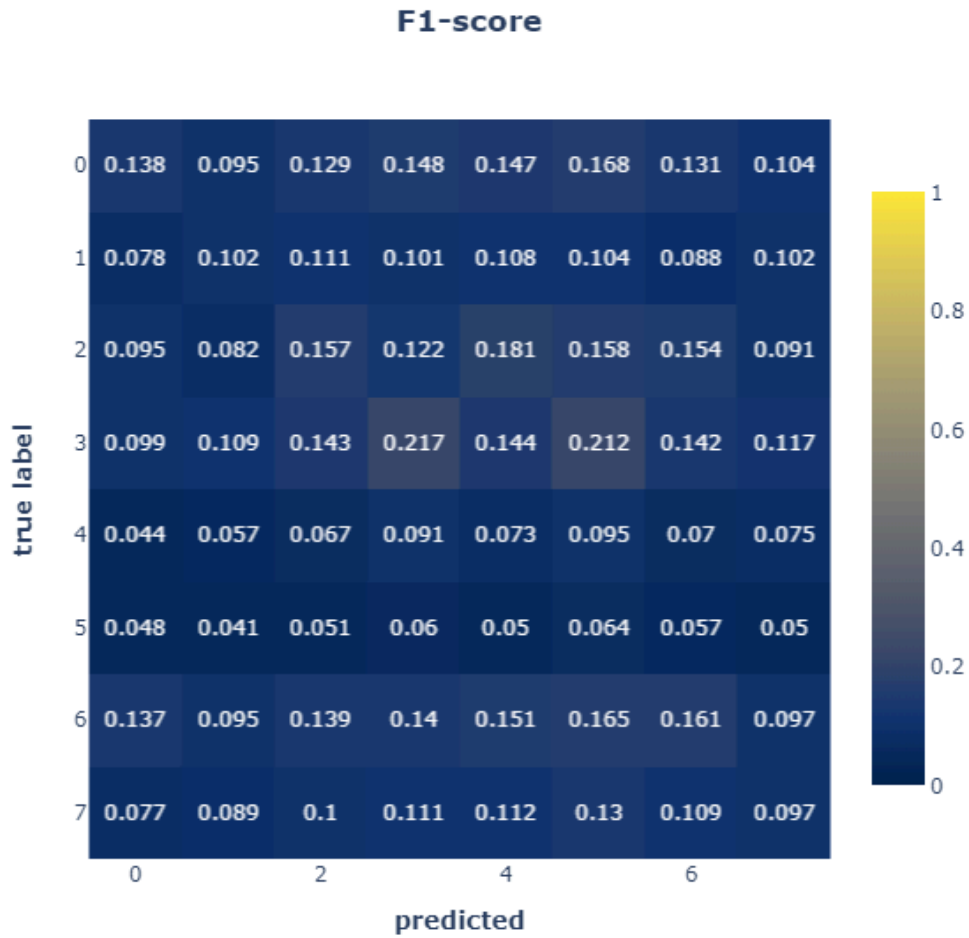
**Figure 24.** Confusion matrices of the classification are based on the 10 most responsive neurons per class - 49 neurons in total (one overlapping cell).

## 4.6. Correlation of kinematic features with neural activity and pose-related feature decoding

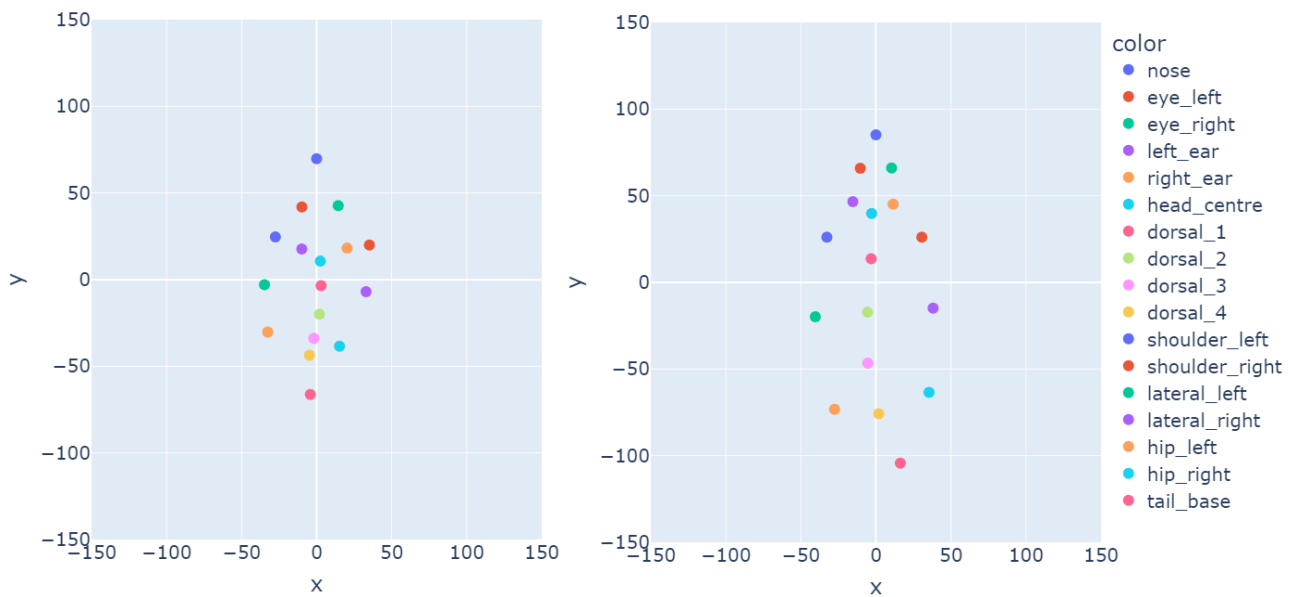
To test whether the encoding was, in fact, for a highly abstract behavioral class rather than just simple kinematic features - such as changes in acceleration (representing starting and ending movement, key components of freezing and rearing) - or merely simple pose clusters that repeated across the recordings, we performed two additional analyses. We showed no significant correlation between neural activity and acceleration [Fig. 25]. Furthermore, when we performed k-means clustering on standardized poses - fixed body direction - and trained our model on the discovered clusters, we found that almost no classes had been decoded above the chance level. A few classes that performed best upon further investigation were found to represent various forms of rearing [Fig. 26]. An example of the cluster with the best decoding performance is presented in Figure 27, showing the central sample of the cluster.



**Figure 25.** The correlation of the neural activity (firing rate) and velocity is presented as the per-neuron correlation value, with the color representing significance.



**Figure 26.** F1-score confusion matrix showing the decoding performance of a model trained on clusters of the animal pose.



**Figure 27.** Visualization of the best-decoded cluster center showing a rearing behavior - on the left. Resting posture for reference on the right.

In sum, to determine whether neural encoding reflected abstract behavioral classes rather than simple kinematic features or repetitive pose clusters, we conducted additional analyses, which revealed no significant correlation between neural activity and acceleration. Furthermore, k-means clustering of standardized poses resulted in minimal decoding above chance levels.

## 5. Discussion

We designed a behavioral paradigm in which the behavior of previously fear-conditioned rats (hereafter called experimental) was tested in the presence of a partner that had undergone fear conditioning followed by fear extinction. This paradigm allowed us to measure neuronal responses in the medial prefrontal cortex (mPFC) to two types of emotional behaviors - freezing and rearing - displayed by the experimental rat or its partner. In this paradigm, freezing reflects a higher level of negative arousal, while rearing indicates a lower level. This setup enabled us to assess the responses of single neurons to both passive (freezing) and active (rearing) behaviors exhibited by the experimental and partner rats.

Our data demonstrate that during social interactions in which both rats had similar prior experiences, i.e., were fear-conditioned, and the partner provided the experimental rat with relevant cues about threat level, the population activity of mPFC neurons reflected not only the behaviors of the experimental animal itself but also that of the partner. Both onsets and offsets of behaviors of the self and the partner have been decoded above the chance level. Notably, the CS offset was not decoded well, implying that other mechanisms are at play in the transition of non-social context-related events. Taken together, we show that behavioral state transitions are encoded in the mPFC at the population level.

Through our experiments, we provide the first evidence of population coding of specific behaviors of conspecifics in the rats' mPFC. These findings have several important implications. First, our data demonstrate that single cells in the mPFC respond reliably to some events, particularly to the animal's own behaviors, suggesting that a subpopulation of cells may serve as preferential coders of these events. In contrast, responses to the partner's behaviors were less distinct, with only a few cells clearly responding to the onsets of the partner's actions. Nevertheless, the partner's behavior could still be decoded from the population activity. Importantly, we found no evidence for mirroring at the population level, as indicated by distinct neuronal trajectories for own vs. partner's behaviors, as well by low confusion level of the classifier when distinguishing between the two.

Notably, the detailed analysis of our model corroborates these observations: decoding accuracy declined sharply when only the most responsive, event-specific cells were used,

leading to strong class confusion, highlighting the challenge of decoding partner-related behaviors from single-cell activity [Fig. 22]. Given the interconnected and integrative nature of the mPFC, along with literature suggesting that stable population coding underpins working memory despite dynamic neural activity (Murray et al., 2016), and evidence for the dynamic coding of multiple stimuli at the population level in the mPFC (Meyers et al., 2008), our findings support the idea of population coding for both self and partner behaviors in the mPFC. This interpretation moves away from a mirror neuron-based explanation and emphasizes the broader integrative function of population-level dynamics in the mPFC, which I discuss below.

A more detailed subdivision of the medial prefrontal cortex (mPFC) is often discussed in this line of research. This is because the prelimbic cortex, infralimbic cortex, and anterior cingulate cortex have all been shown to have distinct properties, fulfill different functional roles, and maintain unique connections to other brain regions. In our experiments, over 75% of the recorded neurons were located within the prelimbic cortex. However, we did not observe any differences in the proportion of responses to specific events or in the manner in which the neurons responded to these events. However, it is worth considering that this lack of observed differences could stem from the relatively small number of recorded cells in our study. Detecting subtle distinctions might require testing a larger sample size or including more animals.

Finally, it is worth emphasizing that this research was only made possible by the development of high-throughput tools and advanced methods of data analysis, such as Neuropixels and DeepLabCut. However, the unprecedented level of detail and the sheer volume of data provided by these technologies present unique challenges, which are discussed below.

### **5.1. Rat mPFC encodes both own and partner's behaviors**

Evidence of observational learning and extracting information from social cues is already present in the literature. For instance, rats have been shown to learn a fear response to a stimulus through observation, a process related to the thalamus-ACC pathway modulated via the basolateral amygdala (BLA) (Keum & Shin, 2019). Additionally, they lower their fear response in the presence of a familiar individual, a phenomenon called social buffering

(Kiyokawa et al., 2004; Górkiewicz et al., 2023). Rats also recognize their conspecifics and their social status (Winslow & Insel, 2004). Furthermore, they modulate their defensive behavior through social cues (Knapska et al., 2006; Knapska et al., 2010). To date, however, little is known about how the brain encodes these social features, particularly the behavior of others.

Recognizing the behaviors of others is performed using multiple modalities. Visual and auditory cues are considered the primary modalities in humans (Haxby et al., 2002). However, in rodents, olfactory signals may play a more significant role (Monfils & Agee, 2019), as studies on social interactions indicate increased nose-to-nose contact and anogenital sniffing (Thor, 1978; Boehm & Aron, 1990; Wolfe et al., 2011). This multimodal nature of social interaction makes the mPFC a prime candidate in the search for the coding of behaviors of others, as it is one of the key brain regions that integrate those multimodal signals.

Previous studies examining other brain areas, such as the secondary motor cortex and posterior parietal cortex, have demonstrated that these regions modulate self-generated behavior in a stable manner but have not identified any coding of observed behavior (Tombaz et al., 2020). This may reflect the possibility that observed behaviors are coded in higher associative areas rather than in secondary cortices, which were the focus of those studies. This highlights the importance of recording neural activity in higher associative areas, such as the mPFC, which may be more involved in the processing of observed behaviors. Another possible explanation for the absence of coding for observed behavior is that this research was conducted on mice, which exhibit social behaviors that differ from those of rats and may be less attentive to the actions of others (Netser et al., 2020). As a result, the observer in those studies may not have been fully attentive to the demonstrator's behavior. In contrast, our use of rats and the implementation of the experiment within the behavioral paradigm we developed provided the experimental rat with a compelling reason to pay attention to its conspecific.

In experiments like ours, a critical control is the clear distinction between the behaviors of the self and those of others, as this plays a major role in drawing meaningful conclusions. To ensure the reliability of our findings, we excluded trials where the onset of behaviors in the two individuals occurred in close temporal proximity. This approach minimizes the likelihood of overlapping signals and ensures that the trials analyzed

correspond to distinct behavioral events. Therefore, when a cell is found to respond to the same behavior in both the experimental animal and its partner, we can be confident that this response is not confounded by simultaneous or near-simultaneous behavior from both individuals. This stringent control strengthens the interpretation of our results.

## **5.2. mPFC encodes behaviors but not simple kinematics**

Through our experiments, we have demonstrated that simple motion features, such as velocity and acceleration - key components of both freezing and rearing - are not encoded in the mPFC in relation to these behaviors. Instead, it seems that the mPFC encodes a general type of behavior rather independently from its kinematics.

While it may seem intuitive to propose that mirror neurons facilitate the encoding of information between individuals - where a subpopulation of cells responds to the initiation of similar locomotor behaviors in both the self and others - our study challenges this notion. We identified only three cells that appeared to respond to the same behavior, irrespective of whether it was executed by the experimental animal or its partner. This finding suggests that, although the movements of another animal could potentially be coded at the neuronal level, they are not represented prominently in the single cell activity of the experimental subject as one might expect.

Our findings also contrast with what mirror neuron literature predicts, particularly in studies conducted in motor areas where neurons are typically grouped based on their preferential coding of specific aspects of movement or behavior (Rozzi et al., 2008). In our case, however, we observed that such low-dimension coding does not apply. Our results indicate that the initiation or cessation of motion - key components of freezing behavior - are not correlated with neural activity and that the kinematic features themselves cannot be reliably decoded. When we clustered the poses of the animals, we found that only a few clusters could be decoded above chance levels, and further analysis revealed that these clusters primarily corresponded to different forms of rearing behavior. This is similar to what has been shown with motion sequencing approaches, which suggest that neural activity in mPFC is more aligned with coding complex behavioral phenotypes rather than individual motion features (Wiltchko et al., 2020; Ehret et al., 2024). This finding raises a significant problem - in studies involving more associative brain areas - analysis related to

low-dimensional kinematic features of the behavior can be insufficient to find this region's neural correlates. Therefore, it is crucial to identify ecologically relevant behaviors that are essential for the animal's survival and well-being, as these behaviors are likely to activate higher associative areas of the brain.

Our findings provide further evidence supporting the integrative and executive function of the mPFC. But why would behaviors be encoded in the mPFC at all? An interesting perspective is provided by Justin Fine and Benjamin Hayden (Fine & Hayden, 2021). They argue that this unique property of the prefrontal cortex as a hub of multimodal processing and integrative processes positions it as a sort of premotor cortex. This does not imply that the mPFC is directly involved in coding motion features but rather that it functions as a constant feedback loop with other brain regions. This approach ensures that no single neuron dominates the decision-making process to the extent that it leads to an erroneous response. According to this framework, the mPFC weighs various signals and provides motor control regions with information about the most appropriate behavioral response based on context and all available information, occupying a top position in a hierarchy of information processing and integration.

As an executive control center, the mPFC offers flexibility in its coding, dynamically adjusting to subtle changes while maintaining an overarching context. For example, it can encode a partner's behavior despite minor variations in how it is expressed as long as the behaviors share sufficient similarity and serve a comparable function. It is especially vital for reliable understanding of behavior, like in our experiment, where the emotional context plays a role in modulating the meaning of the partner's behavior and how the experimental animal can understand it.

### **5.3. Ensemble state rather than specific neurons code animal behavior in mPFC**

We found that behavior in the mPFC is encoded by ensemble states rather than by specific neurons. This does not imply that individual neurons do not exhibit preferential responses to particular events or code for single events outside of social context. In fact, this preferential coding may ensure stability in the working memory through a dynamically changing environment. In other words, a subpopulation of neurons with a stable response stabilizes the otherwise dynamic nature of coding within the mPFC (Murray, 2017). For instance, in our results, the specific population activity that codes for the partner's freezing or

rearing behavior is important for understanding the threat level related to the CS exposure in a new context.

These findings corroborate other studies showing that hippocampal inputs to the mPFC modulate freezing in observers during observational fear learning and that the mPFC coordinates long-range neural circuits through projections to the midbrain periaqueductal gray (Silverstein et al., 2024). Notably, this study also showed that the populations of neurons activated by fear conditioning and observational fear are largely distinct. This further demonstrates that mPFC population coding reflects the presence of a threat based on the observed behavior of a conspecific.

We also observed neurons that appeared to code for multiple functions, including three cells that could be considered mirror neurons. In our decoding analysis, we did not discard neurons that did not show any coding or statistically significant response in order to maintain a larger recorded population. This approach allows us to investigate whether these seemingly unimportant neurons, when considered in the broader context of population activity, could still play a role. This multidimensional approach seems more appropriate, as it does not rely solely on neurons exhibiting evident excitation or inhibition but rather on how, when taken together, they can be used to decode the animal's behavior or perception. Conversely, when important features of the decoder are selectively removed - clearly responsive cells and mirror neurons - the decoding performance is upheld reliably until most of the population is discarded [Figure 19, 23].

The importance of such a solution is evident from the perspective of a working neural network - it is less prone to biases and the influence of a single cell's output, which increases the likelihood of reaching an optimal solution. Additionally, this approach can better differentiate between behaviors that may be similar motorically but serve distinct purposes.

Another implication is that population coding may provide a more robust approach to learning from the behaviors of others. According to the theory of mirror neurons, it is the activation of the same cells during performing the behavior and watching the behavior being performed that allows us or other animals to understand it. We would argue, though, that the behavior can be understood without mirroring on a single-cell level. As we show, the behaviors of others can be decoded from the activity of the population even when most responsive neurons are removed, including the putative mirror neurons [Figure 19].

#### **5.4. Population coding as a means to understand others**

Based on our results and relevant literature on mPFC neural activity, a new hypothesis can be proposed: the behaviors of others are encoded by the neural population in a context- and state-dependent manner rather than by the activity of specific cells. This suggests a dual role for the mPFC:

1. Maintaining a stable population activity that represents high-dimensional features of behavior;
2. Embedding this behavioral representation within the current context.

This dual functionality supports behavioral flexibility through a feedback loop that regulates the initiation and termination of behaviors, akin to a cost function where context sets the threshold for switching behavioral states. Social information from others contributes to the context but is not represented in a stable manner, explaining why few cells explicitly encode it. However, as partner behaviors share common features, the neural population's activity over time reflects these similarities, enabling decoding of a partner's behavior from the experimental rat's neural activity.

Within this hypothesis, understanding others' behaviors is not innate, as the mirror neuron theory suggests. Instead, it involves a dynamic computation of prior experience, current context, and context-specific social information others provide, intentionally or unintentionally.

#### **5.5. The implementation of novel experimental tools: advantages and associated challenges**

New tools are essential for advancing our understanding of large neural populations and their dynamic properties. These populations can be represented in latent spaces using non-linear approaches such as contrastive learning and 1D convolutions, as demonstrated in CEBRA (Schneider, 2023). However, interpreting this data and applying appropriate methodologies remains challenging. Key aspects for studying neural ensembles and information processing include analyzing the geometry of neural population activity, identifying similarities between these geometries, and tracking dynamic changes in the trajectories of information representation over time. These methods bridge the gap between

neuroscience and mathematics, where linear algebra, geometry and mathematical morphology can be used extensively to both analyze and model inner workings of biological systems.

Simultaneously, the field of behavioral neuroscience has undergone significant advancements in recent years. While manual annotation of behaviors remains the gold standard, emerging supervised learning approaches based on transformer architectures offer a new potential, enabling behavior analysis to become more efficient, reproducible, and scalable across studies. Among those innovations are also heuristic or unsupervised approaches like motion sequencing and kinematics based analysis. New hierarchical models (Stoffl et al., 2025) that examine behavior across multiple timescales can further enhance our understanding by providing richer insights than traditional ethological methods, reducing human bias and mitigating anthropomorphic interpretations of animal behavior.

Each method comes with its own set of potential benefits and drawbacks. Though supervised approaches provide more explainability and are easier to adapt by providing straightforward results they are often time consuming to set up and data hungry. Training even a simple gradient boosted random forest model for behavioral classification can take upwards of 30 thousand samples per behavior to achieve sufficient generalization performance. On the other hand, unsupervised approaches like motion sequencing, clustering or self-supervised approaches in neural networks do not require ground truth data which makes them very simple and quick to implement. Their drawback though is that the interpretability of the results may be complicated - very often finding a specific time scale within which we might want to observe behaviors would be crucial. Sometimes classes found by those methods are not distinct enough for us and we might want to combine them into one to increase explainability - striking a balance between the fine differences that the model can find and our need for explainable behavioral types can be extremely challenging.

## **5.6. Limitations and further research**

Our study has several limitations. First, experiments involving fear extinction allow for only one recording before the behavioral response changes, which restricts the number of trials that can be recorded. Additionally, it is essential to control the experimental context with a specific partner in a specific environment, further limiting our ability to increase the

number of trials, as recordings need to be relatively short. Moreover, the freely moving nature of the recorded events introduces variability in the number of observations. One potential solution to address these limitations is to increase the number of animals recorded. This would be particularly beneficial for our analysis approach, which combines data from multiple animals.

Another key limitation is the difficulty in precisely subdividing the mPFC due to the absence of high-resolution brain atlases for rats. This constraint hinders our ability to accurately define subdivisions and pinpoint the exact locations of recorded neurons at a finer anatomical level, in particular at the layer level.

Our results open the door to interesting further research - for instance, would the embedding of the population encoding a specific behavior of a partner remain similar if the context is changed? In other words, is the population state stable across contexts when encoding partners' behaviors, and is it stable for behaviors of self? We would argue that it is most likely not, specifically in the mPFC, since, as we discussed, a major role of the mPFC is the integration of information about the context, which would most likely change its activity. An interesting way to test this would be to check for coding of behaviors in multiple contexts, for instance, by changing the recording conditions and cage layout with the removal of CS to manipulate the emotional context during the recording. One limitation could be that the experimental animal would no longer pay any attention to its partner, but this could potentially be fixed with short social isolation and interaction in a novel context. Another potential experimental design could be a transfer of information about food localization.

# Summary and Conclusions

Understanding how the brain processes the behaviors and emotional states of others is critical to discovering how humans and other animals navigate social contexts, particularly in response to threats. Social dynamics can modulate fear responses, offering potential mechanisms for attenuating fear through interaction.

In this study, we reveal that the medial prefrontal cortex (mPFC) encodes both self-generated and observed behaviors, but not through a fixed set of mirror neurons, as previously speculated. Instead, this process occurs at the population level, suggesting a more distributed and dynamic form of neural coding. This discovery reshapes our understanding of how the mPFC integrates and interprets the behaviors of others.

We also introduce a novel application of the social buffering paradigm, an experimental design particularly well suited for studying social behavior coding. This paradigm enables the multimodal transfer of information between individuals. It leverages its inherent anxiolytic properties to motivate social interactions, which are vital in studies of neural correlates of social behavior.

Our findings suggest that ensemble coding in the mPFC equips animals with a robust and flexible mechanism for interpreting social signals. This adaptability is essential in complex and rapidly changing environments, allowing animals to evaluate threats and refine their responses by continuously monitoring social cues. This dynamic processing underscores the profound role of social interactions in survival and emotional regulation.

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# Publication record of the PhD Candidate

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