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Social transmission of information about the localization of food in rodents and its influence on the hippocampal representation of space

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Abstract

Social partners are an important source of information for animals. By interacting with them, the animals can learn about the properties of the environment such as the presence of mates, food or predators. While we know that rodents have complex repertoires of social behaviors and are capable of transferring information about danger or the quality of food, there is no data on the ability of rodents to gain information about the spatial distribution of resources. The goal of this thesis was to develop a behavioral paradigm, based on the place preference test, to study the ability of rats and mice to detect the source of the food based on the social cues obtained during an interaction with a conspecific.

We show that mice and rats can acquire information about the location of food and use this information to guide their exploratory behavior. We show that the potential channel of the information transfer is related to chemical cues emitted in the anogenital region of a conspecific.

Using single-photon calcium imaging in freely moving mice, we show that the transmission of a social cue leads to the reactivation of the hippocampal cells encoding the food location and hypothesize that this reactivation may be an instance of planning of the exploratory behavior.

Furthermore, we used fiber photometry to measure the responses of the olfactory tubercle, a structure related to the processing of the appetitive aspects of smell. We show that while it responds to nose-nose contacts, its activity increases during interactions with previously fed animals, regardless of whether they provide information about food localization. We also used whole-brain c-Fos expression to find structures potentially engaged in the process of the detection of social cues. We show that two structures - tuberomammillary nucleus and premammillary nucleus may be engaged in the behavior observed in our paradigm. We hypothesize that their role may be related to processing the social cues in the context of the energy balance of an organism and the prediction of energy expenditure.

We propose our paradigm as a tool for studying the relationship between social behaviors, spatial navigation, homeostasis and predictive coding of energy expenditure in mammals.

Streszczenie

Inne osobniki są dla zwierząt ważnym źródłem informacji o właściwościach środowiska, takich jak obecność partnerów, pożywienia lub drapieżników. Chociaż wiemy, że gryzonie mają złożony repertuar zachowań społecznych i są w stanie przekazywać informacje o niebezpieczeństwie lub jakości pożywienia, nie ma danych na temat ich zdolności do zdobywania informacji o przestrzennym rozmieszczeniu zasobów. Celem tej rozprawy doktorskiej było opracowanie paradygmatu behawioralnego, opartego na teście preferencji miejsca, który mógłby posłużyć do zbadania zdolności szczurów i myszy do wykrywania źródła pożywienia na podstawie wskazówek społecznych uzyskanych podczas interakcji z innym osobnikiem.

W niniejszej pracy pokazujemy, że myszy i szczury mogą gromadzić informacje o lokalizacji żywności i wykorzystywać te informacje do kierowania swoimi zachowaniami eksploracyjnymi. Pokazujemy, że potencjalny kanał transferu informacji jest związany z chemicznymi sygnałami emitowanymi w okolicy anogenitalnej drugiego osobnika.

Wykorzystując obrazowanie wapniowe u swobodnie poruszających się myszy wykazujemy, że transmisja sygnału społecznego prowadzi do reaktywacji komórek hipokampa kodujących lokalizację żywności i stawiamy hipotezę, że ta reaktywacja może być przykładem planowania zachowania eksploracyjnego.

Ponadto wykorzystaliśmy fotometrię do pomiaru odpowiedzi guzka węchowego, struktury związanej z przetwarzaniem apetytywnych aspektów zapachu (Munger i in., 2020). Pokazaliśmy, że struktura ta odpowiada na kontakty nos-nos niezależnie od tego, czy w trakcie interakcji dochodzi do transferu informacji o lokalizacji pokarmu.

Wykorzystaliśmy również analizę ekspresji białka c-Fos w całym mózgu, aby znaleźć struktury potencjalnie zaangażowane w proces wykrywania sygnałów społecznych. Wykazaliśmy, że dwie struktury - jądro guzkowo - suteczkowate i jądro przedsuteczkowate grzbietowe mogą być zaangażowane w zachowanie obserwowane w naszym paradygmacie. Stawiamy hipotezę, że ich rola może być związana z przetwarzaniem sygnałów społecznych w kontekście bilansu energetycznego organizmu i przewidywania wydatków energetycznych.

Proponujemy opracowany przez nas paradygmat jako narzędzie do badania związku między zachowaniami społecznymi, nawigacją przestrzenną, homeostazą i predykcyjnym kodowaniem wydatków energetycznych u ssaków.

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Introduction

Foraging is crucial to survival. Many animals, including rodents, spend a significant amount of time foraging. For example, in Korean wood mice (*Apodemus peninsulae*), foraging takes up to 40% of time (Hao et.al., 2020). Efficient foraging poses a challenge for animals. They have to locate the food in the space, navigate to the spot, estimate the time they should spend in the feeding site before the food will get depleted, all the time avoiding detection by a predator (Pyke, 1985). Finding food takes time that could be used for other important activities, such as mating and puts at the risk of predation, which generates a cost for a foraging animal (Kacelnik, 1984); it is thus beneficial for an animal to invest in mechanisms that could allow it to locate food faster and more efficiently.

Laboratory and field studies show that animals indeed develop optimal foraging strategies that maximize food intake and minimize risk from predators. For example, in open habitats mice tend to leave a foraging patch faster than in a forest environment, where they are less visible to predators (Morris, 2000); deer mice and voles make decisions regarding the distance to the foraging site they are ready to cover based on the potential risks (Anderson, 1986). In a task that requires mice to locate food in an environment with dynamic odour plumes, mice develop an efficient strategy that combines stereotyped responses and flexibility, resulting in the reduction of time necessary to locate food (Gire et.al., 2016).

But while personally acquired information is often reliable, it comes at a cost: the animal has to perform a search itself, and can only gain information about one spot at a time (Dall, 2005). Animals living in groups can take advantage of public information about the localization and quality of the food patches in the environment (Danchin, 2004; Galef & Whiskin, 1999; Ward & Zahavi, 1973). Guppies in larger shoals find the hidden food faster than those in smaller shoals (Day, 2001). Hooded crows follow their more successful roost-mates to feeding sites (Sonerud, 2001), starlings observe others to gain

information about the food patch quality (Templeton & Giraldeau, 1996).

The influence of social information on foraging strategies was also observed in laboratory rodents, which opens the possibility of studying those behaviors in a laboratory setting and with the use of neuroimaging methods to explore the neural underpinnings of such behaviors. There is especially vast literature of the social transmission of food preferences in rats and mice. Rats that interact with a conspecific that ate flavored food will develop a preference for food with this flavor (Galef & Wigmore, 1983). The paradigm is called Socially Transmitted Food Preference (STFP). The same effect has been observed in mice (Valsecchi, 1989). What is interesting, the preference was developed even if the Donor was anesthetized, but not if the flavor was sprayed over a piece of cotton (Valsecchi, 1989). It was found that carbon disulfide, a substance present in the breath of animals that consumed food when added to food, leads to the development of food preference (Bean, Galef, & Mason, 1988). Specialized olfactory sensory neurons (OSN) in the olfactory bulb are highly sensitive to carbon disulfide and the blockade of their function in mice leads to a lack of ability to develop food preference (Munger, 2010). Injection of D1-type dopamine receptor antagonists in the dorsal hippocampus impairs food preference learning, suggesting their role in the process (Matta, 2017). Moreover, synaptic connections from the anterior olfactory nucleus (AON) to the olfactory bulb are selectively necessary for the acquisition of the preference (Wang et.al., 2020).

Rodents can also improve their foraging strategies by direct observation of conspecific behavior. Rats can learn to dig for buried pieces of food more efficiently if paired with another rat that was previously trained to dig (Laland & Plotkin, 1990). In other species of rodents, learning how to open nuts by red squirrels is facilitated by observation of experienced conspecific (Weigl, 1980). Golden hamsters learn from others how to use their teeth and forepaws to retrieve food pieces (Previde, 1996).

On the other hand, much less is known about the ability of rodents to gain information regarding the localization of food during a social interaction. Rats develop a preference for bowls marked by excretory deposits of conspecifics (Laland & Plotkin, 1991). Rats coming back from food site deposit a trail that can be used by conspecifics to guide their navigation to this site (Galef & Buckley, 1996).

In an especially interesting experiment, Galef and Wigmore (1983) have shown that rats can transfer information about the location of a distant food source after training. In

their paradigm, rats (observers) were put in a maze with three arms. Pieces of food of different flavors were put at the end of each arm. Rats learned the localization of each food piece during several days of training. Then, during the testing session, food was placed in only one of the arms, and the success rate of rats going directly to the food location was assessed - as rats did not have any information regarding the localization, their success rate was around 33%. In the next phase, rats interacted with a Donor that had eaten food of one of the flavors. A piece of food of this flavor was put in its location and the rats were again tested on their ability to locate food - after the interaction with a conspecific, their success rate increased significantly (Galef and Wigmore, 1983).

In this experiment, rats were able to use social cues to locate food. What is important though, this effect relied on extensive previous personal experience and knowledge about the presence of different types of food in different locations that were reliably found there. There is currently no data showing the ability of rodents to gain information about the new localization of food and by animals without previous knowledge of food localization in the environment. As the availability of food in the environment is often unpredictable and sources of food can appear in new locations (Waltz, 1983), such ability would be especially beneficial - it may decrease the time necessary for foraging.

The development of behavioral paradigms allowing to study the social transfer of information about the localization of food or other resources could be especially useful in bridging the gap between two branches of neuroscience - social neuroscience and studies on spatial cognition.

It was recently pointed out by Parra-Barrero et al. (2023) that while we gained important information about spatial representations in the brain, the knowledge about how those representations are used to guide meaningful behavior is largely missing due to lack of communication between the fields of neuroscience and the study of behavior. On the other hand, studies on social behavior are often conducted in artificial paradigms that do now allow to study how socially acquired information is used to guide ethologically relevant behavior (Kondrakiewicz et.al., 2019). Combining the study of social behaviors with the study of spatial cognition - e.g. how socially acquired information shapes exploratory behaviors and the brain representation of space - could possibly address those shortcomings.

Spatial navigation in rodents

The studies on the neural basis of spatial cognition started with the discovery of place cells in the hippocampus by O'Keefe and Dostrovsky (1971). They have found cells in rats' hippocampi that had spatially-tuned patterns of activity, i.e. were active when an animal occupied a specified place in a cage, and different neurons had different preferred firing locations. Inspired by those results, and by the results of multiple other studies on the function of the hippocampus, they concluded that place cells form an abstract cognitive map of space that allows animals to navigate in the environment (O'Keefe and Nadel, 1978). Muller, Kubie and Ranck (1987) observed that some place cells change their firing locations after changing arena shape or size. Remapping was also observed after changing the colour of a cue placed in the arena (Kentros et al., 1998). Two types of remapping are described: rate remapping (related to changes of the firing rates of the cells) and global remapping (where the cell's place fields and firing rates are shifted between conditions) (Leutgeb et al., 2005).

Interestingly, remapping happens not only as an effect of changes in the physical layout of the environment. Moita (2004) trained rats in two similar (but not identical) areas in a simple foraging task. Place maps of cells in the CA1 region of the hippocampus were established; rats then underwent fear conditioning training in one of those arenas. It was shown that in the area where the conditioning took place, many cells changed their firing locations; the effect was not observed in the control arena (Moita, 2004). Similarly, Wang et al. (2012) exposed mice to the arena to record place cells activity and on the next day placed there a fearful stimulus (a coyote urine). It was shown that place cells in the CA1 remapped and the new representation remained stable for the next several days. Global remapping can be also caused by the presentation of social stimulus to rats (Alexander et al., 2016). Those studies show that the function of place cells might go beyond just the encoding of the physical space.

There are different hypotheses explaining the function of remapping. Some hypothesize (Colgin, 2008) that remapping allows animals to accommodate new information about the known environment or encode different contexts separately (Muller, Kubie, & Ranck, 1987). Interestingly, Saunders et.al. (2020) note that animals don't have

in fact access to the "objective" state of the context, and can only infer it based on sensory information. As they write, "*Key to this account is the idea that the relationship between observable properties of the environment (including context) and remapping is mediated by inferences about unobservable properties of the environment (hidden states). According to this view [...] place fields remap when the animal believes that it has entered a new hidden state. By specifying the animal's internal model of how hidden states relate to observable stimuli, we can make principled predictions about when, why and how place fields remap"* (Saunders, 2020).

In this view, the animal infers the state of the environment - if it assumes that the context remains the same, the hippocampal representation will remain stable. If the context is inferred to be changed, global remapping of cells will occur. On the other hand, partial remapping happens whenever the internal belief of the animal is not set on any of the two possibilities and lies somewhere in between.

As an example of results that may support this hypothesis Saunders mentions a study done by Lever et.al. (2002). Rats were placed interchangeably in previously unknown cages of two different shapes (square or circle), located in the same place in the same experimental room. Interestingly, initially, the level of remapping between those two cages was low; only with experience representations of those two contexts separated and global remapping occurred. It suggests that at the beginning the animals were unable to infer the difference between the contexts, as they didn't know the environment and many cues - the surroundings of the room - were identical between contexts. Only with time they infer that circular and square "contexts" were in fact different. Partial remapping occurred already in the first phase.

The question remains what context *is.* Saunders (2020) notes that it might be treated as an array of sensory cues, properties of the space and the location of the animal, or the task animal is engaged in. According to Kubie (2020), though, each hippocampal map may encode affordances as defined by Gibson (1987), according to whom "*affordances of the environment are what it offers the animal, what it provides or furnishes, either for good or ill".* Context defines then what an animal can *do; a* map encodes actions possible in a given environment: *each map, rather than being a determinant of specific behavioral outcome, is a prediction machine, indicating the range of behavioral possibilities* (Kubie, 2020). The inference performed by animals according to

Saunders (2020), reflected in the remapping of place cells, would be in this view an inference about the range of actions that animals can perform.

This hypothesis is hard to put to the test, though, in most of the existing behavioral paradigms used to study remapping. As mentioned above, they usually involve changes in the physical layout of the environment (Lever et.al., 2002); in this situation, it is impossible to disentangle the effects of the sensory cues and animal's inferences about its behavioral possibilities; remapping observed in such an environment might result from the change of sensory cues and be independent of any estimates about affordances. . One of the exceptions is study by Krishnan et.al. (2022). In this paradigm, head-fixed mice were running through a virtual corridor with a reward (water port) at the end. Three conditions were used: Reward condition, when the reward was present, Unrewarded condition, when the reward was suddenly taken away and Reintroduced Reward, when water was again available. They have observed a remapping of place cells from the Reward to Unrewarded cognition and suggested that it shows the influence of reward expectation influences the spatial map even in the unchanging environment (Krishnan et.al., 2022). There are two caveats, though: the very presence of water at the port is indeed a change in the physical environment, leading to a change in behavior. Moreover, as a head-fixed VR-based study it deviates from the naturalistic conditions and may not reproduce effects observed in the natural environment.

This shortcoming could be solved in a paradigm that would involve changing the belief of the animal about the state of the environment without any change of the physical layout of it. In this situation, animals would be provided with new information about the well-known environment that would otherwise not undergo any modification. Such information would need to relate to an important, behaviorally relevant aspect of the environment, and should be salient enough for the animal to be able to follow it without previous training. Socially acquired information about the localization of distant food or danger would give a perfect opportunity for the development of such a paradigm.

In such a paradigm, animal could receive a new social cue relating to a previously known environment from a conspecific. Such information would be acquired *outside* of this environment and would thus refer to a *distant location,* to ensure that no change in the physical layout of the space would occur. Social cues are especially useful for that purpose, as they are salient and their recognition very often does not require learning (Puścian et al.,

2022). In that paradigm, one could test how acquired information shapes the belief of the animal about the affordances provided by the environment and how this belief is reflected in the activity of place cells.

But the benefits of such a paradigm would extend beyond the development of a tool for studying the encoding of context by hippocampal neural assemblies. It was noted that hippocampal cells encoding a given context can be activated outside of this context in a phenomenon known as hippocampal replay (Ólafsdóttir et.al, 2018). For example, in the experiment conducted by Lee (2002) rats were moving through a space in a fixed trajectory. This trajectory resulted in the sequential activation of place cells encoding consecutive locations. The same sequence of cells activity was later replayed during slow-wave sleep (Lee, 2002). Replay occurs not only during sleep, though. Karlsson (2009) has put rats in two distinct, W-shaped environments. They discovered that sequential activation of place cells typical for one environment was replayed in awake immobile rats present in another environment.

The function of this replay is supposed to be the consolidation of existing memories (Ego‐Stengel, 2010; Gillespie et.al., 2021). There is evidence, though, that the hippocampal replay may also have a function in planning future routes through space. Specifically, Diba (2007) has found so-called forward replay events, when a sequence of hippocampal cells was replayed just before an animal started moving, suggesting a possible role in the planning of action. Moreover, Pfeiffer (2013) observed that when rats were moving towards a known reward location, replay events resembled the pattern representing path towards this location. Ólafsdóttir et.al. (2015) has also found that place cells that will encode a location in the future are activated before the first exploration of this space, during observation of it.

Thus, the paradigm based on the transfer of social information about the localization of food would be an excellent tool for studying also the role of hippocampal replay. Activity of cells encoding the location where food is supposed to be found could be recorded to check if the transfer of information about a given space activates its representation, which may prepare the animal for the exploration of that location.

Hippocampus and olfaction

While classically hippocampus is mostly assumed to be a structure related to memory and spatial cognition (O'Keefe and Dostrovsky, 1971), the first theories of hippocampal function assumed its role in olfaction (Sarnat, 1981). Jacobs (2012) suggests that the primary role of the hippocampus in early mammals was olfactory-guided navigation. For example, it was found that absolute size of the olfactory bulb covaries with the size of the hippocampus among animal groups (Reep, 2007). Another evolutionary argument for the support of this hypothesis is that in whales (e.g. toothed whales), in which navigation is based on echolocation, and which cannot rely on olfactory cues to guide navigation in water as they lack paired nostrils and use single nostril for respiration only (Kishida, 2021), the size of the hippocampus is about 8 - 20% of the size expected for a mammal of this size (Patzke et.al., 2015). Moreover, in humans, olfactory identification and spatial memory are correlated (Dahmani et.al., 2018).

The use of olfaction for spatial navigation is well-documented in rodents. Mice use olfactory cues to quickly learn the distribution of rewards in the environment (Gire, 2016);

In an environment with suppressed visual and auditory cues, the olfactory information is sufficient for the development of place fields of place cells in mice (Zhang, 2015).

But the relationship between hippocampus and olfaction extends beyond purely navigational aspects. The hippocampus may also provide contextual information to the olfactory system, allowing the animal to make sense of olfactory information depending on a broader context (Levinson et.al., 2020) - the same smell in different environments may have different meaning for an individual, just like the presence of smoke smell at the bonfire has a completely different meaning from the same smell at home.

The provision of this information is supposed to be achieved by the connections from the ventral hippocampus (vHC) to the anterior olfactory nucleus (AON) (Levinson et.al, 2020). In the experiment carried out by Levinson et.al., rats were trained to dig for two differently scented cups in two different visual contexts in contextually-cued olfactory

discrimination task. An experimental cage was divided in two parts, one painted white and one painted black. There were two differently scented cups in each part; a food reward was put in one cup in one part and in the other cup in the second compartment. In this setting, rats had to combine the contextual cue (wall colour) and a scent (of a cup) to obtain a

reward. Chemogenetic lesions of the AON or the vHC significantly impaired the performance of animals in this task, while they had no influence on the olfactory discrimination ability per se.

In another study (Aqrabawi, 2018), mice were presented with two distinctive scents (1 and 2) placed on two sides of a cage with a specific visual context (A). After 15 minutes, mice were placed in the same context, but this time only one of the odours was present on both sides of the cage. The sniffing of a substituted place location (e.g. place where scent 1 was replaced with scent 2) was assumed to indicate a discrepancy between the memorized relationship between visual context and olfactory information. The blocking of a connection between the vHC and the AON resulted in an impairment of the performance in this task, suggesting that this connection is indeed necessary for the ability to use contextual information to make meaning of olfactory cues.

Interestingly, the AON has also a role in the processing of olfactory social cues. Deletion of oxytocin receptors in the AON led to a decrease of animals' ability to recognize conspecifics, while not impairing non-social olfactory acuity (Oettl et.al., 2016). Moreover, the impairment of connections between the AON and the olfactory bulb led to the abolishment of the animals' ability to develop socially transmitted food preference without the impairment of basal olfaction (Wang et.al., 2020).

Olfactory tubercle and the motivational valence of smells

There is also a strong association between the sense of smell and the food consumption - olfaction is used, among others, for the assessment of the presence of food and its quality (Lawless, 1991). Moreover, olfactory perception is also influenced by food metabolism - for example, ghrelin, an appetite-stimulating hormone increases olfactory sensitivity and induces exploratory sniffing in rodents and humans (Tong et.al., 2011), what suggests the relationship between olfaction and the motivation for exploratory behavior that aims at locating food in the environment.

Murata et.al. (2020) hypothesized that the structure that may be particularly important in odor-guided eating behavior is the olfactory tubercle (OT), a structure that is a part of the mesolimbic dopaminergic pathway (Ikemoto, 2010) and is associated with a motivational value of odours. In one study, mice were trained to associate the same

olfactory cue with either a food reward or a foot shock (Murata et.al., 2015). The analysis of c-Fos expression has shown that medium spiny neurons of the anteromedial part of the olfactory tubercle that expressed D1 dopamine receptor were activated by a learned cue when it was associated with a food reward. On the other hand, D1-expressing neurons in the lateral domain of the OT were activated by a cue associated with a foot shock (Murata et.al, 2015). Moreover, place and smell preference can be induced by the activation of dopaminergic terminals of the ventral tegmental area - OT pathway (Zhang et.al., 2017). Widespread c-Fos expression was also observed in the OT after the search and consumption of food in mice (Murofushi et.al., 2018).

Murata et.al (2020) claims that those and other pieces of evidence suggest that the OT might be engaged in processing food-related smells and guiding motivational processes aimed at the consumption of food. However, this evidence may also suggest that the OT could be related to the processing of social cues indicating the presence of food in the environment, for example in the paradigm such as Socially Transmitted Food Preference as such cues also motivate animals to consume food. In the paradigm based on the social transmission of food localization, the OT could be engaged in the processing of the olfactory cue and guiding the motivation to explore the environment.

The aim of this dissertation

The study of the ability of rodents to transfer information about the localization of food (especially if guided by social cues) could be used to bridge the knowledge gained by different subfields - the study of social behaviors, spatial navigation and olfaction - in a naturalistic paradigm that would allow to study how the brain uses socially acquired information to guide exploration of the environment. The task would help to overcome the artificiality and low ethological relevance of paradigms used by those subfields separately and allow to study a behavior important from the adaptive point of view.

The goal of this dissertation is to develop a behavioral paradigm combined with brain imaging methods that would allow to answer following questions:

1) Can rodents pass information not only about the quality of food but also about its localization?
2) How is this information transmitted and detected?

3) How does the brain use social information to induce motivational states resulting in the exploration of the environment?
4) How does socially acquired information shape the exploratory behavior of the animal?
5) How does this information shape the neural representation of the space? Can the information about the location of the food be strong enough to induce a change of the internal belief of the animal about the context?
6) Can social transfer of information induce offline replay of cells encoding the context in which food can be found?
7) How the reward system may be engaged in the detection of information about rewards in the environment?
8) What additional brain regions may be engaged in such behavior?

We have developed a modified version of a place preference test, where mice or rats exchange information about the location of food in one of the two distinct compartments. We hypothesized that rats and mice should be able to transmit the information about the localization of food in the environment and that this information should be passed via the olfactory system.

We further hypothesized that the socially acquired information should lead to the changes in the brain representation of space and be observed as a change in the activity of place cells in the CA1 region of the hippocampus. We also expected that the cells encoding the area where food is expected to be found will be replayed during the social interaction. We have implemented single-photon calcium imaging in freely moving animals to record the activity of CA1 cells during the exploration of the environment and during the social interaction to measure the changes in the place fields of place cells evoked by the socially acquired information and to test if the cells coding the context associated with food reward would be replayed during the social interaction.

We have also assumed that an olfactory structure related to the appetitive aspect of an olfactory information - olfactory tubercle - should be engaged in the processing of social information and the anticipation of a reward. We have used fiber photometry to image the activity of olfactory tubercle to test if this structure may encode the rewarding aspect of the food-related information detected during social contacts with a conspecific.

We have also used iDISCO method (Renier, 2014) to obtain a map of c-Fos expressing cells in whole brains of mice that underwent the behavioral procedure and

detect brain regions that may be engaged in the transfer of social information.

We show that rats and mice can indeed exchange information about the location of food in the environment and that this information is used to guide their exploratory behavior. We found that the probable channel of transfer is the olfactory information produced by the anogenital region of the donor of the information. Our experiments suggest that the cells encoding the food location are reactivated during the information transfer, but the effect of the acquired information and the expectations evoked by it are masked by massive changes in the place cells activity evoked by interacting socially with a fed conspecific. We also show that the olfactory tubercle activity seems not to be increased in a group in which mice transfer information. Finally, we show that an activity of structures related to metabolism and homeostasis may be involved in the observed behavior.

Materials and Methods

Animals

All experimental procedures were conducted in accordance with ethical standards of the European Union (directive no. 2010/63/UE) and Polish regulations and were pre-approved by the Local Ethics Committee (approval nr 1530/2023. 740/2018).

Mice

C57BL/6J (B6) male mice were bred at the Animal House of the Nencki Institute of Experimental Biology, Polish Academy of Sciences or Center of Experimental Medicine in Bialystok, Poland and housed at the Animal House of the Nencki Institute.

Animals were housed in groups of 4 in standard IVC cages (GM500 type IIL, Tecniplast, Italy) with added nesting material and paper tubes. IVC cages were individually ventilated with 75 HEPA 14 filtered air exchanges per hour. Before and after the experiment mice were provided with *ad libitum* access to food (standard laboratory chow) and water. The light cycle was 12/12 h. Mice were handled by the experimenter for 10 days before the experiment.

Rats

Male Wistar rats were supplied by the Center of Experimental Medicine in Bialystok, Poland and housed at the Animal House of the Nencki Institute. They were paired randomly and housed in pairs in standard rat home cages (43.0 x 3 25.0 x 18.5 cm) with a 12/12 h light cycle. Food and water were provided *ad libitum* outside of the time of the experiment. Rats were handled by the experimenter for 10 days before the experiment.

Surgical procedures

Endoscopic imaging

Viral injections

Mice (n = 8) were anesthetized before the surgery by isoflurane inhalation (5% isoflurane in inhaled air) and placed in the stereotactic apparatus (Knopf). A heating pad (37.8°C) was placed below the mouse to ensure proper temperature. Analgesic (Butamidor, Richter, 1:20 in saline, 2.5 ml/kg) was injected subcutaneously and the absence of pain was assessed by checking reflexes. Ear bars were placed and eye moisturizing gel (Visidic) was applied to prevent the animal's eyes from drying. The scalp was shaved using scissors and depilation cream (Veet). Skin was disinfected by three alternating applications of disinfectant (octenidinum, Octanisept) and saline. Skin was then cut using scissors (Fine Science Tools) to expose bregma. Unilateral craniotomy was done using a drill (AP - 2.1mm, LM + 2.1 mm). Nanofil 35G needles were used to inject the viral vector (pGP-AAV-syn-jGCaMP8m-WPRE, injected at DV -1.6 mm). The delivery was performed by the Micro435 Syringe Pump (World Precision Instruments, 420 nl of volume, 100 nl/min). The needle was left in the tissue for 5 min after the injection to allow the viral vector's diffusion and was then slowly removed. The incision was sutured using surgical suture (Dafilon, C0935204). Skin on the scalp was once again disinfected. Subcutaneous injections of anti-inflammatory medication (Tolfedine, Vetoquinol, 4 mg/kg) and an antibiotic (Baytril 2.5%, Bayer, 440, 1:3 in saline, 5 ml/kg) were performed. Mice were then removed from the apparatus, placed in a home cage on a heating pad for recovery for 2 hours and singly housed for 5 days to ensure full recovery. Antibiotic and anti-inflammatory drugs were provided for the 2 days after the surgery.

Lens implantations

Two weeks after the injections, GRIN lens (Fig 1a). was implanted above the CA1 region of the hippocampus to enable calcium imaging using a miniaturized endoscope. The initial stage of the surgery was identical to the injection procedure described above, up to the disinfection of the scalp. Skin of the scalp was removed and the edges of the incision were glued to the skull using tissue glue (Vetbond). Skull was scratched using a scalpel to provide better adherence to the dental cement. A circular craniotomy with a diameter of 1.0 mm was drilled on the site of injection. The brain tissue above the hippocampus was then removed using a syringe needle connected to a vacuum pump. The GRIN lens (1.0 mm diameter, ~4.0

mm length, Inscopix) was then placed above the exposed tissue (DV - 1.35 mm). The lens was glued to the edges of the skull with superglue (Kropelka). The surface of the skull was covered with Metabond dental cement. To protect the lens from being bitten by cagemates, it was covered with a cut PCR tube. The tube and the rest of the skull was then covered with Duracryl. Mice were then treated with antibiotics and anti-inflammatory drugs in the same way as after the injection surgery.

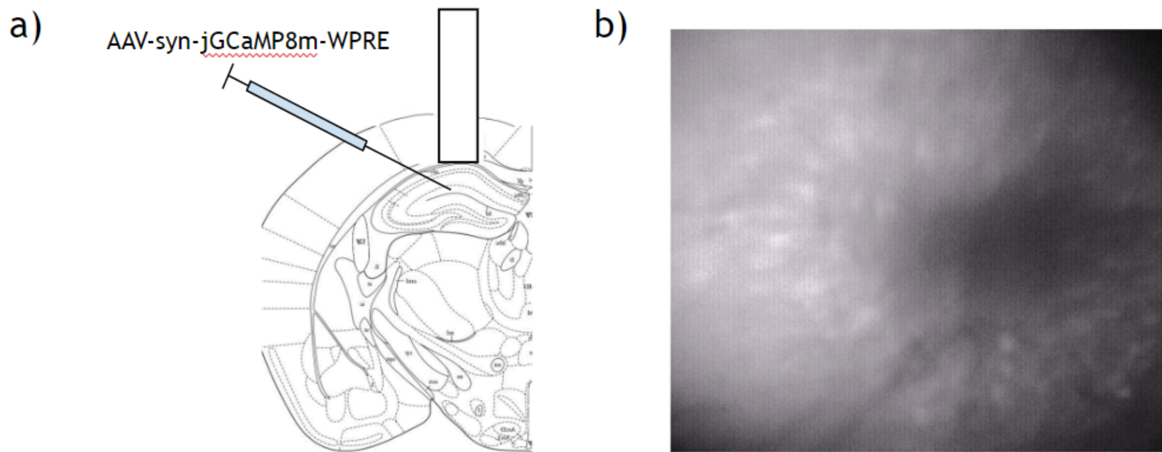

Fig. 1. a) Scheme of stereotactic surgery. Viral vector encoding GCaMP8M calcium indicator was injected in the CA1 region of the hippocampus. GRIN lens was subsequently implanted over the injection site after the aspiration of cortex. b) Example image obtained by UCLA Miniscope imaging.

Baseplatting

After two weeks, the quality of the calcium signal was assessed and aluminum endoscope holders (baseplates) were implanted. Mice were put in the stereotactic apparatus after anesthesia (as described above) and stabilized using earbars. The dental cement was slowly removed from the PCR tube covering the lens. Plastic from the tube was also removed. UCLA Miniscope V.4 placed in the stereotactic holder was used for imaging of the CA1 activity. If the signal was present, aluminum base plate was put on the socket in a position that allowed for the most clear imaging of the tissue, and was then connected to the rest of the socket with metabond dental cement and covered with Duracryl to further stabilize it. A plastic placeholder was then put into the baseplate to secure it. Mice were then returned to their home cages.

Fiber photometry

Viral injections and optic fiber implantations

Viral injections (on n = 6 mice) were performed in a similar way to the endoscopic injection surgeries, with an exception that the viral vector was delivered to the olfactory tubercle (anterior-posterior, +1.5 mm; medial-lateral, 0.7 mm from the bregma; and dorso-ventral, 4.25 mm). Immediately after the injection, an optic fiber with a cannula (400 µm core diameter, 0.48 NA) was slowly implanted 500 um over the target structure. The exposed brain tissue was covered with vaseline. An optical cannula was secured using a light-sensitive cure (Vitrebond, 3M). The surface of the skull and the cannula was then covered with a metabond. The rest of the procedure and the post-surgery care were identical to the procedures applied in GRIN lens implantation described above.

Behavior

Behavioral apparatus

The behavioral apparatus was based on the classic Conditioned Place Preference test cage (Huston, 2013). It consisted of a white rectangular box (93 x 34 x 37 cm) divided by transparent Plexiglass walls with open 8-cm-wide passages into three equal-sized compartments (31 x 34 cm). The middle (neutral) compartment was the place where the animals were put at the beginning of the experiment.

Two side compartments were equipped with distinct cues: vertical or horizontal black stripes and olfactory cue A or B, respectively. Before each session the walls and the floor were scrubbed with a tissue paper covered with 0.5ml of a scented oil (pine or lemongrass, Nowa S.A).

The apparatus was placed in a dimly-lit room in a plastic frame with LED strips around the rim that provided dimmed, equal illumination of the floor of the cage.

The apparatus was identical in the rat and mice experiments.

Behavioral protocols - Mice

Experimental (F+L) and control (F) groups

Mice were randomly divided into pairs composed of a Recipient (R) and the Donor (D) of socially transmitted information. In the experimental group, the Donor mice had information about the presence and the localization of the food in the environment. This group was accordingly called Food + Localization group (F+L; n = 14). In the control group, the Donors were fed in their home cages immediately before being transferred to the experimental cage. Thus, they had information about food presence but not about its localization within the test cage (Food group, F, n = 15). Such design allowed for controlling the effects of interaction with a fed conspecific.

The experiment in both groups lasted for 4 days.

Habituation (Days 1-3)

For three days mice were habituated to the place preference cage by putting them in the apparatus for 10 min (Fig. 2). After the first day of habituation, mice were put on a food restriction scheme with an access to food limited to 4 hours daily. On two consecutive days, food was placed in the homecage right after the return of the animal from the experimental apparatus.

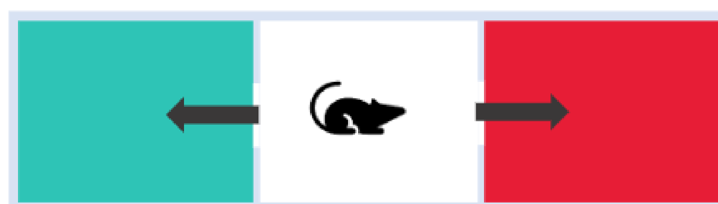

Fig. 2. Habituation - for the first 3 days of the experiment mice were put in the apparatus and allowed to freely explore it for 10 min.

On the third day of habituation (baseline condition), place preference of the Recipient mouse was assessed by measuring time it spent in two cue-distinct compartments (Fig. 3). The less preferred compartment was selected as a Target compartment.

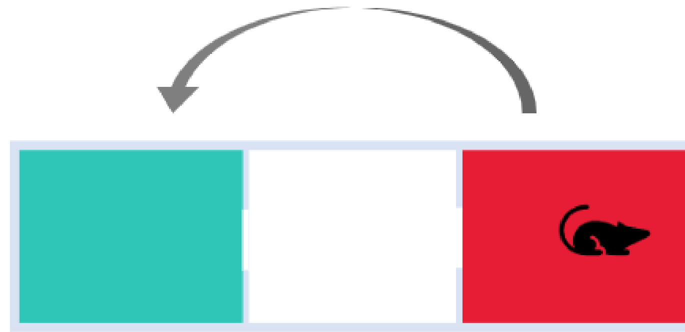

Fig. 3. Selection of the target compartment. The compartment where the mouse spent less time during the last day of habituation (green) was selected as a Target compartment.

Test (Day 4)

The goal of the test day was to assess if the Recipient mice in the F+L group change their preference from the Non-Target to the Target compartment after interacting with the Donor fed in the Target compartment. To this end, on the fourth day of the experiment, the Donor mouse was placed in the Target compartment with a piece of food; the door to the compartment was then blocked with a plexi door. After 8 minutes, the mouse was transferred to the second compartment for 3 minutes to ensure that it will have the scent of both compartments on its skin and the information could not be extracted based solely on the distinct smell of one of the compartments (Fig. 4, upper row). In the F group, the Donor was first placed in an otherwise empty, previously unused homecage with a piece of food for 8 minutes and was then transferred to the Target and Non-Target cage for 3 minutes in each (Fig. 4, bottom row). The olfactory cues used to make the compartments distinct and position of the Target and Non-Target compartments were balanced across the tested animals.

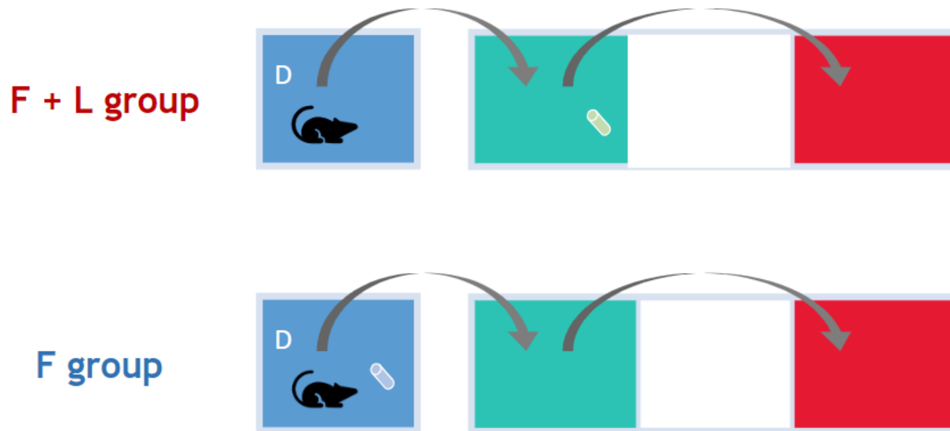

Fig. 4. Experimental procedure in the F+L (upper row) and the F group (bottom row). The detailed explanations are provided in the text.

The rest of the protocol was identical for both groups. Right afterwards, the Donors were placed in the middle (neutral) compartment of the apparatus with doors to both side compartments closed. The floor and the walls of the Target and Non-Target compartments were cleaned three times with a 70% alcohol solution to ensure that there were no crumbs of food left on the floor.

The recipient mouse was then placed in the middle compartment together with the Donor (Fig. 5). They were allowed to interact freely for 10 minutes. Afterwards, the Donor was removed, the entrances to both side compartments were unlocked and the Recipient was allowed to explore the apparatus freely (Fig. 6).

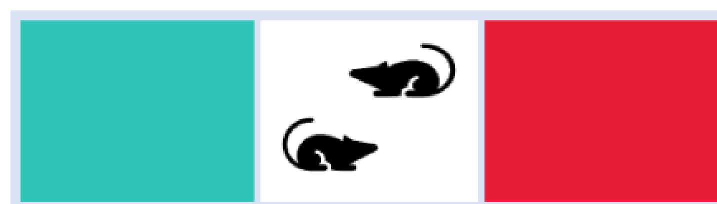

Fig. 5. Social interaction of the Donor and Recipient took place in the neutral middle compartment.

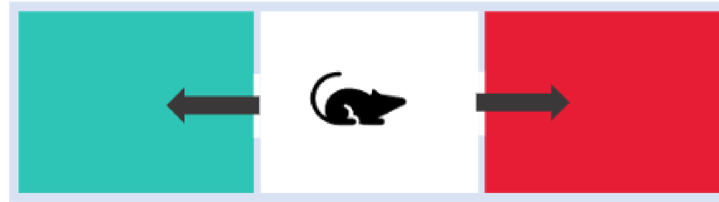

Fig. 6. The Recipient was allowed to freely explore the apparatus after the interaction.

After the experiment, the place preference of the Recipient was calculated and compared to the preference expressed on day 3 of the habituation.

No Interaction group

Even though the floor of the Target compartment was carefully cleaned after the consumption of food by the Donor, some traces of food or its smell might have been left. To control for that possibility, another control group - No Interaction, NI group - was designed (n = 9). The experimental procedure in this group was identical to the one described in the previous section for the F+L group with the exception that there was no social interaction between the Donor and Recipient. This group allowed for testing whether the social interaction is necessary for the change of the preference to the Target cage in the Recipients.

No Olfactory Cue group

To test for the role of the olfactory cues provided by the experimenter on the floor and walls of the side compartments, the No Olfactory Cue group was designed (n=12). The protocol was similar to the one used in the F+L group, with an exception that identical olfactory cues (pine or lemongrass) were placed in both side compartments.

iDISCO c-Fos mapping

For the purpose of mapping brain regions engaged in the reception of social information during the interaction, a behavioral paradigm identical to this introduced in F

and F+L groups was used, with the exception that the exploration phase was omitted and mice were sacrificed 1.5 h after the interaction with the Donor.

Behavioral protocol - Rats

Analogous experiment was carried out on Wistar rats. The procedure was similar to the one developed for mice (the F and F+L groups, both with n = 19 rats), with the following changes: the rats were separated after the 1st day of habituation; the social interaction took place in a separate home cage in order to provide animals with enough space to interact. In a subgroup of animals ultrasonic vocalizations (USV) were recorded with a single microphone hanging over an interaction cage.

Minicope imaging behavioral protocol

The behavioral protocol for miniscope imaging was a modified version of the protocol for testing the F and F+L groups (described in detail above). Modifications were introduced to protect the miniscope from the biting by the Donor and to provide a sufficient length of the recording to allow reliably associate neuronal activity with spatial location of the animal. The adaptations also aimed at habituating the animals to the weight of the miniscope on their heads.

Following modifications were introduced:

1) **Habituation to the miniscope**. For three days before the experiment, Recipients were taken to the experimental room. They were accustomed to the room for 20 minutes. They were then taken out of the cage;the miniscope was placed in the baseplate and the animals; they were then placed in an empty Plexi box of the size of 70 x 40 cm and let to freely explore it for 10 minutes daily.
2) **Connection to the miniscope.** UCLA miniscope was placed on the heads of mice during all three days of habituation and the test day, including the interaction phase. It was connected via coaxial cable to a data acquisition board via a rotary commutator (OEPS Electronica) to prevent the cable from entanglement. The length of the connector cable was matched to allow the animal to freely explore the cage. Manual adjustments of the cable were performed in case of entanglements.
3) **Extension of the time spent in the behavioral apparatus.** As the length of the recording sessions for place cells recordings is usually longer than 10 minutes, as the

space must be evenly explored by the animal, the time of the exploration phase during the three days of habituation and the experiment was increased to 20 minutes. The length of the interaction was the same as in the standard behavioral protocol, i.e. 10 minutes.

4) **Placement of the Donor in the mesh cage.** To prevent the Donor mouse from biting parts of the miniscope, it was placed in the cylindrical plexi tube (12 cm diameter and 26 cm height) with 1-cm-wide holes in the walls, allowing contact between the mice. The tube was placed in the middle of the neutral compartment. The tube was placed there for all days of the experiment to habituate mice to it. If the new element would be introduced during the test day, it may cause place cells remapping.

Fiber photometry imaging behavioral protocol

The behavioral protocol for the optic fiber imaging was similar to the standard behavioral paradigm for the F and F+L groups, with the following modifications:

1) **Habituation to the fiber.** For three days before the experiment, the Recipients were taken to the experimental room. They were accustomed to the room for 20 minutes. They were then taken out of the cage and the; connector was attached to the cannula.; They were then placed in an empty Plexi box of the size of 70 x 40 cm and let to freely explore it for 10 minutes daily.
2) **Attachment to the optic fiber.** During each day of the habituation, implanted cannulas on mice’s heads were attached to the recording system with a rotary joint connector (FRJ_1x1_FC-FC Doric). The rotary joint prevented the fiber from entanglement. The length of the fiber was matched so that animals could have explored the cage freely.

Data acquisition

Behavior

The exploratory behavior of the animals in the last habituation session, social interaction and cage exploration afterwards was recorded with a camera (BlackFly, F+LIR)

placed above the apparatus using custom-made Bonsai (Lopes et.al., 2015) recording script. USV recordings in the rat experiment were acquired using Avisoft UltraSoundGate 416H DAQ and analyzed using RatRec software.

Calcium Imaging

Single-photon calcium imaging was performed with UCLA Miniscope v.4 miniaturized endoscope system (OEPS). Data was acquired with a custom-written Bonsai script using UCLA Miniscope library, with a sampling rate of 20 Hz and LED intensity matched to the signal strength. The signal from the camera was synchronized with Arduino as an external clock.

Fiber photometry

Custom-made setup was used to record calcium signal using fiber photometry. The excitation light source was 470 nm LED (M470F1 Thorlabs). It was collimated using an aspheric lens (F240FC-A Thorlabs) and passed through excitation filter (ET470/24M Chroma) and then passed thorough dichroic mirror (T495LPXR Chroma). The beam was launched into a 400µm core, 0.48 NA fiber patch cable using an aspheric lens (F240FC-A Thorlabs). Excitation and detection of fluorescent signal was achieved through multimode optic fiber, connected to the cannula implanted on the animal's head using a fiber-optic rotary joint (FRJ_1x1_FC-FC Doric). Emitted fluorescence was passed through dichroic mirror, emission filter (ET525/50M Chroma), focused with aspheric condenser lens (ACL25416U-A Thorlabs), and acquired with amplified photodiode (Newport 2151). Data was acquired using PCIe-6321 National Instruments data acquisition board (sampling rate: 6100) that was also used to modulate the frequency of the diode signal with a frequency of 531 Hz and to synchronize the photometry and camera data.

Histology

Perfusion and injection site inspection

Mice from calcium imaging with miniscopes and fiber photometry were perfused to check for injection and implantation sites. The deep anesthesia of mice was performed using

sodium pentobarbital (50 mg/kg intraperitoneal). Mice were then perfused transcardially with PBS (Phosphate-buffered saline; POCH, Poland) and then 4% paraformaldehyde (PFA; Sigma-Aldrich, Poland, in 0.1 M PBS). This solution was also used to store brains at 4°C overnight. Brains were then transferred to the solution of 30% sucrose in 0.1 M PBS (pH 7.5) and stored for another 72h. Coronal sections were cut from tissue frozen at -20°C and transferred to PBS. Slices were washed three times for 6 min in PBS and mounted on microscopic slides, which were then covered with mounting dye with DAPI (Invitrogen, 00-4959-52). Microscopic images were obtained with a fluorescent microscope (Nikon Eclipse 80) and checked visually for the sites of injections and implantations.

iDISCO c-Fos stainings

Ninety minutes after the social interaction, mice were anesthetized with pentobarbital and perfused transcardially with PBS and 0.1% Heparin, and then perfused with 4% PFA in PBS. The brains were removed and fixed overnight in 4% PFA (at 4°C). On the next day they were washed 3 times in PBS and afterwards stored in PBS with 0.02% NaN3 at 4ºC.

For iDisco procedure, brains were put in separate 5 ml tubes. Firstly, brains were dehydrated with a solution of methanol in water; the concentrations of 20%, 40%, 60%, 80% and 100% were used, for one hour each. The tubes were put on a rotator, at room temperature (RT). The brains were then incubated overnight in 66% DCM / 33% Methanol solution, on a rotator, at RT. After the incubation, the samples were washed twice in 100% methanol at RT, and then cooled at 4ºC. Bleaching was performed afterwards in cooled fresh 5% H_2O_2 in methanol, overnight, at 4ºC, on a rotator. After the bleaching, the brains were rehydrated with methanol/H_2O series: 80%, 60%, 40%, 20%, 1h each, at RT, on a rotator. Then the brains were washed twice in PTx.2 solution (0.2% TritonX-100 in PBS), 1 h each wash and incubated in permabilisation solution (400 ml PTx.2, 11.5 glycine, 100ml DMSO) for 2 days, at 37ºC, on a rotator. Afterwards, the samples were incubated in a blocking solution (42 ml PTx.2, 6 g BSA, 5 ml DMSO) for 4 days, at 37ºC, on a rotator. Following that the brains were incubated for 7 days with a primary anti c-Fos antibody (1:1000), at 37ºC, on a rotator. Primary antibody solution was prepared in PTwH [1000 ml PBS, 2 ml Tween-20, 1 ml heparin (10mg/ml)], 5% DMSO and 3% BSA and centrifuged 20K, for 10 min, at 22ºC. Then, the samples were washed five times with PTwH (0.5 hour per wash), followed by additional washing overnight (at 37ºC, on a rotator). Secondary antibody solution (1:500) was prepared in PTwH, 5% DMSO and 3% BSA and centrifuged 20K, for 10 min, at 22ºC. The

samples were incubated with the secondary antibody for 7 days at 37ºC on a rotator. They were then washed five times with PTwH (1 hour per wash), followed by additional washing overnight (in the same conditions). Next, the samples were dehydrated in methanol/H2O series: 20%, 40%, 60%, 80%, 100%, ; 1 h each, and then kept in 100% methanol until next day (all washings on a rotator, at RT). On the last day the brains were incubated in 66% DCM / 33% Methanol at RT for 3h and then dehydrated in 100% DCM for 15 minutes, twice, at RT, all incubations on a shaker. In the final step, the brains were incubated overnight in DBE, at RT.

Cleared brains were imaged with a light-sheet microscope (Pawlowska et al., 2019).

Data analysis

All statistical analyses were performed in custom-written scripts in Matlab and Python.

Behavioral data

Animal tracking and preference estimation

Animal tracking (center of mass position) was performed using a custom Bonsai script. It was then analyzed in Matlab. On the last day of the habituation phase, the time spent by the animal in two side compartments was measured. Time spent in every compartment was calculated as a percent of time that mice spent in that compartment, where 100% was the time spent in two side compartments in total, with the exclusion of the neutral (middle) compartment, as in the formula:

$$P_1 = \left(\frac{T_1 * 100}{T_1 + T_2} \right)$$

Where P1 is the percent of time spent in compartment 1, and T1 and T2 are times spent in compartment 1 and 2, respectively. The compartment where the animal spent less time during the last habituation session (baseline) was designated as the Target compartment. After the test phase, the preferences were calculated once again. The preference of the Target compartment on the last day of the habituation was compared for each animal to the preference measured on the Test day using Mann - Whitney test.

Analysis of social interactions

Behavior of animals during social interactions was manually scored using BORIS software (Friar, 2016). Two behaviors: nose to nose contacts, and nose to anogenital contacts were analyzed, and the total duration time, mean duration of a single event and number of events were scored. Both behaviors were scored when the nose of a mouse touched the nose or the anogenital region of the other.

Calcium imaging data analysis

Videos obtained from microendoscopes were cropped in Fiji software (Schindelin et.al., 2012) to contain only active cells, in order to minimize memory load. Python implementation of CaImAn software was then used to perform motion correction of the videos, identification of the cells, and extraction of the calcium signal (Giovannucci et.al., 2019).

Motion correction

Non-rigid motion correction was performed with the NoRMCorre algorithm (Pnevmatikakis, 2017) implemented in CalmAn. NoRMCorre estimates motion vectors over a set of overlapping patches on the high pass spatially filtered image. A smooth motion field is then inferred based on those estimates.The inferred motion fields are applied to the original videos.

Source extraction

Cells were automatically identified in CalmAn using CNMF algorithm. This algorithm expresses spatio-temporal activity of each source as a rank one matrix given by the outer product of a component in space (spatial footprint - location and shape of a putative cell) and a component in time (the activity trace of the putative cell). An additional visual inspection was performed to detect cases of misidentification (non-cell shapes, duplicates); parameters (noise to signal ratio threshold, size of the region of interest, resemblance to the shape of the neuron as identified by algorithm implemented in CalmAn) were fine-tuned to filter identified cells.

Between-days cell identification

Identity of cells between the day 3 of habituation and the Test day was established using the algorithm implemented in CalmAn. The algorithm calculates distance between cells

in different sessions and automatically assesses the identity by solving a linear assignment problem.

Deconvolution and signal processing

The deconvolution of the neural activity was performed using the OASIS algorithm implemented in CalmAn (Vogelstein et al., 2010). Obtained signal was smoothed with a Gaussian filter (s = 75 ms). Calcium events were identified; a calcium event was detected whenever the cell's deconvolved signal crossed 20% of its maximum value. Cells activity was z-scored for normalization; example traces are depicted on Fig.7.

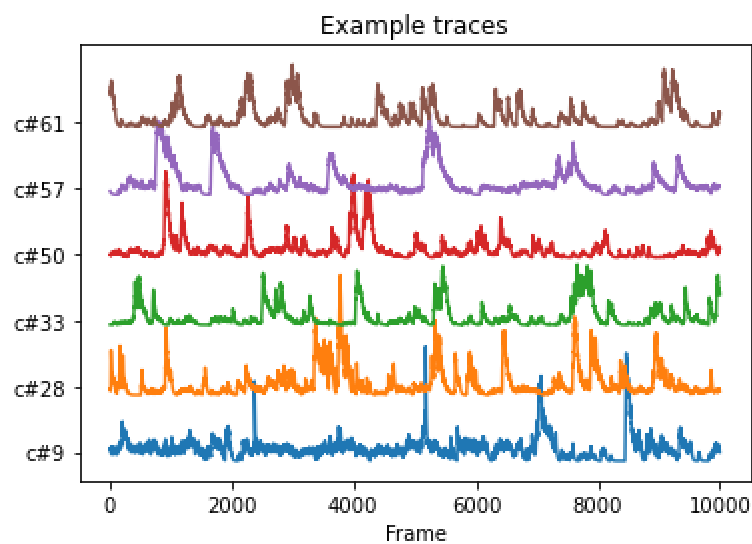

Fig. 7. Example calcium traces of the identified cells.

Identification of place cells

To obtain reliable spatial tuning of cells and select place cells, only instances of mice moving were selected for analysis. This approach allowed us to avoid replay events. The cage was divided in 3 x 3 cm bins and occupancy of each bin was calculated, forming occupancy matrix; bins in which mice spent less than 1s were excluded from analysis. Total activity matrix was calculated within each bin to form an activity matrix. Both matrices were then smoothed using a gaussian filter with s = 6 cm.

Mean activity in each bin was calculated as a ratio of the total activity to the occupancy in a given bin. Spatial information content was then calculated for each cell according to a formula:

$$\text{Spatial information content} = \sum P_i(R_i /R)log_2(R_i/R),$$

Where i is the bin number, Pi is the probability of occupancy for bin i, Ri is the mean activity for a bin i, and R is the overall mean firing rate of the cell.

Spatial information was then compared to the value expected by chance. Activity and occupancy matrices were shuffled 1000 times, and the spatial information was calculated each time. All cells whose spatial information exceeded 95% values calculated in this way were assigned as place cells. The Target Place Cells were identified as those whose peak bin activity was placed in the Target compartment; the Non-Target Place Cells were selected as those with peak bin activity in the Non-Target compartment.

Selection of Target and Non-Target Context Cells

More general selection of Target and Non-Target cells was performed to include cells whose activity was highly correlated with the presence of the animal in a given compartment, but which did not fulfill the criteria for place cells. For each cell identified by the CalmAn algorithm, the mean activity matrix was used in the analysis of context-specificity. Mean activity of all bins in every compartment (Target, Neutral, Non-Target) was calculated. Cells were marked as the Target Context Cells if their mean activity in the Target cage exceeded the mean activity in two other compartments at least 10-fold. An analogous procedure was performed to identify the Non-Target Context Cells.

Place and Context Cells stability assessment

To assess the stability of place- and context cells between days and measure remapping, mean activity matrices were flattened to 1-dimensional vectors. Pearson correlations between vectors coming from the 3rd day of habituation and the Test day were then calculated and used as a measure of cell spatial stability.

Cell activity during social interactions

To check for the activity of Target or Non-Target place and context cells during social interactions, videos from the interactions were manually analyzed and frames of the nose-nose contact initiation were identified. The signal of cells was then aligned to this onset, with 2-second baseline period before the interaction and 3-second period of post-initiation

activity. Mean activity of the Target and Non-Target place and context cells after the initiation of interaction was calculated and compared between the F and F+L groups.

Fiber photometry data analysis

Fiber photometry signal was demodulated before the analysis. Signal was expressed as dFF (relative fluorescence), according to the formula:

$$dFF = \Delta F / F_0 = (F - F_0)/F_0$$

Where F_0 is the median of signal lasting for 2 s before the onset of the event.

Initiations of nose-nose and nose-anogenital contacts were identified manually. The photometry signal was aligned to the onsets of the contact. The peak of z-scored activity post-onset (3 s) was calculated for each trial, and the peak amplitude of all events was calculated for each mouse.

iDISCO whole brain c-Fos activity mapping

The output of the lightsheet imaging was a series of single-channel TIFF stacks: one per channel and per tile at the native resolution of the camera, 1.45 µm/pixel, with a few hundred slices separated by 4 µm for the c-Fos channel and 10 µm for the autofluorescence channel, respectively. Removing of the vignetting artifacts was performed using the BaSiC plugin (Peng et al., 2017). Brain slices were stitched using non-rigid stitcher, WobblyStitcher plugin implemented in ClearMap2 software (Kirst et.al., 2020). It works by aligning pairs of stacks in the wobble axis using a maximal intensity projection (MIP), determining alignments for the individual planes, tracing the best alignment for them and aligning all the planes in a way that is globally optimal.

The CellMap pipeline was then applied to the data. The pipeline is based on seven consecutive steps. First, the correction of illumination is performed. Then, the background signal is removed and the image is equalized. Difference of the Gaussian filter is then applied to improve the contrast of blob-like objects. In the next step, cell centers are detected by finding local maxima in the images and the cell shape detection is implemented to measure the volumes of the detected cells. In the last step, cell intensity measurements are made of various expression levels and intensities of cells.

Data is then aligned to a brain atlas and annotated, enabling the identification of the presence of c-Fos positive cells in different brain regions. We used the number of active cells in a given structure divided by the whole number of active cells in each brain to normalize for the staining quality.

Statistical analysis

For data with normal distribution (as checked with the Kolmogorov–Smirnov test) dependent samples t-tests were used for intra-group comparisons of the change of preference between days and independent samples t-test for the comparison between groups. In the case of data that did not meet the criterion of normal distribution, the Wilcoxon matched pairs test was used for the intra-group comparisons and the Mann–Whitney U test was applied to inter-group comparisons. Performed tests were two-tailed tests, unless stated otherwise. The significance level was $p <0,05$. One star on a plot means $p < 0.05$, two - $p <0.005$, three - < 0.0005.

Results

Mice are able to detect food localization during an interaction with a conspecific

We have measured a change of place preference in mice from two groups, F (Food exposed) and F+L (Food associated with Location). In both groups baseline preference for two compartments of a standard preference test cage was established, and the less preferred compartment was assigned as the Target one. On the test day, mice (Recipients) interacted with a fed conspecific (Donor) and were then let to freely explore the place preference cage. In the F group, the Donor was fed in a home cage just before being put into the place preference cage; in the F+L group, the Donor received food in the Target compartment. We hypothesized that the mice in the F+L group would develop a preference for the Target compartment. In line with this hypothesis, we have found that the preference for the Target compartment was significantly higher in the F+L group after the interaction with the Donor (M=57.26; SD = 13.78; n = 15) as compared to the baseline preference (M = 39.93; SD = 7.42; n=15) measured during the last day of the habituation (t-test for dependent samples, $t(14) = -4.809$, $p = 0.0002$, Fig. 8b). In contrast, we did not observe any change of cage sides preference after the interaction with the Donor in the F group (M = 49.03; SD = 11.81; n = 13) as compared to the baseline preference (M = 41.45; SD = 5.3; n = 15) measured during the last day of the habituation (t-test for dependent samples, $t(12) = -2$, $p = 0.06$), Fig. 8a. Moreover, there was a significant difference between the change of preference (measured as the preference of the Target cage on the test day minus the preference of this compartment on the last day of habituation) between the F and F+L groups, with a higher change observed in the F+L group; one-tailed t-test for independent samples, $t(26) = 1.835$, $p=0.039$, Fig. 8c.

This result suggests that mice can detect the localization of food found by another mouse and use this information to guide their exploratory behavior.

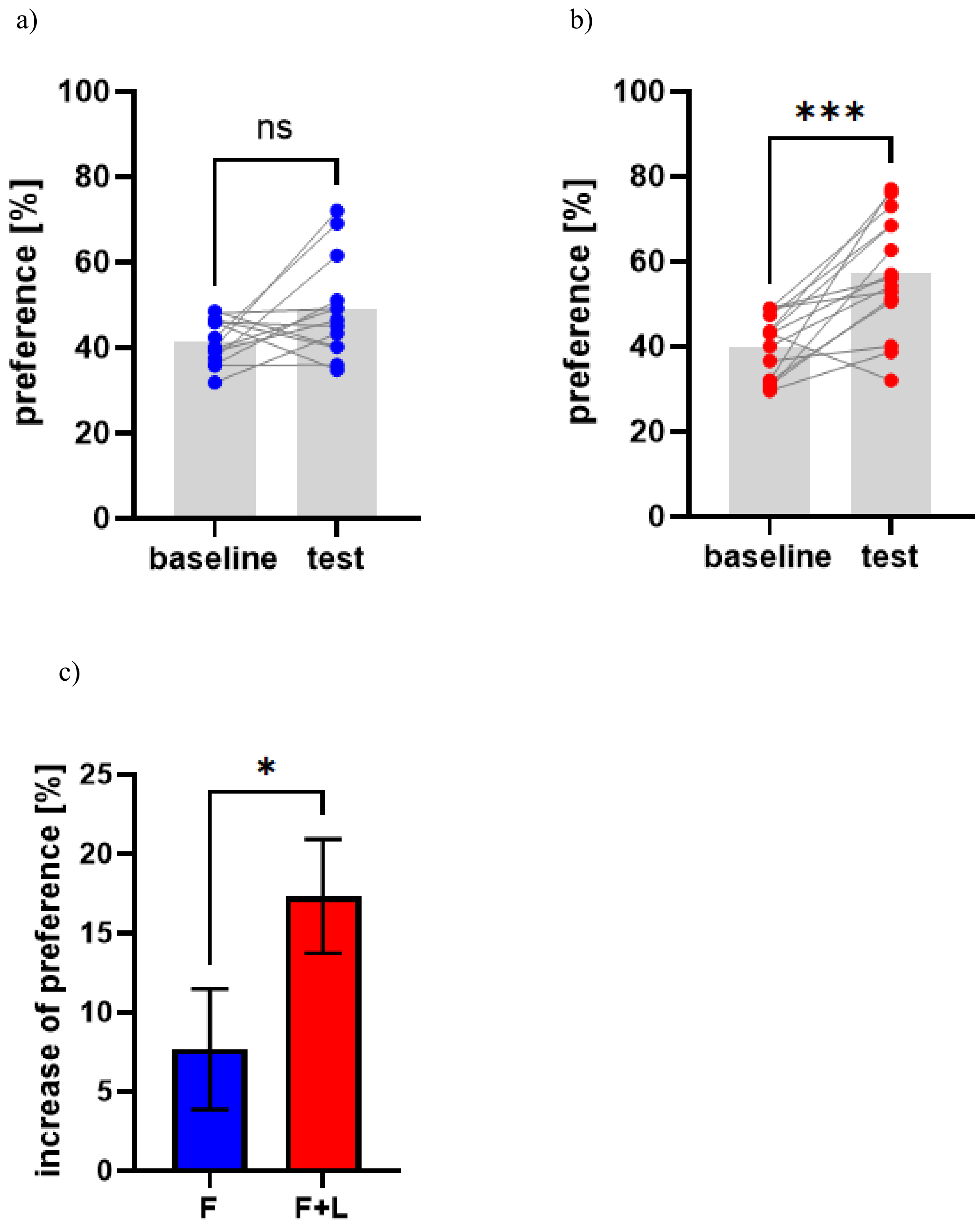

Fig. 8. Change of preference between the last day of habituation (baseline) and the test day, a) F group, b) F+L group; c) the comparison of the change of preference between the F+L and F groups.

Social interaction is necessary for the change of preference

To test whether the observed preference change was not caused by any traces of food left in the Target compartment by the Donor, we have deployed a No Interaction (NI) control group. The animals in the NI group were treated as mice in the F+L group, except the interaction between the Donor and Recipient was omitted. Suppose there was a trace of food left on the floor; the Recipient should develop a preference for the Target compartment even without an interaction. We did not observe any significant preference change in this group (MD = 47.18; SD = 11.58) as compared to the baseline (MD = 40.52; SD = 5.95) (t-test for dependent samples, $t(8) = 1.697$, $p = 0.128$; Fig. 9a). There was also a significant difference between the change of preference between the F+L and No Interaction group, one-tailed t-test for independent samples, $t(22) = 1.920$, $p=0.0340$ (Fig. 9b), which suggests that the possible remaining olfactory traces are not sufficient to evoke the behavior change.

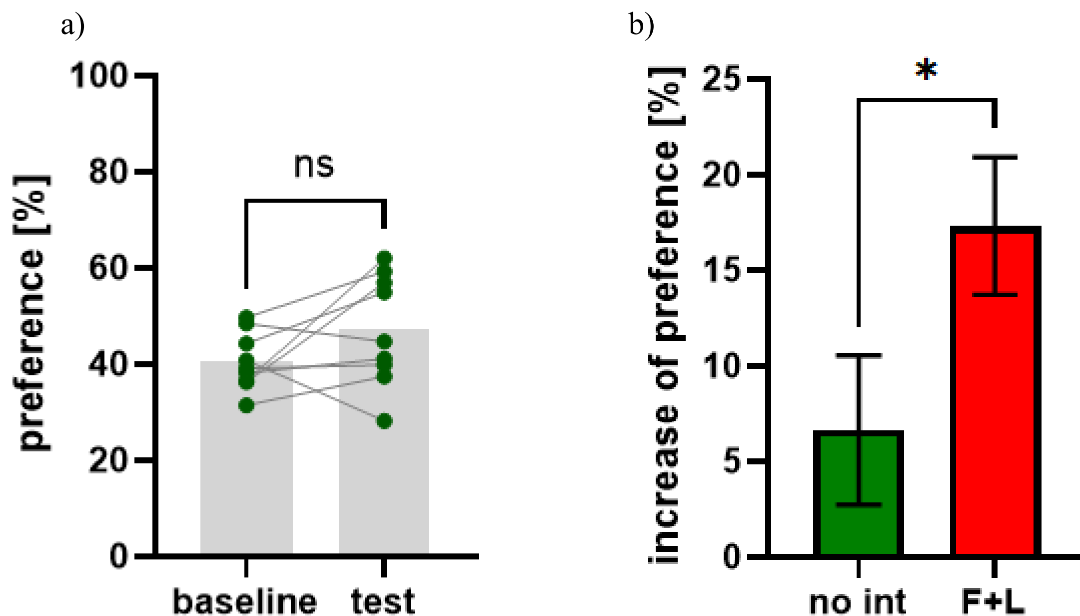

Fig 9. a) The change of preference in the No Interaction group. b) the comparison of the change of preference between the baseline and test day in F+L and No Interaction groups.

Channels of information transfer may include chemical cues emitted in the anogenital region

We hypothesized that the Recipient can detect the smell of the food and the scent of the Target compartment in the breath of the Donor. To test this hypothesis, we have measured the number of nose-to-nose contacts during the interaction. We expected to observe an increased number of those contacts in the F+L, as compared to the F group. Contrary to this hypothesis, we did not observe a significant difference between the F (n= 15) and F+L (n=14) groups in the number of nose-to-nose contacts (Wilcoxon rank-sum test, $Z = 1.1361$ $p = 0.2559$, Fig. 10a).

Interestingly, even though we did not observe any differences between the F and F+L groups in the number of the nose to nose contacts, we have found a significant difference between the F (MD = 17.714; SD = 9.722; n= 15) and F+L (MD = 25.666; SD = 11.806; n=14) groups in the number of nose to anogenital contacts (Wilcoxon rank-sum test, $Z = 2.0322$, $p = 0.0421$, Fig. 10b). This result may imply that the information regarding food localization is transmitted by scents excreted by the Donor in the anogenital region during the interaction.

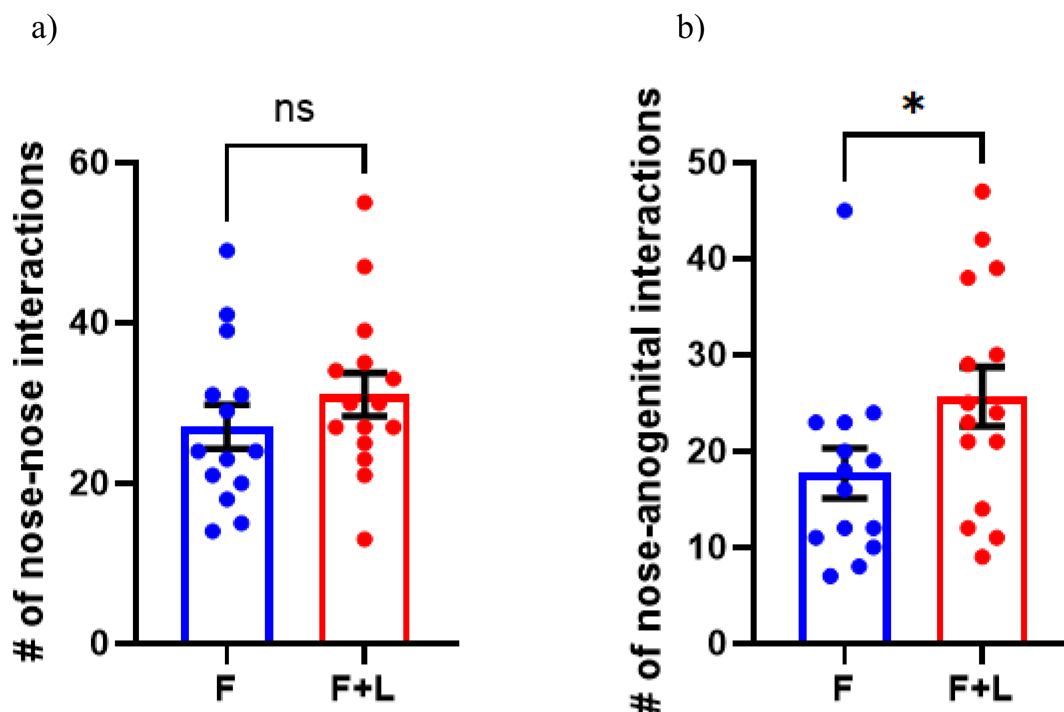

Fig. 10. Number of nose-nose (a) and anogenital (b) interactions in F and F+L groups.

On the other hand, even a few contacts might be sufficient for a mouse to detect the scent of the food and the scent of the target compartment and use this association to guide its exploration of the environment. To test if the specific scent of the Target compartment influences the detection of food localization, we have deployed a No Scent Cue group (NSC), where both the Target and Non-Target compartments were covered with the same olfactory cue (lemongrass). Interestingly, even in the absence of differentiating scent cues mice were able to detect the localization of food and increase their preference for the Target compartment (MD = 53.07; SD = 10.64; n = 12) as compared to the baseline (MD = 42.30; SD = 4.27; n = 9; t-test for dependent samples, $t(11) = -3.254$, $p = 0.003$, Fig. 11).

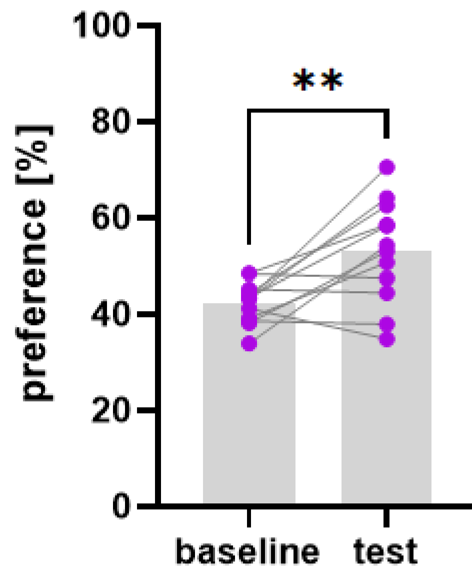

Fig. 11. The change of preference in the No Scent Cue group.

An alternative hypothesis is that the information could be passed between mice during the interaction if the Donor spent more time close to the Target compartment's door. Then, the transfer would be just a result of the Recipient observing the Donor during the interaction and following him.

To test this possibility, we analyzed the time spent by the Donor and Recipient in two parts of the middle compartment of the place preference cage, where interaction took place; in one half located closer to the Target compartment, and another half, located closer to the Non-Target compartment. We hypothesized that, if the position of the Donor guides the behavior of the Recipient, in the F+L group both mice should spend more time together

closer to the Target compartment. Contrary to this hypothesis, we did not find a significant difference in the time spent on the two sides of the compartment by both mice simultaneously between the F (n= 15) and F+L (n=14) groups (Wilcoxon rank-sum test, $Z = 0.1091$, $p = 0.9131$, Fig. 12).

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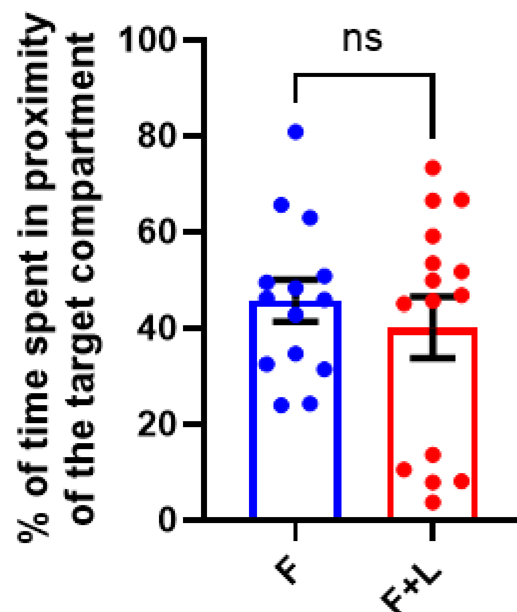

Fig. 12. Time spent in the proximity of the Target and Non-Target compartments during interaction.

Rats are also capable of transferring information regarding the localization of food

To test if the behavior observed in mice is an ability present in other members of the Muridae family, we have conducted an analogous behavioral experiment in rats (*Rattus rattus*). There was one difference between the paradigms for rats and mice, namely, due to their size, rats were interacting in a home cage instead of the middle compartment of the place preference cage.

Just like in the case of mice, we did not observe a significant change of preference between the baseline (M=43.170; SD=4.60; n=19) and test conditions (M=47.772; SD=18.017; n=19) towards the Target compartment in the F group; t-test for dependent samples showed that this difference was not statistically significant, t(18) = -1.199, p = 0.246), Fig. 13a. On the contrary, a significant change between the baseline (M = 41.581; SD = 6.187; n=19) and the test (M = 56.102; SD = 11.970) conditions was observed in the F+L group, just like in the case of mice; t test for dependent samples, $t(18) = -4.342$, $p = 0.0003$, Fig. 13b.

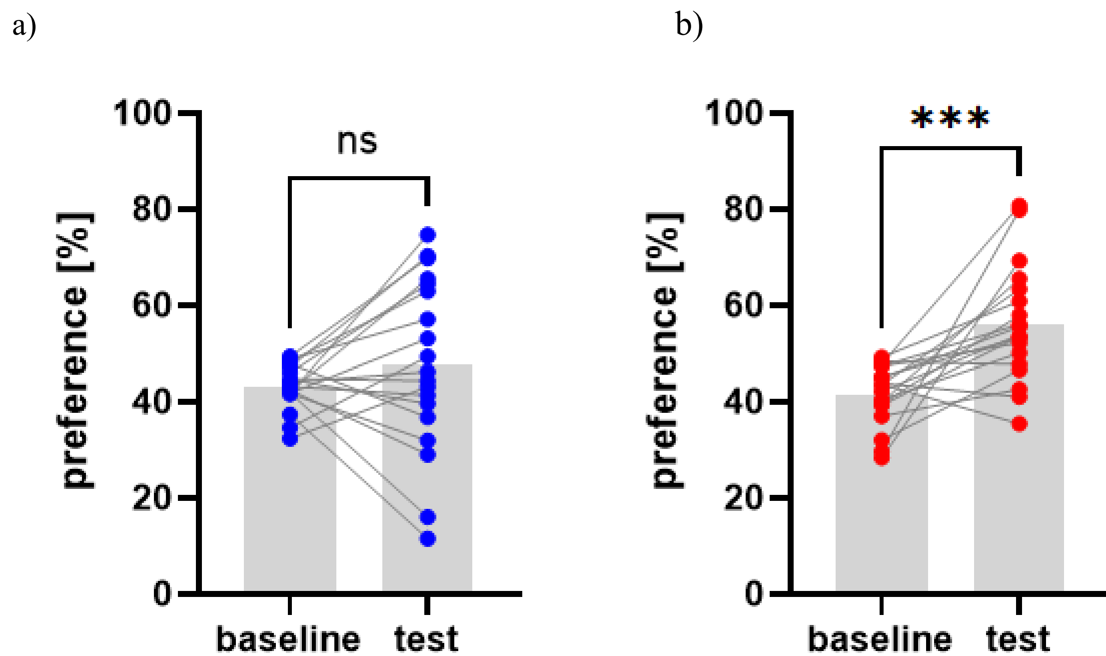

Fig 13. The change of preference in the F (a) and F+L (b) groups in rats.

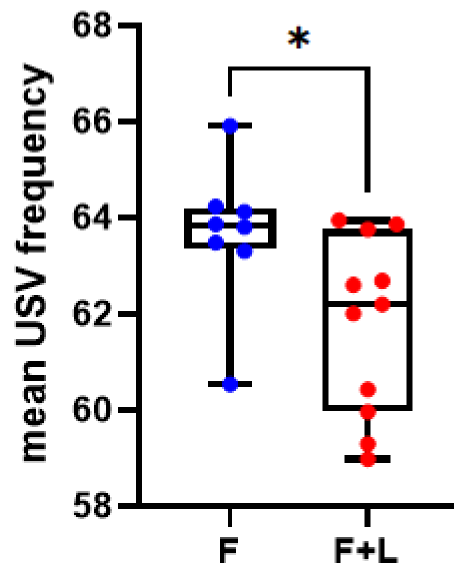

Fig. 14. The mean frequency of USV in the F and F+L groups during the first 2.5 min of interaction.

We have also recorded ultrasonic vocalizations of a subgroup of animals during the first 2.5 minutes of the interaction. Interestingly, we have found that there was a small but significant difference in the mean frequency of vocalizations between the F (M = 63.658; SD = 1.491; n=8) and the F+L (M = 61.794; SD = 1.839; n=11) groups, with vocalizations of a slightly lower frequency recorded in the F+L group; t-test for independent samples, $t(17) = -2.436$, $p = 0.026$), Fig 14.

Socially acquired information does not influence spatial representation stability

We have used UCLA Miniscope recording to identify place cells in the CA1 region of the hippocampus, track their identity between the last day of habituation and the test day, and measure the stability of their spatial tuning. 99 place cells were identified in the F+L group (in 4 mice) and 60 in the F group (in 4 mice).

Correlation in mean activity maps was used as a measure of representational stability. We hypothesized that socially acquired information should lead to the intensified remapping of place cells in the F+L group. Contrary to this hypothesis, the comparison of stability between the F (MD = 0.12, SD = 0.35) and F+L (MD = 0.126, SD = 0.37) groups showed no

significant difference between them (t-test for independent samples, t(157) =-0.023, p = 0.98) (Fig. 15a).

In addition to analyzing the activity of place cells, we have calculated the stability of spatial tuning of all recorded CA1 cells in the F (n = 345) and F+L (n = 176) groups. As with place cells, comparison yielded no significant differences in the stability of cells between the F (MD = 0.06, SD = 0.38) and F+L (MD = 0.12, SD = 0.37) groups, (t-test for independent samples, t(519) =0.583, p = 0.560), (Fig. 15b).

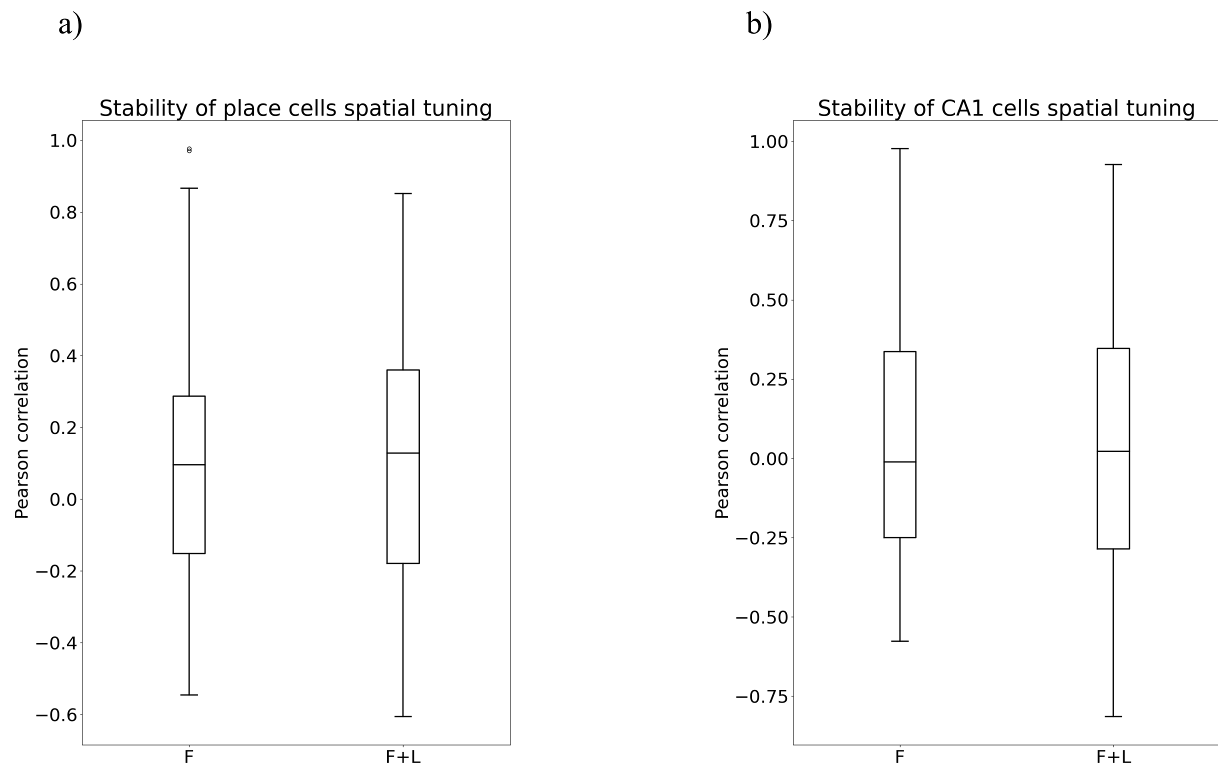

Fig. 15. The comparison of place cells (a) and all hippocampal cells (b) stability between the last day of habituation and the test day.

Reactivation of context cells during the nose-nose interaction

To check if the direct interaction between the Recipient and Donor leads to the reactivation of cells encoding the context of the Target compartment, we have identified

context cells, defined as cells whose mean activity in the Target compartment is at least 10 times higher than in the Non-Target compartment (examples of the context cells are shown on Fig. 16). We have aligned the activity of those cells to the beginning of the interaction (defined as the onset of the nose-to-nose contact).

Comparison of the mean activity of the Non-Target (n=24, MD = -0.02, SD = 0.20) and Target (n=106, MD =-0.029, SD = 0.15) cells recorded through the 3 sec after the onset of the interaction (heatmap of all cells activity is shown on Fig. 17 and 18, traces on Fig. 21) showed no significant difference in the F group (t-test for dependent samples, $t(128) = 0.121$, $p = 0.90$), (Fig. 23). Importantly, though, in the F+L group (heatmap of all cells activity is shown on Fig. 19 and 20, traces on Fig. 22), an increased activity of the Target cells (n = 47, MD = 0.02, SD = 0.17) as compared to the Non-Target cells (n = 70, MD = -0.09, SD = 0.25) was observed (t-test for dependent samples, $t(115) = 2.86$, $p = 0.005$), (Fig. 24).

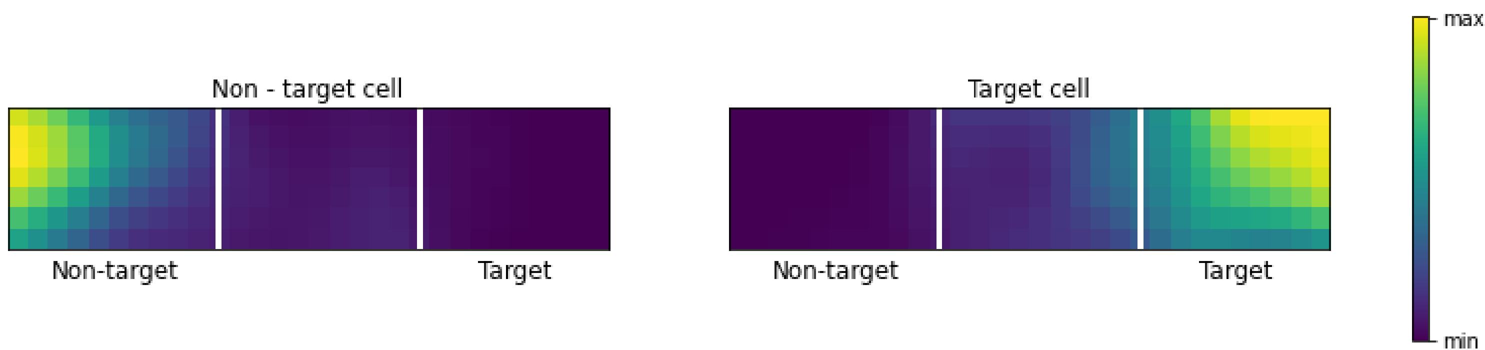

Fig. 16. Examples of Non-target and Target cells mean activities. Mean activity for each spatial bin is color-coded.

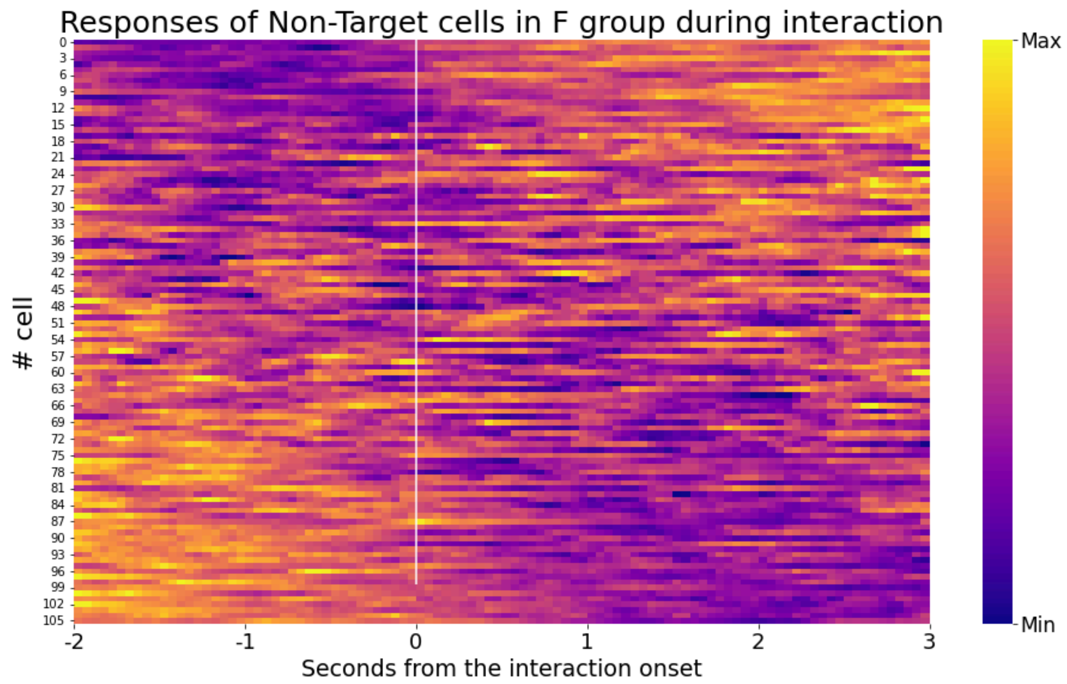

Fig. 17. Responses of cells encoding the Non-Target context to nose-nose contacts in the F group.

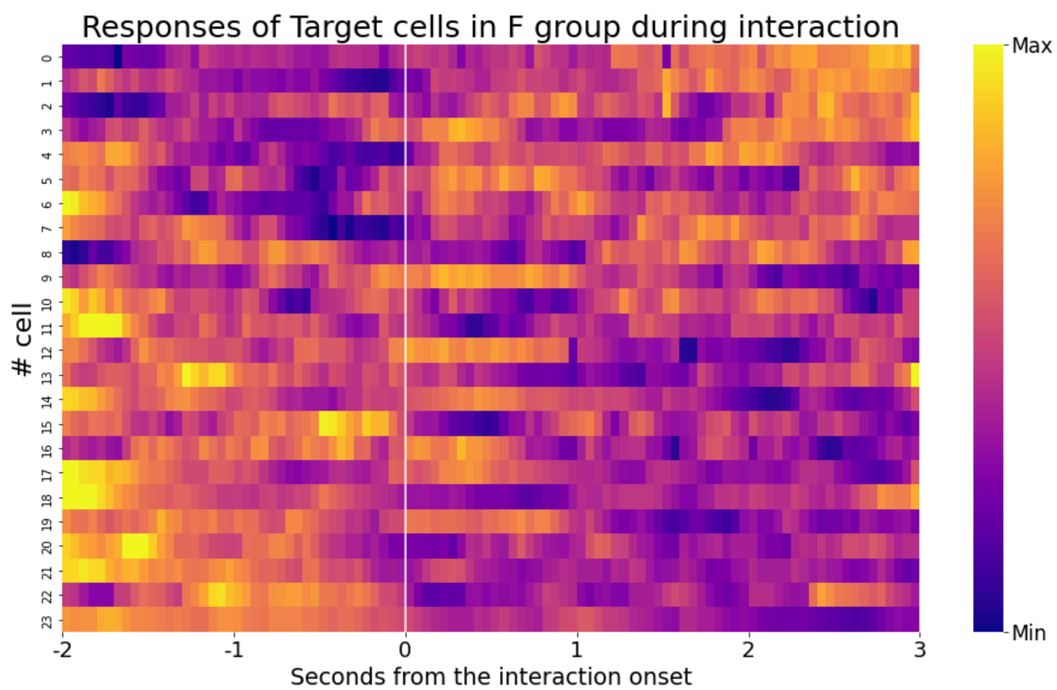

Fig. 18. Responses of cells encoding the Target context to nose-nose contacts in the F group.

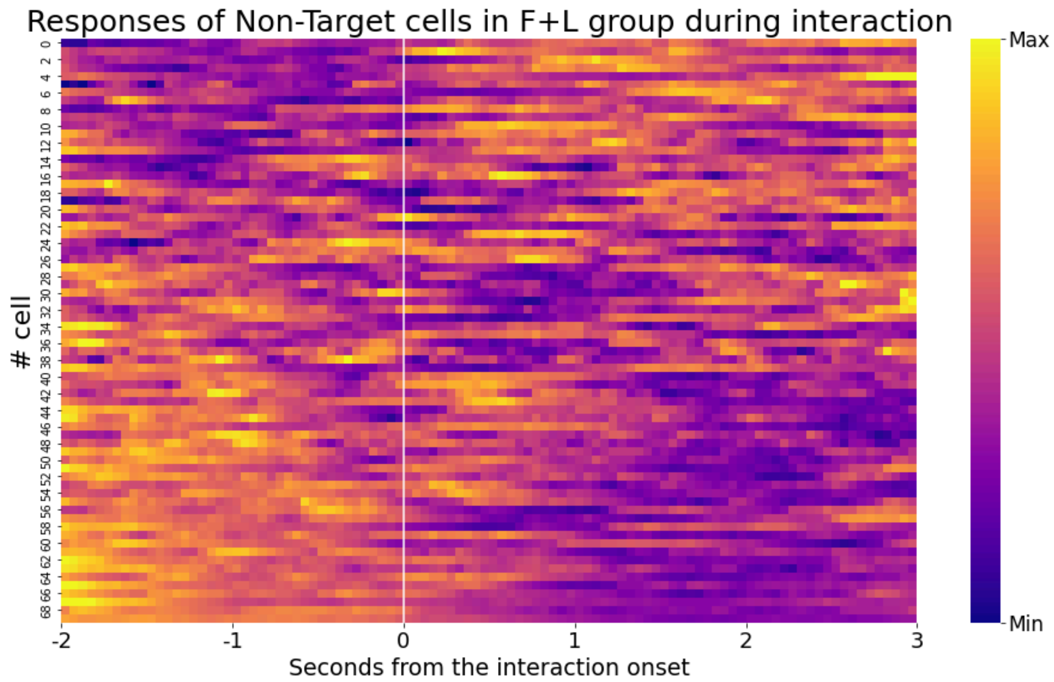

Fig. 19. Responses of cells encoding the Non-Target context to nose-nose contacts in the F+L group.

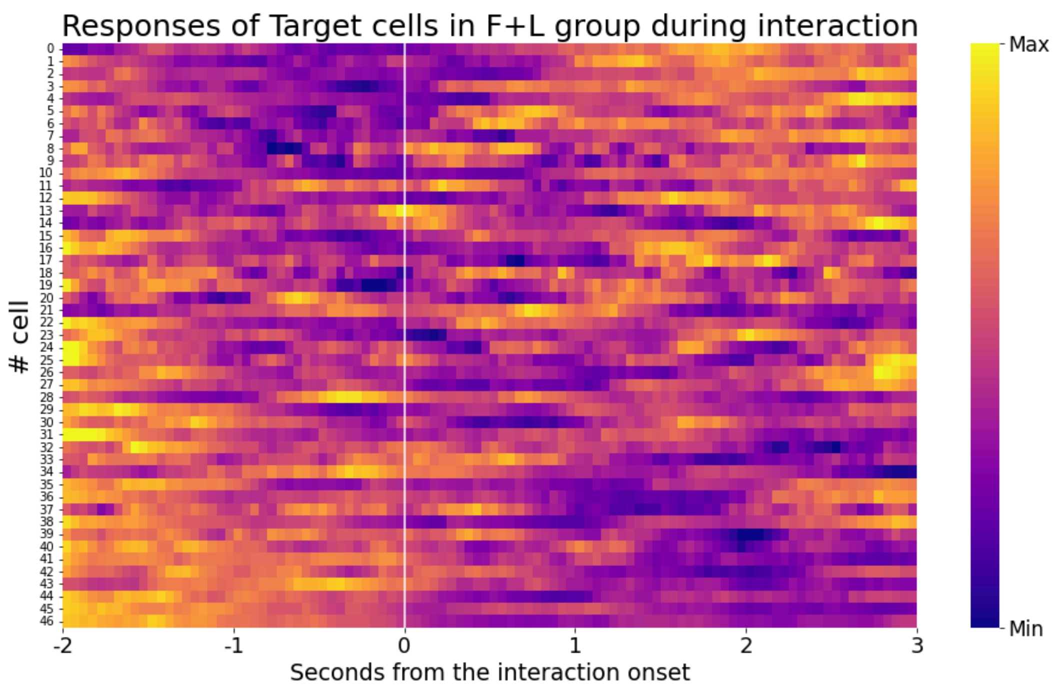

Fig. 20. Responses of cells encoding the Target context to nose-nose contacts in the F+L group.

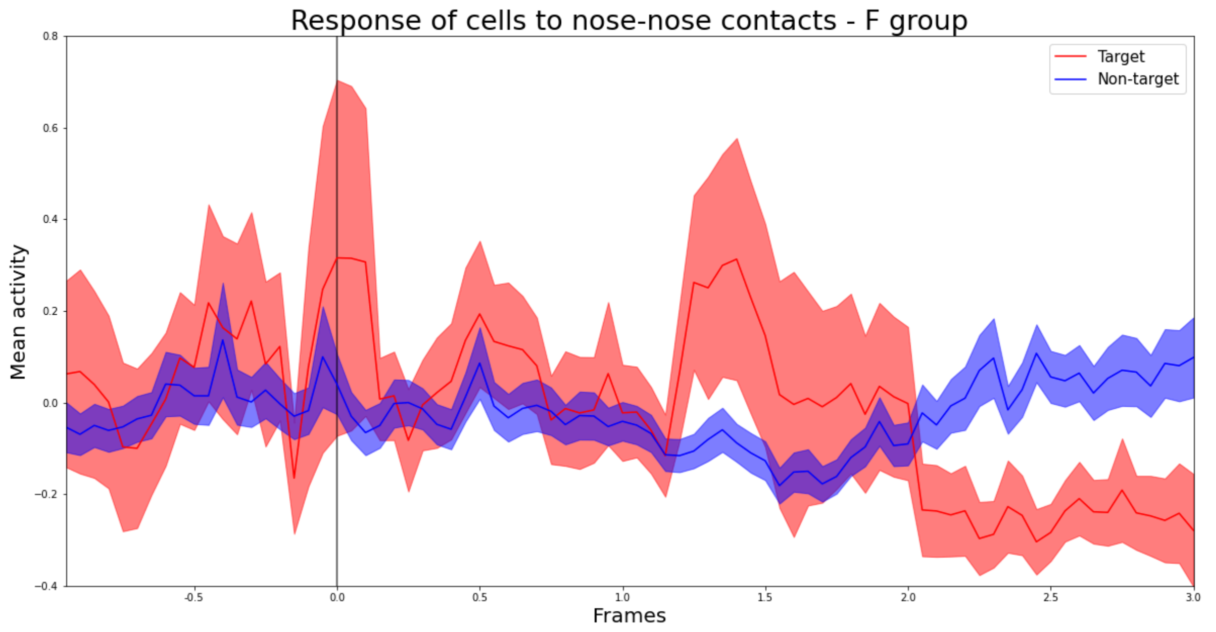

Fig. 21. Traces of activity of the Target and Non-Target cells during the interaction in the F group. 0 marks the onset of the interaction.

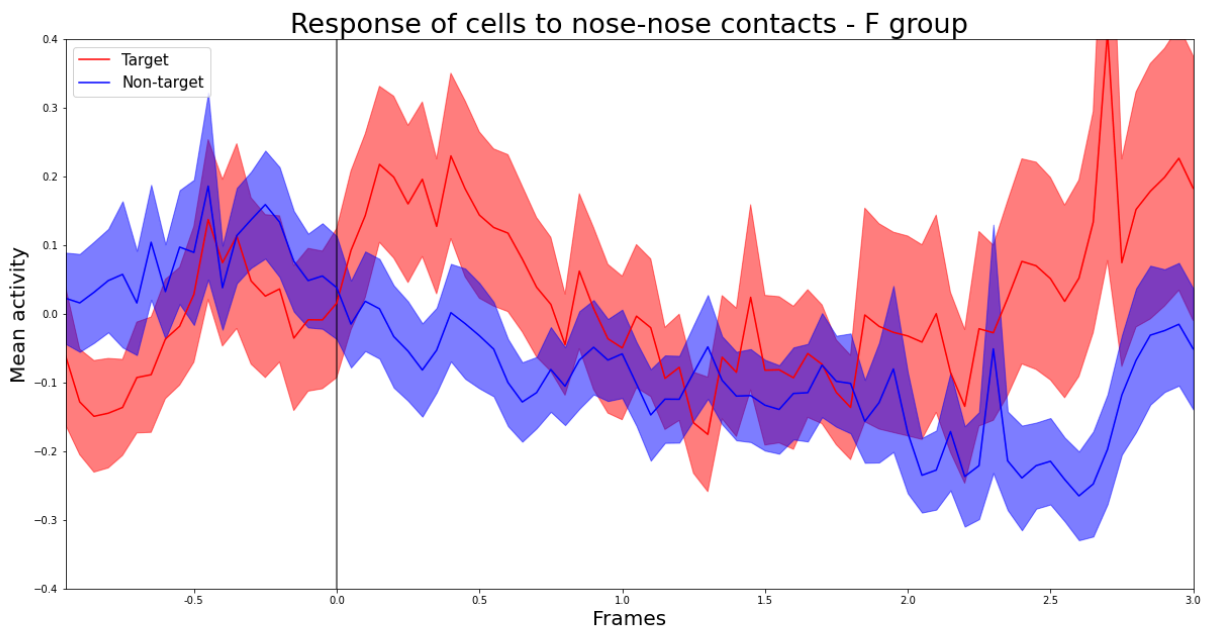

Fig. 22. Traces of activity of the Target and Non-Target cells during the interaction in the F+L group. 0 marks the onset of the interaction.

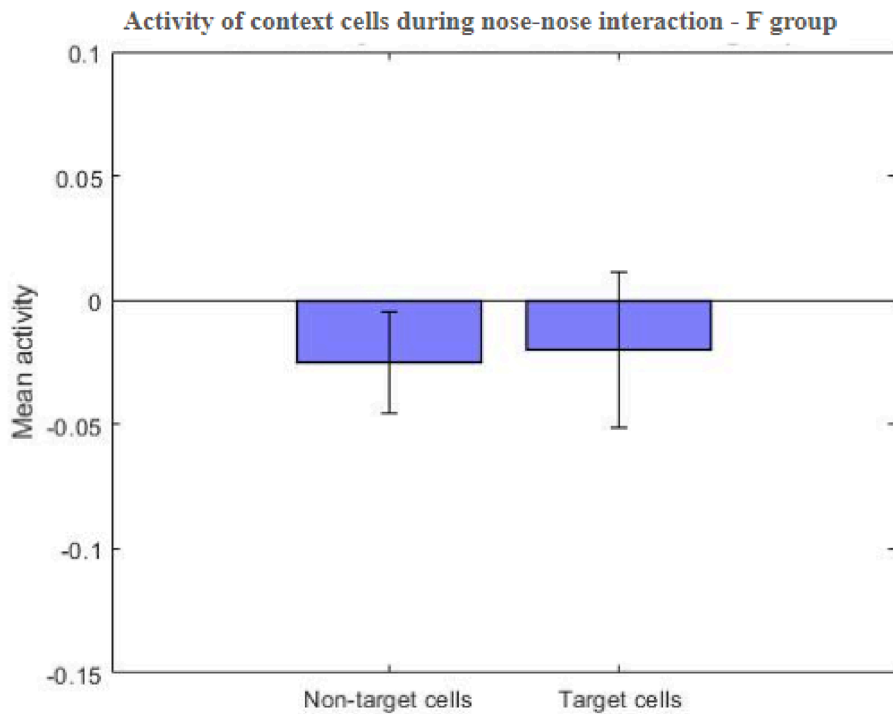

Fig. 23. The comparison of activity of the Target and Non-Target cells after the onset of the interaction in the F group.

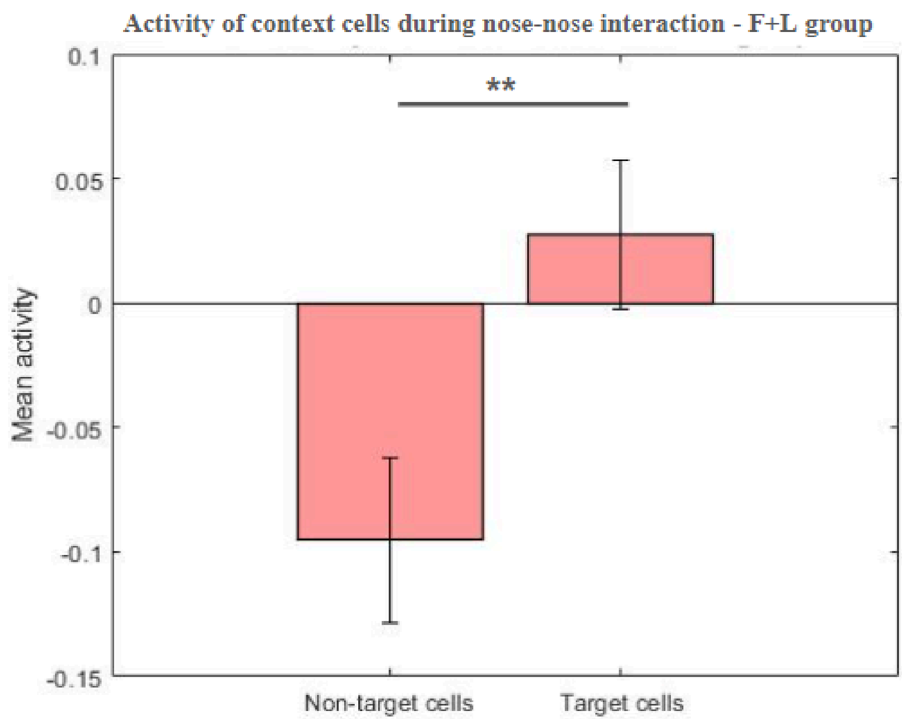

Fig. 24. The comparison of activity of the Target and Non-Target cells after the onset of the interaction in the F group.

Olfactory tubercle activity during interactions does not differ between the groups

To test if olfactory tubercle, a structure related to the processing of the rewarding value of a scent, may be engaged in the detection of olfactory information associated with food location, we have used fiber photometry to measure activity of olfactory tubercle neurons during the onsets of the nose-nose and nose-anogenital contacts, defined as moments when Recipient touched the nose or the anogenital region of the Donor during the interaction. We expected to observe an increased OT activity in the F+L group as compared to the F group (respective traces are shown in Fig. 25). Contrary to our hypothesis, there was no significant difference in the post-interaction onset peak activity between the groups (F: MD = 8.758; SD = 0.818; n = 3; F+L: MD = 8.560; SD = 0.791; n = 3), Wilcoxon rank-sum test, Z=9, p=0.7, Fig. 26a. A similar analysis for the area under the curve also did not show significant differences (F: MD = 263362.7; SD = 318204.4; F+L: MD = 270054.3; SD = 24008.53), Wilcoxon rank-sum test, Z=9, p=0.7, Fig. 26b.

Similarly, no difference was found in OT activity between the groups during nose-anogenital contacts (traces are shown on Fig. 27), either measured as post-interaction onset peak activity (F: MD = 8.8410; SD = 4.275; n = 3; MD = 7.69; SD = 3.88; n = 3, Wilcoxon rank-sum test, Z=1854, p=0.343, Fig. 28a) or comparing the area under the curve (F: MD = 284629.8; SD = 23469.86; F+L: MD = 274979; SD = 19632.25, Wilcoxon rank-sum test, Z=10, p=1, Fig. 28b).

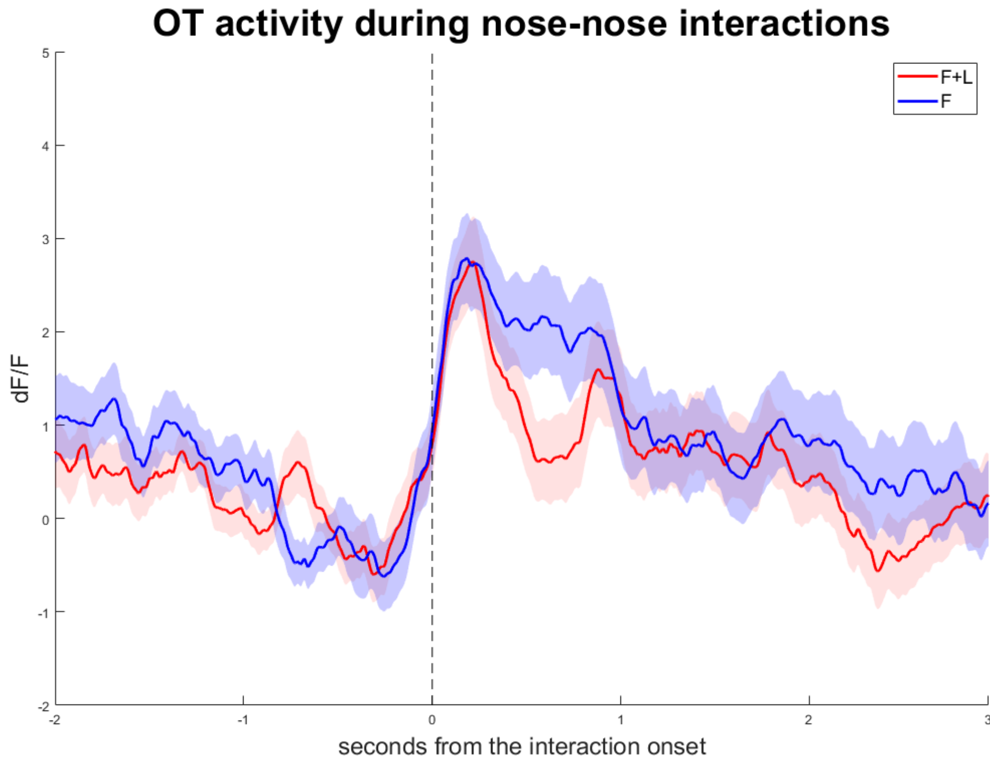

Fig. 25. Olfactory tubercle activity during nose-nose interactions; 0 indicates the interaction onset.

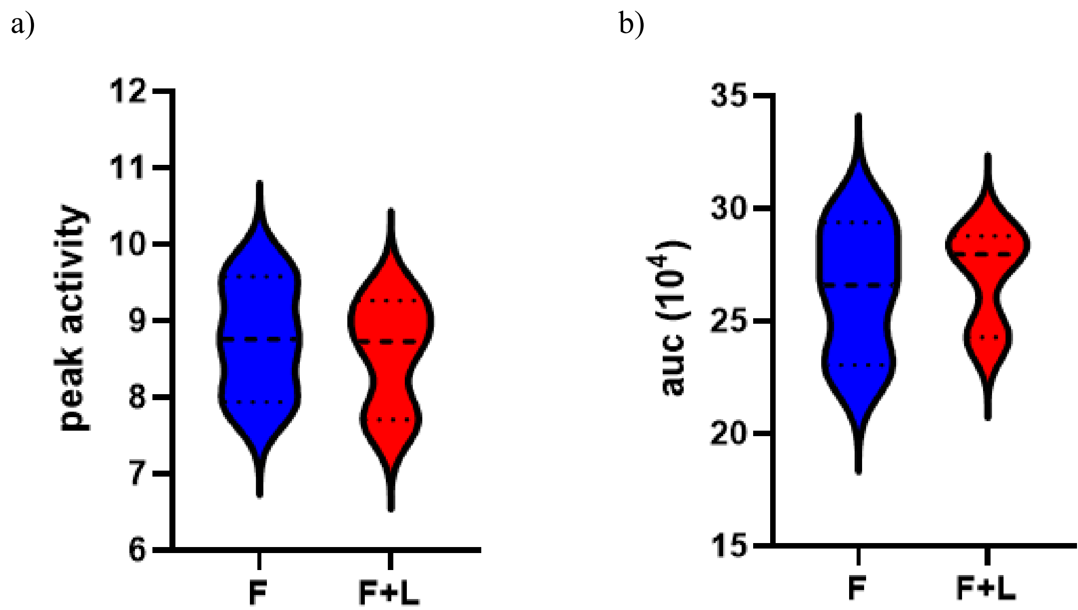

Fig. 26. Peak activity (a) and AUC (b) of post nose-nose interaction OT activity in the F and F+L groups.

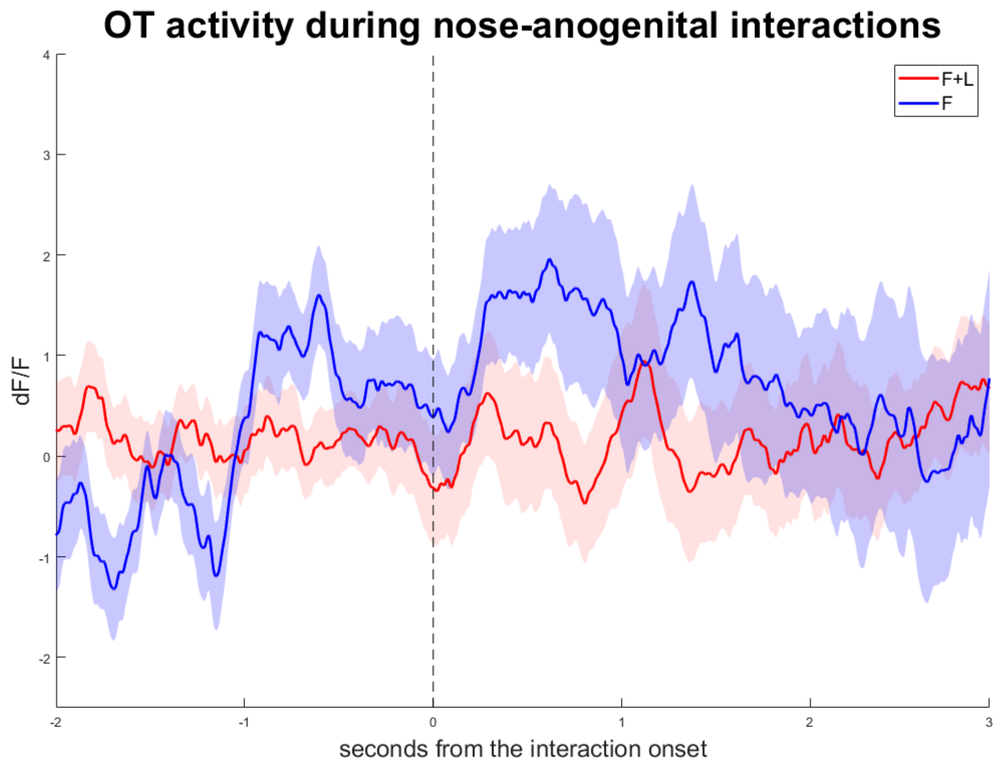

Fig. 27. Olfactory tubercle activity during nose-nose interactions; 0 indicates the interaction onset.

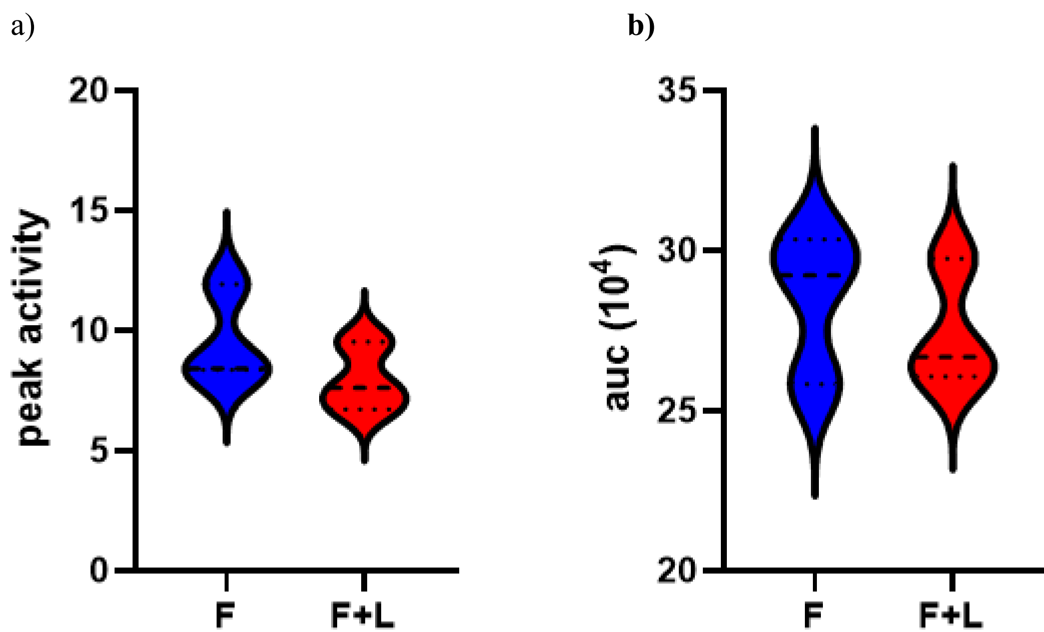

Fig. 28. Peak activity (a) and AUC (b) of post nose-anogenital interaction OT activity in the F and F+L groups.

Structures with an increased activity during social transfer of information

We have used an exploratory whole-brain c-Fos mapping to tentatively map structures that may exhibit upregulation during the social transfer of information about the localization of food. We have found an increased activity in two structures.

We have found an increased normalized number of c-Fos positive cells in the tuberomammillary nucleus in the F+L ($M = 1.6*10^{-4}$, $SD = 0.282*10^{-4}$) group as compared to the F group ($M = 0.310* 10^{-4}$, $SD =0.103*10^{-4}$), t-test for independent samples, $t(6) = -4,34$, $p=0.0048$, Fig. 29.

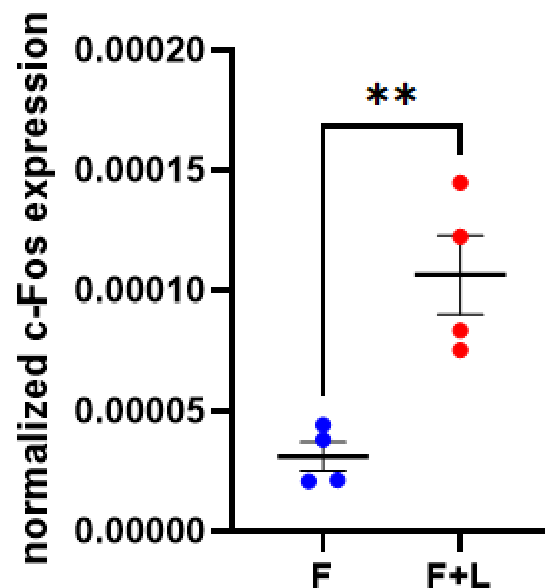

Fig. 29. Normalized c-Fos expression in the tuberomammillary nucleus.

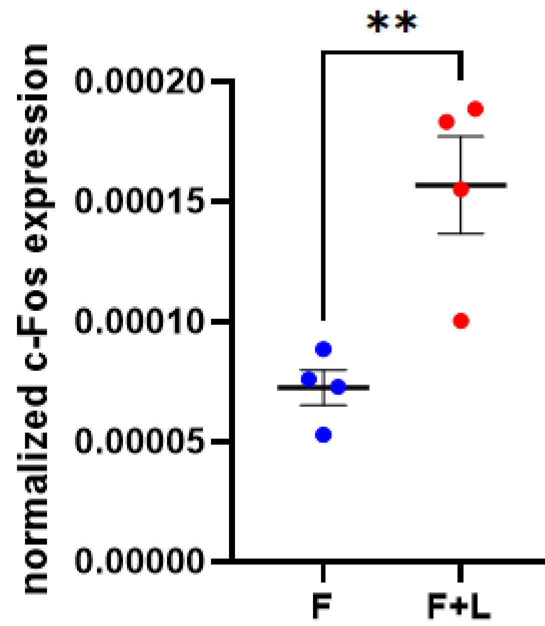

Fig. 30. Normalized c-Fos expression in the ventral premammillary nucleus.

An increased normalized number of c-Fos positive cells in the ventral premammillary nucleus was also observed in the F+L (M= $3.245*10^{-4}$, SD = $0.69*10^{-4}$) group as compared to the F group (M = $1.192*10^{-4}$, SD = $0.875*10^{-4}$), t-test for independent samples $t(6) = 3.183$, $p=0.018$, Fig. 30.

Discussion

Mice can transfer information about the localization of food

In a series of behavioral experiments, we show here that mice and rats can extract information about the possible localization of food in an environment during social interaction with a conspecific. This result raises many questions.

The ecological relevance and evolutionary context of the finding.

The first issue is the ethological relevance of our results. Social behaviors in mice are variable complex and strongly context-dependent (Kondrakiewicz et al., 2019). Typically, mice living in an open space will form territories, with a dominant territory owner showing mostly aggressive behaviors toward other males (Crowcroft, 1958). Females live more peacefully among each other, sometimes with multiple females living together on the territory of a dominant male (König et.al., 2012). In this setting, one would not expect many opportunities for the exchange of social information between males; competition between them would also make any exchange of information potentially maladaptive.

It is known, though, that male mice can exchange information about the palatability of food (Valsecchi, 1989). It could be argued that this behavior might be only observed in laboratory animals that were domesticated and display modified social behaviors (Kondrakiewicz et.al., 2019) and have no ethological relevance. Contrary to this claim, similar behavior was observed in wild populations as well (Valsecchi, 1996).

It may be explained by the aforementioned context dependency of the social organization of mice populations. In confined spaces, when food is abundant and there are no strong reasons for competition, males may not form territories and live peacefully instead (Brown, 1953; Davis, 1958). This social setting may encourage social interactions and the possible transfer of information. Moreover, the social exchange of information may have evolved in the context of mother-offspring interactions and then be generalized to all conspecifics.

The ethological relevance of my results poses a similar challenge. First question that needs to be addressed is to what extent information about the localization of food in the

environment could be useful for animals living in the wild. The territories of males can be very small, and animals may not stray from their nest more than a meter away. Small territory, occupied for a long time may not pose a challenge in terms of localizing food. The size of the territory may vary, though, depending on food availability and the habitat. Range size of mice in wild populations may reach from 365 square meters in a field in feral mice (Quadagno, 1968) up to 80 000 square meters in the wheatlands in Australia (around 280 x 280 meters) (Chambers, 2000). For a rough analogy, such size would correspond to a territory size of around 370 x 370 km for a human being. Mice eat around 20% of their body weight daily (Berry, 1970), and visit up to 30 food sites daily (Potter, 1994). Such high energetic demand, combined with a large territory size, may promote behaviors such as obtaining information about the localization of food sources from conspecifics, as they may reduce time necessary for foraging. On the other hand, as we have mentioned previously, large areas promote territorial behaviors, and thus mostly aggressive interactions between individuals.

It is thus most probable that the ability to detect the localization of food during the social interaction may have evolved in the context of interactions between males before maturity. Young males stay on their father's territory until maturity, when they start to be attacked by him (Bronson, 1979) - during this period that may benefit from the social exchange of information. Another context that might have promoted evolution of such behavior is dispersal - after reaching maturity, males disperse to establish their own territories; social information about the potential localization of food might be important for the decision regarding the establishment of the territory (Berry, 1970). Alternatively, it might have been a behavior initially specific to females, as many of them can occupy one territory and interactions between them are less aggressive (König et.al., 2012). A result partially confirming this hypothesis was observed by Forestier at al (2020). In this experiment, mice developed a food preference for a given food type by sniffing feces of an individual that consumed it. It was found that females develop this preference while sniffing feces of familiar or unfamiliar males and females. Males, though, developed a preference only if the feces belonged to an unfamiliar juvenile male or an accustomed female.

Behavioral experiments conducted in this thesis were done in an impoverished, largely unstructured environment, with an artificial social context. Further analysis of this behavior should be conducted in a more realistic setting, preferably in naturalistic or semi-naturalistic conditions. One could envisage a large area occupied by a population of mice in which every animal can be individually tracked. Food would be randomly placed in different locations. By giving an access to a given food location only to a selected mouse, and

by tracking its interactions with others and the spread of the information (measured as the number of visits of other animals in a given spot), one could trace how this information is exchanged, and which animals take benefit of it. It could be tested, for example, if the information is mostly shared by young males, females or all mice independently of age. One could also test the role of the position in the social hierarchy on the usage and spreading of social information.

The dependence of this behavior on the environmental context would be also an interesting question that could be tested in this setting. Abundance of food and/or the size of the environment could be manipulated to induce either territorial behavior or the development of social hierarchy - it would allow us to test how different social settings in mice influence the transfer of information. Developmental questions could be also addressed: behavior observed in our paradigm doesn't require any previous learning. Testing for its presence at different stages of development, and controlling the social environment, would allow us to check if the trait in question is learned at any stage or is rather a case of robust, unlearned behavior.

A more elaborated version of this paradigm could be also used to test predictions of social foraging theory (Giraldeau, 2000). By manipulating the cost of relying on social or personal information, i.e. by inducing potential predator risk (Galef, 2009) one could study when animals rely on which source of information. The parameters such as reliability of Donors, the stability of food sources, availability of food in a food source, food quality could be also measured and could be implemented in a model predicting mouse's foraging decisions (Giraldeau, 2000). Such a paradigm would be a great improvement over the existing tests of social behaviors in mice, e.g. three - chambered test (Jabarin, 2022).

As mice in my paradigm don't need training to exhibit behavior in question, it most probably is a part of a natural repertoire of mice behaviors, and may be more ecologically relevant than just simple assessment of social preferences. Moreover, when done in a more complex setting, just as described above, it would allow a more fine-grained phenotyping of impairments of social behavior. Avoidance of other conspecifics in a three chambered test may be related to many factors (e.g. increased anxiety, impairment of olfaction, pain, etc). In a paradigm that relies on a complex and spontaneous exchange of social information it would be possible to disentangle many aspects of possible impairment. One could imagine possible impairments in sensitivity to social cues, ability to use socially acquired information, context-sensitivity to social cues (e.g. using or not using social information depending on the reliability of the information source) and more, that could be assessed separately.

The channel of information transfer

It is now well established that mice develop a preference for a novel food consumed by a conspecific by exposure to a breath odour, containing the scent of the food and the social cue, namely carbon disulfide (Galef, 1988) and there is a specific subsystem in the olfactory system that is sensitive to this cue (Munger, 2010). We have expected that this may be also the way of transfer of information in the behavior observed in our paradigm. Contrary to this hypothesis, We have not observed increased nose - nose interactions in the experimental group as compared to the control group. This observation alone, though, cannot exclude breath odours as the channel of information transfer. One could argue that just a brief contact with a Donor is enough to sense the scent of food and the scent of the environment the food was found in. One way of testing this hypothesis more thoroughly would be to enforce nose - nose interactions by e.g. constraining the donor mouse. It would induce strong stress, though, and may impair the information transfer, making the interpretation of a negative result impossible. On the other hand, a blockade of specific cells responsible for the detection of carbon disulfide would allow us to check if this molecule takes part in the transfer of information not only about the palatability of food, but also its location (Munger, 2010).

It is an interesting observation that mice in the F+L (experimental) group had more intensive nose-anogenital contacts. The role of such interactions in the context of this paradigm is unclear.

Rats increase their sniffing rate during nose-anogenital interactions (Wesson, 2013) and it is hypothesized to be a form of gathering information about the conspecific (e.g. identity, sex, reproductive state); androgens influence intensity of anogenital sniffing and are hypothesized to increase sensitivity to social cues (Thor, 1978). It was found, for example, that an alarm pheromone is produced in the perianal region of a stressed rat; it can induce stress response in a recipient rat (Kiyokawa et.al., 2004). The nature and role of social cues in the context of our paradigm is hard to guess, though. Mice can develop a preference for a food type via interaction with feces (Forestier et.al., 2020). The gastrointestinal transit (time from the consumption of food to defecation) in mice is more than 6hr (Padmanabhan et.al., 2013); as the time between consumption and social interaction in our paradigm is maximally around 10 minutes, it is impossible that any traces of consumed food reached the rectum.

One possible explanation of the effect is the motivation of the Recipient mouse to gain more information about the mouse that provides possible information about the location

of food in a known environment. In this scenario, the mouse learns that there may be food in the environment, and may need to assess e.g. reliability of the Donor - is it a known or an unknown mice or what is its position in the social hierarchy, as the hierarchy structure of the population may influence social learning (van Boekholt, 2021). Alternatively, Recipient may follow the Donor to gain more cues regarding the localization of the food.

While we have assessed basic variables that may exclude the usage of visual cues (e.g. time spent by both animals on the Target side during the interaction), more subtle forms of the usage of visual cues cannot be excluded.

The most probable channel of the information transfer is still probably the breath smell, possibly supported by other cues, most probably visual.

To precisely establish the channel of information transfer, one could deploy more precise behavioral analysis methods. Specifically, moSeq, an unsupervised behavioral classification software could be used to detect different behavioral “syllables” that are used only in the F+L (experimental) group, and then analyze those syllables to check if they may correspond to some forms of e.g. visual cues (Wiltschko et.al., 2015). It would require, though, an adaptation of the behavioral setup to enable precise recording with 3D cameras.

The transfer of social information about the localization of food by rats

In an additional experiment we show that rats are also capable of transferring information about the localization of food in the same paradigm.

Rats are highly social animals (Kondrakiewicz et.al, 2019); the social transmission of food preferences was initially observed in rats (Galef and Wigmore, 1983). Rats usually live in groups of 15 (up to 100) individuals (Lore, 1977). They establish a stable hierarchy, and after its establishments live mostly peacefully (Barnett, 2017). Rats are sensitive to many social cues - they are capable of emotional fear contagion, and respond to olfactory, vocal and visual social cues (Masuda et.al., 2013; Litvin, 2007).

The presence of a similar behavior suggests that it was present in a common ancestor of rats and mice (that lived around 16 million years ago), and may be an ancestral behavioral trait of all Muridae species (Steppan, 2017), a hypothesis that could be tested on more distant Muridae species used in laboratories, such as *Peromyscus* mice.

Interestingly, while we have observed a consistent and almost universal shift of preference in the experimental group with low variability, there is a striking variability in rat preferences in the control group. The preference of control rats during the test seems to be completely reset, with animals randomly shifting their preferences without consistency with the previous day's preferences.

It could be potentially explained by one procedural difference between procedures used in rats and mice in our paradigm. Rats were separated for three days during the experimental period to enhance social interaction between them (and possible information transfer) (Varlinskaya, 1999). This interaction, and a strong positive affect related to it, might have completely changed the assessment of context in control animals; on the other hand, in the experimental group, the motivation to find food overcame this effect.

We have also observed a subtle, but significant difference in properties of ultrasonic vocalizations used in rats in two groups. Interestingly, differences in vocalizations were also detected in the social transfer of food preferences experiments. Galef (2004) has found that female rats emitted vocalizations at higher rate when interacting with a conspecific that consumed unfamiliar food before the interaction. Devocalized rats, though, were as efficient in information transfer as control rats (Galef, 2004), making those results hard to interpret. Similar differences were also observed in mice (Moles, 2000).

Knutson (2002) hypothesized that ultrasonic vocalizations may indicate the anticipation of reward or punishment. Aforementioned effect - and possibly the effect observed in our paradigm - could be potentially explained by the anticipation of animals to find new palatable food (in the case of STFP paradigm) or food in general in the state of hunger (in the case of our paradigm). More in depth analysis of produced vocalizations, and their precise classification, could be performed to find more cues as to the role of emitted vocalizations, especially when paired with the simultaneous behavioral tracking of the animals and the identity of vocalizing individual - it could be testes which rat (Donor or Recipient) is producing vocalizations that differ between groups and in which context they are emitted.

An alternative explanation could be based on findings of Blumberg (1992) who suggested that at least some vocalizations used by rodents might be a side effect of body movements. If rats in the experimental group are more agitated, they might produce different types of vocalizations related to different body movements.

Global remapping of place cells and all hippocampal cells

We have expected to observe an increased change of place cells activity to happen in the F+L group, hypothesizing that the socially acquired information will change internal beliefs of an animal. Contrary to that assumption, we observed complete remapping of place cells in both groups. This finding could be explained by different factors.

The first possible explanation of the phenomenon is that just the presence of the Donor in the test condition is a change of the context strong enough to induce remapping. Hippocampus is hypothesized to encode a map for social navigation in humans (Tavares et.al., 2015) and its functions related to social cognition in general are recently being more widely studied (Montagrin, 2018).

Place cells remapping evoked by the change of the social context would be inconsistent with recent findings, though. In a study by Wu et.al. (2023) cells in the CA1 region were recorded in rats in a paradigm based on changing social context; rats were exposed to unfamiliar and familiar conspecifics in wire cages (similar to ones used in our paradigm) in a familiar environment. The change of conspecifics did not lead to place cells remapping, in contrast to changes in the color of the experimental compartment (Wu et.al., 2023). Authors hypothesize that the social context may not be encoded in the hippocampus.It is important to note, though, that the baseline condition in the paper included the presence of conspecific, and there was no reference to a context without any other rats present in the environment. In another study, though, remapping of spatial cells related to the presentation of conspecific was observed in the CA2, but not CA1 region of the hippocampus (Alexander et.al., 2016).

In this light, the presence of remapping in the F group, in which no information regarding the localization of space is unexpected. Another reason that may explain this result, though, is the potential presence of a reward. Mice in our paradigm are hungry and the scent of food can be expected to be rewarding. While we hypothesized that the information about the localization and the expectation formed by it should be potent enough to differentiate two groups in terms of changes in CA1 cells activity, the scent of food might be a stimulus strong enough to induce global remapping in both groups.

Cells selectively responsive to rewards had been found in the CA1 (Gauthier, 2018), interestingly, though, their responsiveness is stable and is preserved even in the case of a

change of the environment leading to the global remapping of place cells. Place cells seem also to overrepresent reward locations (Mamad et.al., 2017; Kauffman, 2020).

The study by Krishnan et.al. (2022), mentioned in the introduction, has shown that the lowering of the reward expectation led to the place cells remapping, indicating that changes in reward expectations may indeed result in the change of the representation of the context. Thus, the scent of the food, present in both groups, might in fact be a strong stimulus changing the activity of cells in two groups, overshadowing the potentially weak effect of the additional information about the localization of food.

This hypothesis could be tested in at least two ways. First of all, additional control groups could be introduced to eliminate the factor of the rewarding scent of food. First group could be called No Food (NF), where the Recipient would interact with a hungry Donor. It has a disadvantage of potentially changing the interaction dynamics: the Recipient might be less interested in the interaction with a hungry conspecific, and the reduced number of social contacts might have an additional effect on brain activity.

Alternatively, another group, called Low Motivation (LM) could be implemented in which Recipients would not be food deprived, but would interact with a fed Donor. Here the scent of the food pellet is present, but its rewarding value is diminished. This condition, though, is also not devoid of potential confounding factors; animals might be less motivated to explore the environment, which may make the identification of place cells problematic; just like in the previous group, their motivation to interact with the Donor might be also compromised.

Krishan (2022) has observed that the inhibition of the ventral tegmental area dopaminergic axons in the CA1 replicated the effect of the reduction of reward expectation on the place cells remapping. Manipulation of this connection (e.g. its activation) could be also potentially used to check if the modulation of the influence of the reward system in our paradigm may result in similar remapping effects.

Another hypothesis that could potentially explain the presence of strong remapping is methodological. While one-photon microscopy was used multiple times to identify cells between days and assess their stability (Sheintuch et.al., 2020; Zeva, 2023), there is some doubt regarding its limitations in that regard (Masset, 2022). A good example is the study on the stability of the activity of cells in the HVC nucleus in the brain of a zebra finch (Liberti et.al., 2016). HVC is a structure responsible for maintaining the timing of a bird's song (Long et.al., 2010). In the study of Liberti et.al (2016), where single-photon microscopy was used, it was found that the activity of around 40% HVC cells is insatiable in time. Interestingly,

though, two-photon imaging of HVC activity by Katlowitz (2018) indicated that the neuronal activity in this structure is very stable. This surprising difference might be explained by the technical limitations of the miniaturized single-photon imaging, such as lower resolution, much stronger background activity and motion artifacts much stronger than those expected in a head-fixed setup (Chen et.al., 2020) that may lead to impaired ability of algorithms to properly identify cells between sessions.

One thing that needs to be noted is that this study used a v4 version of UCLA Miniscope, developed in 2021 (https://github.com/Aharoni-Lab/Miniscope-v4/wiki). Up to this point, this exact method was not used for the purpose of studying stability of place cells, in contrast to its most recent version (Guo et.al., 2023; Blair et.al., 2023), so we lack a benchmark for the assessment of its reliability. We have noted several problems with the device, ranging from frequent disconnections to complete malfunctions; the stability of the image was also worse in comparison to the older version. Older versions, on the other hand, lacked the regulated zoom and had to be adjusted manually; their use required having one microscope per animal to ensure that the same field of view is being imaged.

This drawback could be avoided either by developing a head-fixed, VR-based version of our paradigm, or the usage of a miniaturized two-photon imaging system with a much better image quality that has recently become available (Madruga et.al., 2024).

To sum up, the global remapping observed in both groups may stem from the strong effect of an appearance of a conspecific in the known context, the effect of the presence of a rewarding cue or from the limitations of the single-photon miniaturized imaging system.

Context cells encoding food localization are reactivated during the social interaction

We have identified sets of cells that encode the context associated with the Target and Non-Target compartments and measured their activity during the social interaction. We have found that in the F+L group, where the transfer of information about the localization of food takes place, context cells encoding the Target Compartment are indeed more active as compared to the cells encoding the Non-Target compartment. This pattern of activation was not observed in the F group.

As discussed in the Introduction, one of the hypothesized roles of the replay of hippocampal cells is planning of future actions (Diba, 2007; Pfeiffer, 2013; Ólafsdóttir et.al.,

2015); the other hypothesis assumes that its main role is memory consolidation (Ego‑Stengel, 2010; Gillespie, 2021).

The result obtained in this dissertation could be interpreted in the light of both hypotheses. The most intuitive explanation of the activation of the cells representing the Target compartment during social interaction would be that it reflects the planning of the exploration of this location. The animal may 1) recognize the potential source of food, 2) associate it with an encoded memory of this location and 3) use this recalled information to plan the route or exploratory strategies.

One possible way of verifying this hypothesis would be to check if the temporal order of the replayed context cells reproduces later trajectories of the animal in the experimental cage. The shape of the cage used in our paradigm is, however, not well suited for such an analysis, as it enables movements in all directions. A linear track that constraints trajectories to a one-dimensional vector could be applied to test this hypothesis. Moreover, machine learning algorithms could be used to test if the more complex aspects of a mouse behavior (e.g., its exploratory behavior like sniffing, rearing, or movement speed) can be predicted based on the recorded activity of context cells during the social interaction.

On the other hand, an activation of the Target cells during the interaction may be an indication of an enhanced consolidation of the memory of that space by an animal. In fact, though, those two hypotheses are not mutually exclusive. An animal can do both things simultaneously, consolidating the information and at the same time using it for planning.

One thing that needs to be noted is that the identification of the onsets of the nose-nose interactions was done manually, based on camera recordings. The recorded videos do not allow to assess the exact moment when an animal began sniffing of the snout of a Donor, and the onsets for different trials might be slightly misaligned. Thismay explain a peak of activity observed around 2.5 seconds after the onset of the interaction in the F+L group. To precisely identify the start of the interaction, thermistors implanted to the animals' noses could be used. They allow the sniffing patterns of freely-moving mice (McAfee et.al., 2016). However, they are invasive, and in combination with invasive GRIN lens implantation, and the necessity to put more weight on the head of the animal, they may exert many negative effects on the mice's well-being and their spontaneous behavior. Alternatively, a head-fixed version of our paradigm could be developed. In this way, sniffing patterns could be assessed from high-quality video recording of an immobile mouse.

The fact that animals had to interact via a mesh surrounding the Donor disabled the possibility of recording context cells during episodes of sniffing of the anogenital region of

the Donor by the Recipient. As our results from social interactions suggest, this behavior clearly distinguishes the F+L group and suggests that information could be transferred also this way. Development of a setup in which mice could interact without such a barrier could enable testing of this hypothesis as well.

The activity of olfactory tubercle does not differ between the groups

Contrary to our hypothesis, we did not observe any differences in the activity of the olfactory tubercle (OT) between the F and F+L groups. As the OT encodes the affective value of olfactory information (Murata et.al., 2015), we hypothesized that the reward expectation in the F+L group, in which the information about the localization of food is being transmitted, will induce changes in the activity of this structure.

The possible explanations of the lack of the expected effect are few. First of all, the OT includes separate populations of cells encoding aversive and appetitive aspects of the stimulus (Murata et.al., 2015). The dopamine receptor 1 (D1)- expressing neurons encode positive and dopamine receptor 2 (D2)- expressing neurons encode negative values. As the photometry enables to measure only the net activity of multiple cells, the effect of the reward expectation depending on D1-expressing neurons might be lost. This could be overcome by using a calcium indicator expressed under a D1 promoter to measure the activity of the D1-expressing neurons.

Alternatively, an effect of the food cue might be so strong - just as in the case of place cells remapping - that it overshadowed the subtle effect of the information about the localization of food on the activity of the OT. The OT clearly responded to the nose-nose contact onsets, but its response to the nose-anogenital contacts is less marked, and in the F+L group is almost not observable. It might suggest that the OT indeed strongly responds to the scent of food of the Donors in both groups and is not engaged in the processing of social cues produced in the anogenital region.

The control groups similar to those suggested in the remapping section of the Discussion could be employed to test this hypothesis.

As discussed earlier, a possibility to record sniffing patterns of the Recipient would improve the identification of sniffing onsets during the social interaction, making interpretation of the data easier.

Exploratory whole-brain activity mapping suggests engagement of structures related to metabolism and social interactions

We have performed an exploratory whole-brain c-Fos immunostaining to identify structures that may be engaged in the social transfer of information about the localization of food. Interestingly, we did not observe differences in the structures that we hypothesized to play a role in the investigated behavior - the CA1 and CA1 regions of the hippocampus, anterior olfactory nucleus or olfactory tubercle.

Instead, we have found indications of differences in two nuclei related to metabolism, the ventral premammillary nucleus and tuberomammillary nucleus.

The ventral premammillary nucleus (PMv) is a structure engaged in linking energy metabolism to reproduction (Audzijonyte, 2018) and sexual maturation (Donato et. al., 2011); it is also activated in male rats in response to the olfactory cues of females (Calvacante, 2006) and belongs to a network of brain regions related to aggression (Motta et. al., 2013; Stagkourakis, et. al, 2018). Receptors for multiple energy level-related hormones are expressed there, including orexin, urocortin-3, leptin, and ghrelin (Leshan, 2014),

The increased activation of this structure in the hungry Recipients in our paradigm suggests that its role in linking metabolism with social cues about food may extend beyond reproduction-related cues. It might serve a more general function, integrating social information with estimates of the organism's energy levels to guide behavior and development.

On the other hand, the PMv is active during aggressive interactions between males and its optogenetic activation evokes an attack in male mice (Stagkourakis, et. al, 2018). While it may suggest that the observed difference between the groups may stem from differences in aggressive behaviors during social interactions, we did not observe any aggressive behaviors between the Donors and Recipients in our experiments.

The tuberomammilary nucleus (TMN) is a structure consisting of histaminergic neurons (Sherin et. al., 1998). Especially interesting from the perspective of the current study is the role of this structure in motivated arousal. For example, in the study by Valdés et. al. (2010), in hungry rats trying to obtain food from a wire-mesh enclosure, increased activation of the TMN was observed and its lesion led to a strong reduction of motivated behavior. An increased activity of the TMN was also observed before feeding in a scheduled food intake

regime, with a dramatic decrease after a meal (Inzunza et. al., 2010). Thus, it is hypothesized that this structure may encode the expectation of feeding (Umehara et.al., 2011). Interestingly, activation of the TMN was also observed in a study on the influence of physical exercise on learning. Increased performance in the object recognition task in a group that exercised was correlated with the activity of the TMN (Grinspun et.al., 2019). Thus, the structure may also play a role in reinforcement learning (Huston, 1997). Its increased activity in the F+L group may mean that mice in this group formed an expectation to find food in a known environment that was stronger than in the F group, additional information about the localization of food increased the motivated arousal in the Recipients.

An experiment could be performed to test this hypothesis. Mice could be trained in a regime where they obtain food rewards with different probabilities. One could test if there is a relationship between the food reward probability and the activation of the TMN.

Additionally, an expanded version of our paradigm could be implemented. Mice could be trained in an X - shaped maze with food rewards present randomly in one of the four arms of the maze in each trial. Cues regarding the localization of the piece of food would be provided during an interaction with a Donor, who ate food in the target arm. The reliability of a Donor could be then manipulated, with a social cue providing correct information only in some percentage of the trials; then it could be tested if the activity of the TMN encodes the reliability of this cue.

In conclusion, the two structures found to be upregulated in the current study are related to homeostasis and metabolism. The social transfer of information may thus engage the structures involved in prediction of food intake, and integration of social cues with the information about the energy state of the organism.

c-Fos mapping provides very good spatial resolution (at the level of single cells) and, when combined with brain clearing, is a high-throughput method of analyzing neuronal activity in the whole brain. One should remember, however, that studies based on mapping the brain activity with c-Fos have multiple limitations. They have low temporal resolution and do not show all activated cells (Hudson, 2018). Our analysis, due to a relatively small group and a large number of comparisons, can be treated only as an exploratory study. To test the engagement of all the aforementioned structures in the transfer of social information, fiber photometry could be applied during social interaction to probe the activity of neurons in real time. Then, the recording of the activity of single neurons would be helpful to understand the role of these structures. The fact that the structures are localized deep in the brain may make use of endoscopic imaging problematic, as it requires the implantation of GRIN lenses.

Silicon probes could be used instead to measure the activity of single neurons in the structures of interest to check what parameters of the social interactions the neurons are tuned to.

Homeostasis, allostasis and social cues

It was hypothesized that one of the main functions of the brain may be to predict and satisfy metabolic demands; the brain in this framework is viewed as the nutritional prediction system (Walker, 2017). Walker (2017) points out that a purely homeostatic system - one in which a parameter (e.g., energy levels) is measured, deviations from the set point are detected, and corrections are applied to counterbalance the deviation - may not be optimal. Organisms may need to predict their future energy demands and the availability of nutrients in the environment.

An example of such a situation would be if nutrients would not be available and a significant additional effort would be necessary on the side of an animal to obtain it. A purely homeostatic system would fail in this scenario, as it is required to push the parameters even further from the setpoint to obtain energy. The ability to predict the availability of food in a distant location would drastically improve its performance.

Walker names such effect *predictive homeostasis*; it was defined in the past as *allostasis* by Sterling (1988). This view fits in a broader theoretical framework of predictive coding (Keller, 2018). The results obtained in our study and the paradigm of the social transfer of information about the localization of food in the environment thus open a possibility to bridge studies on social behaviors, predictive coding and physiology.

It could be hypothesized that during social interaction, the scent of food and its appetitive aspect are processed in the reward system, including the OT. Information from social cues is combined with an animal's estimates of its energy levels in structures such as the PMv. Predictions about the location of food, its energetic value, and the effort necessary to obtain it could be computed in structures including the TMN and the hippocampus. The role of the hippocampus would also be to plan the route and the search behavior. Imaging the structures of interest in our paradigm at different points of the behavioral sequence could be used to test and expand this hypothesis.

Conclusion

In this study, we have found that mice and rats, through social interaction, are capable of detecting the location of food found by a conspecific. The channel of information transfer remains unknown, but it is likely that it includes the scents produced in the anogenital region of the conspecific. We have found that cells in the CA1 region of the hippocampus, which encode the space where the mouse is expecting food, are reactivated during the social interaction. This suggests that planning of future exploration may be one of the functions of hippocampal replay. We have also found evidence that a subset of nuclei related to homeostasis and metabolism is activated during the information transfer.

The behavioral paradigm designed in our study opens the door for further research on social learning. It could be especially useful for studying the decision-making process in social contexts, the relationship between socially acquired information about space and its brain representation, the interplay between social cues, metabolism, and predictions, and the way the value of a reward is encoded in the olfactory system.

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Publication record of the PhD Candidate

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