Social Influence on Rat Preferences and Communication

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Irina Noguer Calabús
from Torroella de Montgrí, Catalonia

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from the Comparative Psychology Laboratory,
Institute of Experimental Psychology
at the Heinrich Heine University Düsseldorf

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Supervisor: Prof. Dr. Tobias Kalenscher
Co-supervisor: Associate Prof. Dr. Marijn van Wingerden

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INDEX

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This thesis aims to describe part of the neural substrates of social influence and some features of communication in rats, both of which are everyday phenomena. To me, the study of normal behavior represents the beauty of generating new knowledge that extends beyond a purely functional focus on immediate applicability. The pursuit of knowledge for its own sake is a value that must be protected. Nevertheless, in the medium to long term, the results of basic research become essential foundations for theory building and applied science. Support for the further development of basic research is crucial, and with this in mind, I would like to express my gratitude for all the support I have received along the way.

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SUMMARY

Rats live in groups and engage in a wide range of social behaviors. This thesis focuses on two key aspects of social behavior – the social influence on food preferences and communication – and their impact on decision-making.

We investigated social influences on food choices using an adaptation of the socially transmitted food preferences (STFP) paradigm. In this task, an observer rat develops a preference for an initially non-preferred food after detecting its odor on the breath of another rat, known as the demonstrator. First, we proved that the nucleus accumbens shell (NAcSh), a brain region central to reward processing and motivation, is essential for STFP acquisition. We then examined the role of oxytocin (OXT), a key modulator of social behavior, in STFP by assessing the effects of systemic OXT administration. Additionally, we included familiarity with the demonstrator (known or novel) as a variable that modified the strength of preference change. Our findings indicate that STFP is shaped by OXT, familiarity, and their interaction.

In addition, we investigated ultrasonic vocalizations (USVs), a form of vocal communication in rats, and their subtypes in different contexts. To this end, we designed a novel paradigm that juxtaposed social and non-social rewards. Our results revealed a trade-off between social interaction and sucrose consumption, with preference for sucrose increasing as the sugar concentration rose. Rats produced significantly more USVs during social interaction. However, vocalization patterns did not fully align with behavioral preferences, indicating that USVs reveal additional information beyond choice behavior.

Taken together, these findings highlight the salience of social interactions in rats, which are processed and modulated by distinct neural mechanisms in a context-dependent manner. Moreover, USV analysis offers a powerful tool for deepening our understanding of how social information is integrated into decision-making.

With this work, I hope to make a small but meaningful contribution to the vast field of social neuroscience, helping to develop tools that deepen our understanding of human interactions and improve social dynamics.

ZUSAMMENFASSUNG

Ratten leben in Gruppen und zeigen ein breites Spektrum an sozialen Verhaltensweisen. Die vorliegende Dissertation konzentriert sich auf zwei Schlüsselaspekte dieses Sozialverhaltens – den sozialen Einfluss auf Nahrungspräferenzen und Kommunikation – ebenso wie deren Auswirkungen auf die Entscheidungsfindung.

Wir untersuchten den sozialen Einfluss auf die Nahrungsauswahl mit Hilfe einer Adapation des socially transmitted food preference (STFP)-Paradigmas. Bei dieser Aufgabe entwickelt eine Ratte, der Beobachter, eine Präferenz für ein zunächst nicht bevorzugtes Futter, nachdem sie dessen Geruch im Atem einer anderen Ratte, dem sogenannten Demonstrator, wahrgenommen hat. Zunächst wiesen wir nach, dass der Nucleus accumbens shell (NAcSh), eine für Belohnungsverarbeitung und Motivation zentrale Hirnregion, für den STFP-Erwerb essenziell ist. Anschließend untersuchten wir die Rolle von Oxytocin (OXT), einem wichtigen Modulator des Sozialverhaltens, im STFP, indem die Auswirkungen einer systemischen OXT-Verabreichung analysiert wurde. Darüber hinaus haben wir die Familiarität des Beobachters zu dem Demonstrator (bekannt oder fremd) als eine Variable einbezogen, die die Stärke der Präferenzänderung beeinflusst. Unsere Ergebnisse deuten darauf hin, dass STFP durch OXT, Familiarität und deren Interaktion beeinflusst wird.

In der dritten Studie untersuchten wir eine Form der vokalen Kommunikation bei Ratten, die Ultraschallvokalisationen (USVs), und ihre Subtypen in verschiedenen Kontexten. Dafür entwarfen wir ein neuartiges Paradigma, das soziale und nicht-soziale Belohnungen gegenüberstellte. Unsere Ergebnisse zeigten einen Kompromiss zwischen sozialer Interaktion und Konsum einer Zuckerlösung, wobei die Präferenz für diese mit steigender Zuckerkonzentration zunahm. Ratten produzierten signifikant mehr USVs während sozialer Interaktion. Die Vokalisierungsmuster stimmten jedoch nicht vollständig mit den Verhaltenspräferenzen überein, was darauf hindeutet, dass USVs zusätzliche Informationen über das Wahlverhalten hinaus offenbaren. Zusammenfassend unterstreichen diese Ergebnisse die Bedeutung sozialer Interaktionen bei Ratten, die durch verschiedene neuronale Mechanismen in einer

kontextabhängigen Weise verarbeitet und moduliert werden. Darüber hinaus bietet die USV-Analyse ein leistungsfähiges Instrument unser Verständnisses zu vertiefen, wie soziale Informationen in die Entscheidungsfindung integriert werden.

Ich hoffe, mit dieser Arbeit einen kleinen Beitrag zum weiten Feld der sozialen Neurowissenschaften leisten zu können und zur Entwicklung von Methoden beizutragen, die unser Verständnis von menschlichen Interaktionen vertiefen und die soziale Dynamik verbessern.

INTRODUCTION

We live in a highly connected world in the age of social networking. Our desire for social interaction has driven technological development to see and talk to each other instantly from opposite sides of the world. Our desire for social information has popularized its use. New jobs focused on communication have emerged, including influencers who share their lifestyle, influencing followers' preferences in food, music, clothing, travel, and more. This rapid change we have seen in the last years has its roots in an intrinsic human drive: social contact.

The study of human social behavior and motivation is an expanding field (Stanley & Adolphs, 2013), necessarily complemented by animal research. Animal models are fruitful tools that provide valuable insights into human psychology and neuroscience (Kalenscher & van Wingerden, 2011; Necka et al., 2015). They enable the direct manipulation of brain structures homologous to those implicated in human social behaviors, allowing for a more precise investigation of underlying neural mechanisms (Kalenscher & van Wingerden, 2011). In this thesis, we use rats as an animal model to investigate the rewarding effects of social interaction. Rats are particularly well-suited for this research due to their naturally complex social organization. In the wild, they live in mixed-sex colonies that can reach more than 150 members including multiple generations (Schweinfurth, 2020). Empirical evidence has demonstrated that rats display social behaviors such as helping others in distress (Bartal et al., 2011). Additionally, they show prosocial biases – defined as choices that benefit others but not oneself (Hernandez-Lallement et al., 2015; Márquez et al., 2015) – and reciprocal interactions, in which individuals alternately help each other access food (Schweinfurth, 2020).

This thesis aims to expand our understanding of the rewarding nature of social interactions in rats and their neural basis by investigating the following questions:

1. *Study I:* Rats' food preferences are influenced by other rats (Galef et al., 1984). To investigate this phenomenon, we adapted the socially transmitted food preferences (STFP) paradigm. In this adaptation, the observer rat first establishes individual food preferences. Next, the observer interacts with a demonstrator rat

that has recently consumed the observer's non-preferred food. Immediately afterward, the observer adjusts its food preferences influenced by the social interaction. Several brain regions involved in reward and social cue processing contribute to STFP. In this study, we lesioned the nucleus accumbens shell (NAcSh) in observer rats to assess its specific role in this process.

2. *Study II:* STFP may be influenced by the familiarity between the observer and the demonstrator, that is, whether they know each other (Agee et al., 2019; Galef et al., 1984; Galef & Whiskin, 2008b). Therefore, we examined the effects of familiarity and investigated whether oxytocin (OXT) modulates this influence, given its established role in social recognition (Choleris et al., 2009; Oettl et al., 2016; Popik et al., 1992).
3. *Study III:* Rats show motivation for social interaction and sucrose consumption (Ikemoto & Panksepp, 1992; Kirkman et al., 2022). We aimed to assess how they allocate time between these two stimuli presented simultaneously on opposite sides of a maze. This task allowed us to determine the value assigned to social stimuli relative to sucrose reward. These rewarding experiences elicited ultrasonic vocalizations (USVs), a key form of rat communication (Brenes & Schwarting, 2014; Brudzynski, 2015; Burgdorf et al., 2008), and we evaluated the relationship between these vocalizations and their choices.

Beyond the social component, which serves as the backbone of this thesis, our research questions share two fundamental concepts: reward valuation and motivation. Neither reward valuation nor motivation can be measured directly. Yet in scientific literature, they are usually analyzed using decision-making paradigms. Motivation and valuation are often inferred from the effort a rat is willing to make to obtain a reward or by the rat's choice between two rewards, assuming that the one chosen is more valued and therefore more motivating. Traditionally, reward value and motivation are manipulated by changing magnitude or introducing effort or time delays (Bissonette et al., 2013; Chong et al., 2016; Roesch & Bryden, 2011; Tang et al., 2016). In this thesis, however, the first two studies investigate how social information modifies food reward value, while the third uses a decision-making task that juxtaposes social vs. caloric rewards. Social stimuli have the capacity to either

enhance the value of a non-social reward or compete with it (van Gurp et al., 2020; Yates et al., 2013). Thus, the core of this thesis lies in exploring how social stimuli influence rats' decision-making.

1. Mechanisms of social and non-social reward processing

Rats continuously encounter and evaluate alternative rewards within their environment, acquiring knowledge about them. They assigned relative values to familiar rewards to make choices and display preferences. Over time, rats dynamically adjust these reward valuations in response to a combination of changing internal and external factors. Internal factors may include physiological states such as hunger or the need for social contact, while external factors include environmental changes, such as reduced resource availability (Burke et al., 2014; Dwyer et al., 2017; Huh et al., 2009; Piet et al., 2018; S. R. White et al., 2024). This ongoing updating of reward values, mediated and complemented by various cognitive processes, facilitates adaptive choices and promotes survival in dynamic ecological contexts. Such updates can occur through direct experience or social learning (Heyes, 1994; Reader, 2016; Zentall, 2006). In the latter case, information is acquired by observing demonstrators or detecting their byproducts, including scent marks, excrement, or behavioral outputs (Heyes, 2012).

A substantial body of research in humans and non-human mammals supports the idea that psychological and neural mechanisms underlying social learning are analogous to non-social learning (Heyes, 1994, 2012; Joiner et al., 2017). For instance, it has been hypothesized that species with high non-social cognitive capacities should show similar levels of social capacities and vice versa, a hypothesis supported by empirical data (Heyes, 2012). This author argues that the distinction lies not in the learning processes themselves but in the nature of the input. However, other studies have indicated the presence of specialized neural mechanisms that have evolved to facilitate learning from conspecifics (Galef, 2012; Gariépy et al., 2014; Insel & Fernald, 2004). Human data also suggests subregional differences (Tso et al., 2018). Yet these differences can range from being separated brain regions to subcircuits situated in close proximity (Behrens et al., 2008; Klein & Platt, 2013; Watson & Platt, 2012).

Despite their initial presentation as antagonistic theories, these perspectives are not fully incompatible. Social information is biologically relevant for rats, leading to extensive processing of social cues. Specialized social-processing subcircuits can develop ontogenetically due to highly social contexts but might not be encoded phylogenetically. Social learning presents advantages in changing environments, where genetic change is too slow, and individual learning is too costly (Reader, 2016).

1.1. Mesolimbic dopaminergic system

Certainly, specific neural subcircuits selectively process social information, yet the mesolimbic dopaminergic system is key in encoding any form of reward value (Fig. 1, Bromberg-Martin et al., 2010; Lammel et al., 2014; Schultz, 2013). The system originates in the ventral tegmental area (VTA) and projects to the amygdala, ventral pallidum (VP), hippocampus, and nucleus accumbens (NAc). These interconnected regions collectively contribute to the processing of rewards, motivation, and context-dependent behavior (Albanese & Minciacchi, 1983; Domesick, 1988; Hamilton et al., 2010; Ikemoto, 2010; Yim & Mogenson, 1983). Nevertheless, I will highlight the key functional role of each of these regions in the following paragraphs.

The amygdala integrates sensory information and is equipped to detect salient stimuli (Uwano et al., 1995), modulate motivation, and increase arousal to facilitate a response through its connections with other regions (Baxter & Murray, 2002; Janak & Tye, 2015; Phelps & LeDoux, 2005). Although traditionally associated with fear processing, the basolateral amygdala (BLA) is essential for encoding associative and motivational significance of rewards and dynamically updating their value based on context (Janak & Tye, 2015; Murray, 2007). Beyond associative learning, the BLA is implicated in reinforcement learning by encoding the positive or negative valence of stimuli, shaping emotional states, and driving appropriate behavioral responses. However, the BLA is not essential for the general processing of rewards, such as stable food preferences (Baxter & Murray, 2002). In conclusion, concerning the subject of this thesis, the BLA plays a pivotal role in dynamically mediating the relative and context-dependent value of rewards and outcomes, as well as in encoding the

emotional significance of rewards and punishments, guiding adaptive behavioral responses (Baxter & Murray, 2002; Grace et al., 2007; Janak & Tye, 2015).

The VP is a key output node of the mesolimbic dopaminergic system. It processes both positive and negative valence, with some neurons responding to rewards and others to aversive stimuli. These responses depend on inputs from the NAc and other regions (Richard et al., 2016), helping the VP balance motivation and pleasure (Berridge & Kringelbach, 2015). In turn, the VP sends outputs to the thalamus and motor areas, transmitting motivational and hedonic signals (Richard et al., 2016; K. S. Smith et al., 2009; Zahm, 2000). Through its connections, the VP integrates diverse inputs to regulate motivation and the pursuit of rewards.

Although the hippocampus is not a component of the mesolimbic dopaminergic system, it plays a critical modulatory role in learning, memory, and contextual processing (Jarrard, 1993; D. M. Smith & Mizumori, 2006). Its connections with the VTA and NAc enable the detection of novelty and the association of rewards with environmental context (Lisman & Grace, 2005). These interactions ensure that rewards are properly linked to situational factors, enhancing the adaptability of behavioral responses.

While the prefrontal cortex (PFC) is neither a direct component of the mesolimbic dopaminergic system, it plays a crucial integrative role in evaluating and synthesizing information from this system to guide decision-making and goal-directed behaviors. The PFC projects to the NAc and the VTA, forming bidirectional connections with the last (Carr & Sesack, 2000; Gorelova & Yang, 1996; Hou et al., 2024). These pathways modulate reward processing and motivated states by integrating dopaminergic signals with cortical processing (Carr & Sesack, 2000). In fact, reward-related processes are distributed across PFC subregions: the orbitofrontal cortex prevents impulsivity and the anterior cingulate cortex encodes action-reward associations contributing to effortful behavior (Rudebeck et al., 2006; Walton et al., 2003). By integrating higher-order cognition with reward processing, the PFC enables context-dependent, adaptive behavior through its coordination with connected regions.

Finally, the NAc is proposed to act as an integrative hub in the mesolimbic dopaminergic system, incorporating goal-directed information from the PFC, contextual inputs from the hippocampus, and emotional salience from the amygdala. Dopaminergic input from the VTA enhances its ability to assign motivational salience, ultimately influencing motor planning and action execution through its outputs to the VP and midbrain. By integrating these reciprocal connections, the NAc is ideally positioned to translate motivation into action (Goto & Grace, 2005; Kalivas & Nakamura, 1999; Mogenson & Yang, 1991). Therefore, in study I we hypothesized that NAc is essential for STFP. Specifically, we lesioned the NAcSh, a subregion that integrates relative reward value, hedonic value, and motivational signals with rewards among other functions (Jang et al., 2017; Katsuura & Taha, 2014; Peciña & Berridge, 2005; Saddoris et al., 2013; Wyvell & Berridge, 2000).

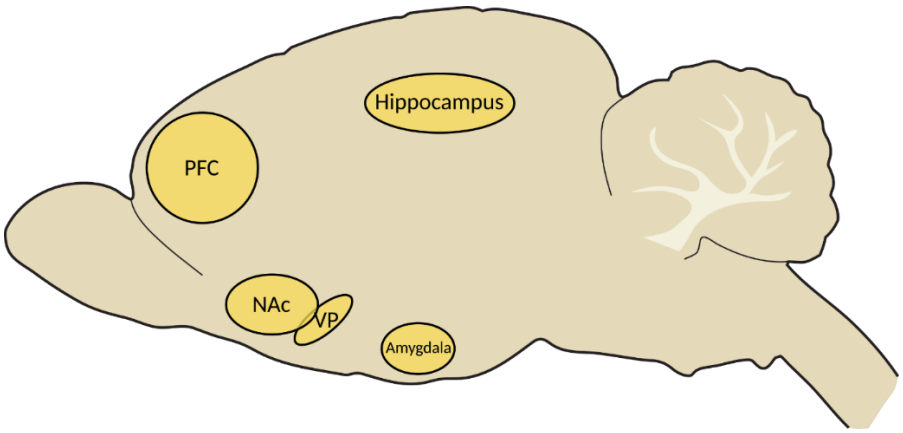

Figure 1. Schematic representation of the rat's amygdala, ventral pallidum (VP), hippocampus, nucleus accumbens (NAc), and prefrontal cortex (PFC).

1.2. Dopamine

As its name suggests, the mesolimbic dopaminergic system relies on dopamine (DA) as a critical neurotransmitter involved in reward processing and motivation. Initially, the first recognized function of DA was its role in the regulation of motor activity, particularly in relation to Parkinson's disease (Hornykiewicz, 1966). Later, Fibiger and Phillips (1979) demonstrated DA's role in reinforcement, showing that it was

necessary for intracranial self-stimulation of the VTA and NAc. Their findings contributed significantly to establishing DA's importance within the reward system. Supporting these findings, Salamone and Correa (2002) reviewed evidence indicating that administration of DA antagonists in rats significantly reduced their motivation to exert effort for rewards. This evidence stresses the role of DA in motivation beyond motor activity, suggesting that DA is essential for the motivation to pursue rewards rather than just consumption. Building on these ideas, Berridge and Robinson (1998) further clarified DA's role in reward processing. They postulate that DA does not mediate pleasure or "liking" of reward but instead signals the incentive salience or the "wanting" of reward. This distinction highlights that DA drives the motivational aspect of seeking rewards, while other systems, such as OXT and endocannabinoids, are involved in the hedonic response once the reward is consumed. Furthermore, Berridge's research suggests that DA is involved in learning and updating the value of rewards over time. This updating process is influenced by other neurochemicals, which help solidify the value of a reward based on experience (Berridge & Kringelbach, 2015).

Schultz and colleagues (1997) made a major contribution to the understanding of the mesolimbic dopaminergic system by showing how DA neurons, particularly those in the VTA, respond to reward prediction errors. Their findings revealed that DA neurons increase their firing when an outcome is better than expected and decrease firing when an outcome is worse than expected. When the reward is exactly as predicted, DA neurons remain stable. This process, known as reward prediction error signaling, is critical for learning and updating expectations about rewards. Thus, DA plays a key role not only in motivating effort toward rewards but also in updating the value of rewards when new information arises. It acts as a driver of motivation and a signal for learning about reward contingencies (Roitman et al., 2004; Salamone & Correa, 2002; Syed et al., 2015).

This thesis addresses key research questions concerning reward value and motivation. Specifically, study I focused on impairing the NAcSh to demonstrate its pivotal role

in integrating social information into the valuation of food rewards. This work highlights the role of the mesolimbic dopaminergic system, and concretely the NAcSh, to mediate complex motivational and reward-driven behaviors, advancing our understanding of its function.

1.3. Oxytocin and social information

The social component of rat behavior forms the spinal cord of this thesis, as highlighted at the beginning of the introduction. OXT is a neuropeptide essential for various social-specific functions, including pair bonding, maternal care, and social recognition (Keebaugh et al., 2015; Oettl & Kelsch, 2018; Young et al., 1998). Originally, OXT was identified for its roles in reproduction and maternal behaviors (Higuchi et al., 1986; Wakerley et al., 1990). In male bulls, OXT is released into the bloodstream after ejaculation, indicating a role in sexual function (Sharma & Hays, 1973). The first central infusion of OXT into the brain demonstrated its capacity to stimulate maternal behavior in female rats, highlighting its central neuromodulatory effects (Pedersen et al., 1982; Pederson & Prange, 1979). From these investigations on, more socially complex functions have been identified.

Currently, it is known that OXT is synthesized in the paraventricular and the supraoptic nuclei of the hypothalamus projecting to several other brain areas and the posterior pituitary gland, where it is subsequently released peripherally (Anacker & Beery, 2013; Gimpl & Fahrenholz, 2001). The oxytocinergic projections from the hypothalamic paraventricular nucleus extend to areas critical for social and emotional processing, such as the NAc, amygdala, and PFC. Nevertheless, OXT's functionality depends primarily on the distribution of its receptors, which varies among rodent species (Anacker & Beery, 2013; Insel & Young, 2001). For example, prairie voles, known for their monogamous behavior, have higher OXT receptor densities in the prelimbic cortex and NAc compared to non-monogamous montane voles. These receptor distributions are thought to underpin differences in social organization, as OXT in the NAc facilitates pair-bonding behaviors (Keebaugh et al., 2015; Liu & Wang, 2003; H. E. Ross et al., 2009; Young et al., 1998). Furthermore, OXT in this region interacts with the dopaminergic system, with the DA D2 receptors promoting

partner formation and DA D1 receptors maintaining established bonds (Aragona et al., 2006; Liu & Wang, 2003; Romero-Fernandez et al., 2013). Strikingly, Bosch (2016) has shown that the OXT system in prairie voles can be affected by social changes such as partner loss. In addition, the distribution of OXT receptors can change rapidly in montane voles too, increasing their density following parturition to allow temporary affiliative behavior (Insel & Shapiro, 1992). The role of OXT in encoding social reward is not exclusive to voles, demonstrating a conserved function across mammalian brains. For instance, research in mice has demonstrated that OXT and serotonin in the NAc are necessary to encode social reward (Dölen et al., 2013).

OXT in rodents is primarily released during social interactions (Lukas et al., 2013; Salvi et al., 2018). In olfactory regions, the suppression of OXT receptors selectively disrupts social recognition while preserving the recognition of non-social odors in mice (Oettl et al., 2016). Likewise, experimental manipulations of OXT have been shown to modulate social recognition without affecting non-social memory (Choleris et al., 2009; Ferguson et al., 2000, 2001; Oettl et al., 2016; Popik et al., 1992). Socially specific OXT functions extend to learning processes, as OXT appears to modify social information at each stage, from perception to memory, in order to facilitate adaptive behavior (Salvi et al., 2018). Therefore, familiarity with a conspecific – the recognition of the other – is significantly influenced by OXT.

The functions of OXT are not limited to (positive) social interactions; they also encompass stress regulation. As previously stated, partner loss in prairie voles resulted in alterations to the OXT system, increasing depressive-like behavior. However, OXT infusions into the striatum showed preventive effects (Bosch et al., 2016). The phenomenon of social buffering, which refers to the reduction of stress responses in the presence of conspecifics, often relies on oxytocinergic mechanisms. For instance, an OXT antagonist infused into the anterior cingulate cortex, an empathy-related region, prevented comforting a familiar in distress in prairie voles (Burkett et al., 2016). There is evidence to suggest that OXT has an anxiolytic and antidepressant effect, typically driven by the presence or actions of a conspecific (Li et al., 2019; Martinetz et al., 2019). Accordingly, anxiety and depressive behaviors were reduced after OXT administration (Han et al., 2018; Slattery & Neumann, 2010). Nevertheless,

OXT was also released as an early response to stressors in the absence of a familiar conspecific; whether the function of this mechanism is to seek social support remains an unresolved question (Salvi et al., 2018).

This thesis employs two tasks related to feeding behavior. Therefore, it is necessary to account for the anorexigenic effects of OXT, which reduces food intake, particularly carbohydrate consumption, in rats (Herisson et al., 2014; Olszewski et al., 2010). However, the paradigms commonly used to demonstrate OXT's impact on food intake, motivation, and seeking behavior test isolated rats (Wald et al., 2020). In nature though, food is mostly consumed in social environments (Inglis et al., 1996). Herisson et al. (2016) showed that a social setting prevents intra-accumbens OXT effects on food intake: OXT-injected rats placed in a cage allowing partial interaction with a conspecific ate as much as those injected with saline. The authors propose that social interaction triggers OXT release in the NAc, subsequently down-regulating OXT receptors expression, and as a result, NAc OXT infusions have null effects due to the low OXT receptors availability. OXT release after interaction is a mechanism observed across species (Crockford et al., 2013; Jurek & Neumann, 2018).

Considering scientific literature, OXT is a compelling candidate for addressing the questions raised in this thesis. Therefore, we tested the influence of OXT on the STFP paradigm. Given OXT's critical role in signaling social recognition and promoting bonding, we included a familiarity factor in the experimental design of study II. We predicted that familiarity with the demonstrator (known or novel) would lead to differential outcomes modulated by OXT, providing insights into the mechanisms underlying socially transmitted food preferences in rats.

2. Socially transmitted food preferences paradigm

Rats rely on social information from conspecifics to guide their behavior just like many other social species (Allen, 2019; Galef, 2012). Thus, food choices are not only based on individual experiences but also incorporate observations of others' feeding behavior. The STFP paradigm, originally introduced by Galef and Wigmore (1983), establishes this naturalistic form of social learning in laboratory settings. In the adaptation of the STFP task carried out in this thesis (Fig. 2), observer rats revealed a

preference for one of two flavored food items. After interacting with a demonstrator rat that consumed the non-preferred food, observers typically increased their consumption of the demonstrated food. This phenomenon displays a socially driven revaluation of food preferences.

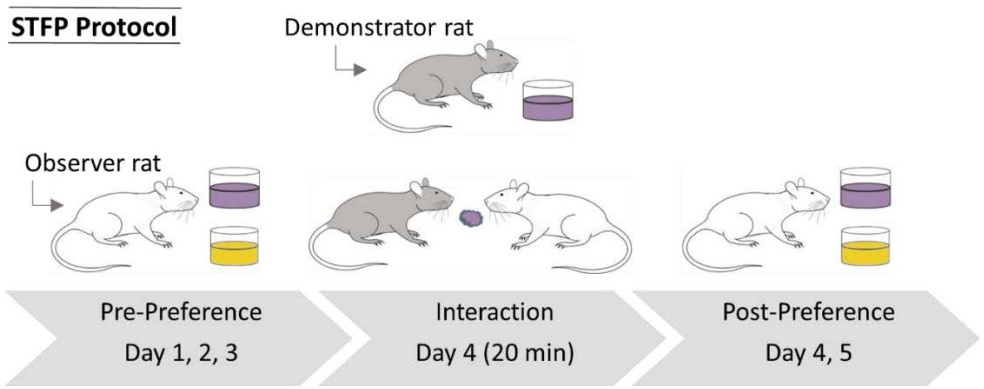

Figure 2. Schematic representation of the socially transmitted food preferences (STFP) protocol used in this thesis. Over three days, the observer rat (white) reveals a preference for one of two food types: grape- or banana-flavored sugar pellets. On the fourth day, the observer interacts with a demonstrator rat (gray) that has been fed the non-preferred food. Immediately after this interaction and on the following day, the observer is given access to both food types to reassess its preferences.

Decades of research on the STFP paradigm have built our understanding of this behavior. Crucially, the acquisition of socially transmitted preferences requires exposure to food odor combined with the presence of carbon disulfide – a chemical in rats' breath – rather than exposure to food odor alone (Galef et al., 1988). Importantly, this behavior is robust across various conditions, such as the observer's motivational state (food-deprived or fed ad libitum) and the demonstrator's health or age. Food preferences are transmitted even from anesthetized demonstrators, which highlights the critical role of olfactory cues, particularly in the breath (Galef et al., 1984; Galef & Wigmore, 1983). Still, the STFP phenomenon is not a simple recency effect; instead, it reflects a complex decision-making process where rats integrate socially acquired information into their existing preferences. Although food preference

transmission is generally robust under controlled settings, as summarized above, ecological contexts such as mutual influence during joint foraging or fluctuations in feeding schedules can modulate the extent of this behavior (Damphousse et al., 2019; Galef & Whiskin, 2004). This adaptability highlights the ecological relevance of social learning in rats.

The adaptation of the STFP employed in this thesis is rooted in a previous design by Galef and Whiskin (2008a) to assess conformity, defined as the tendency to override personal knowledge with that of others. In their studies, the authors concluded that observer rats assign more value to social than individual information. This phenomenon has also been studied in humans. However, such studies have used alternative stimuli including music (Zaki et al., 2011) and facial attractiveness (Campbell-Meiklejohn et al., 2010). Nevertheless, they consistently report that preference changes correlate with activity in the ventral striatum, an evolutionarily conserved region that includes the NAc (Cartmell et al., 2019; Izawa et al., 2003). Indeed, Nook and Zaki (2015) showed that human group norms can shift individuals' food preferences, with the NAc encoding revaluation rather than mere compliance. This aligns with findings in rats reported in study I, suggesting common mechanisms underlying social learning and conformity across species.

Neurobiological studies on the STFP task in rodents have implicated multiple reward-related brain regions previously discussed in this thesis. Specific subregions of the PFC, including the orbitofrontal (R. S. Ross et al., 2005; but see C. A. Smith et al., 2010) and prelimbic cortices (Boix-Trelis et al., 2007; Gold et al., 2011; Portero-Tresserra et al., 2013), play a crucial role in STFP. Additionally, the BLA is necessary to acquire socially transmitted food preferences (Carballo-Márquez et al., 2009; Y. Wang et al., 2006). However, despite extensive investigation, the role of the hippocampus remains a topic of debate (Alvarez et al., 2001; Clark et al., 2002; Thapa et al., 2014). Given the nature of the task, olfactory structures such as the anterior olfactory nucleus and the olfactory bulb are essential for acquiring food preferences from demonstrators' breath (C. Y. Wang et al., 2020). Moreover, recent evidence suggests that the piriform cortex-to-mPFC-to-NAc network plays a role in both the

acquisition and expression of STFP in mice (Loureiro et al., 2019). This aligns with our hypothesis regarding the involvement of the NAcSh in this process.

This body of work demonstrates the value of the STFP task for investigating the mechanisms of social learning and preference modification. In particular, the adapted version of the STFP is an optimal tool for studying how individual and social information are combined, rather than relying on one or the other, in guiding decision-making, choices, and behavioral outcomes (Reader, 2016).

3. Rats' communication

Rats communicate motivational states, emotional conditions, and physiological needs through the production of USVs (Brudzynski, 2013; Opiol et al., 2015). They use USVs as affective signals for social communication (Wöhr et al., 2015). Furthermore, USVs are heterogeneous, with specific subtypes being context-specific. For instance, the trill subtype is predominantly emitted during positive social interactions, whereas flat USVs are more commonly produced to initiate social contact or during feeding behavior (Mulvihill & Brudzynski, 2018; Wright et al., 2010).

In early mammals, vocal signals are hypothesized to have played a critical role in mother-infant interactions, with offspring vocalizing to express needs. It has been suggested that, over time, this communication evolved and extended to other social contexts, including play and mating behaviors (Brudzynski, 2015). The transition from audible sounds to ultrasonic frequencies may have served as an adaptive strategy, potentially reducing the risk of detection by predators while enhancing communication among conspecifics. This evolutionary adaptation, along with others, is thought to have contributed to increased sensitivity to high-frequency sounds in rats, which signal arousal in both threatening and affiliative contexts. As communication facilitates cooperation and survival, USVs likely evolved in response to the selective pressures associated with social living (Kolacz et al., 2018).

USVs are sounds beyond the range of human hearing, typically between 20 and 100 kilohertz (kHz). Adult rat USVs are classified into two categories based on their peak frequency and duration: 22-kHz and 50-kHz calls. 22-kHz calls are long (300-

3,400ms) and their peak frequency range is 20-30kHz, as illustrated in figure 3 (Brudzynski, 2013). These calls are predominantly emitted in aversive situations, reflecting negative emotional states such as fear, anxiety, or discomfort. For example, 22-kHz calls are triggered by negative affective states related to pain, drug withdrawal, social defeat, or post-ejaculatory states in which they signal the termination of the sexual interaction (Barfield & Thomas, 1986; Berger et al., 2013; Brudzynski, 2013; Kroes et al., 2007; Oliveira & Barros, 2006). These calls are also associated with defensive behaviors, including hiding, highlighting their role as aversive signals (Inagaki & Ushida, 2021). 50-kHz calls, in contrast, are shorter (10-150ms) and range between a frequency of 35-80kHz. Often referred to as "rat laughter", these calls are associated with positive emotional states (Panksepp & Burgdorf, 2000). In adults, 50-kHz calls facilitate the establishment of social hierarchies and sexual interactions. In juveniles, 50-kHz calls play a pivotal role in rough-and-tumble play. This form of play, characterized by simulating fighting, helps juveniles develop social and communicative skills (Burgdorf et al., 2008; Pellis et al., 2018). As mentioned, further categorization of 50-kHz calls into subtypes, such as trills and flat calls, proves their context-specific functions, with certain subtypes linked to social play and others to non-social exploratory behaviors (Wright et al., 2010).

Although specific USV subtypes have been linked to certain behaviors, to the best of our knowledge, no study has examined the production of USV subtypes in a task where rats have free access to both social and sucrose rewards. In the task we developed in study III, rats can freely allocate their time interacting with an unknown juvenile rat or consuming sucrose water at 2%, 5%, or 10% concentrations.

These vocalizations are underpinned by distinct neural circuits. Positive emotional arousal involves the mesolimbic dopaminergic system, particularly the VTA and NAc. DA release in the NAcSh is essential for 50-kHz call production and is linked to increased approach behavior and motivation (Brudzynski, 2013; Brudzynski et al., 2018; Burgdorf et al., 2001). This system, extensively studied for its role in motivation and reward processing, is particularly relevant to our research, as the NAcSh was the primary target of study I. Conversely, negative emotional arousal engages the

mesolimbic cholinergic system, originating in the laterodorsal tegmental nucleus and projecting to regions such as the anterior hypothalamic-preoptic area and lateral septum. This system drives the production of 22-kHz calls during aversive states, including fear and anxiety (Brudzynski, 2013; Brudzynski et al., 2018). Additional brain regions, such as the amygdala and periaqueductal gray, further modulate USV production, linking them to context-specific emotional responses (Kroes et al., 2007; Parsana et al., 2012; Sadananda et al., 2008).

Taken together, USVs are a quantifiable output with which we can further study complex social interactions. The evidence suggests that USVs are an intentional means of communication between rats to signal, for example, their willingness to initiate an interaction (Knutson et al., 2002). Thus, they can provide insight into social interactions that are complementary to the repertoire of unintentional social behaviors, usually analyzed on the basis of movement.

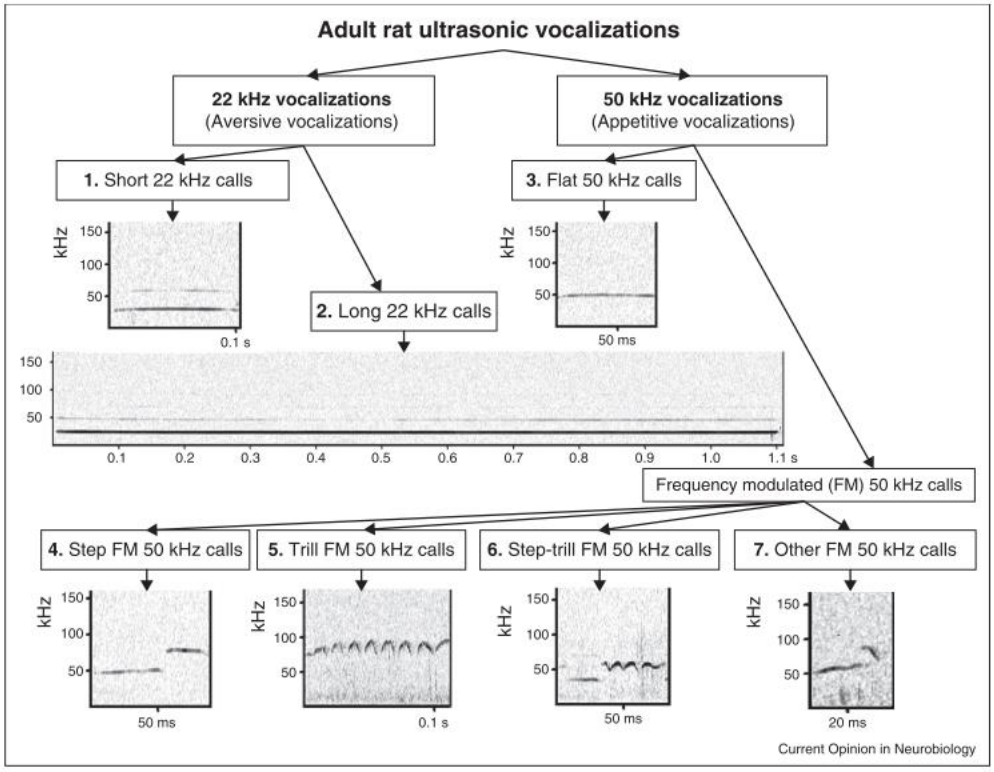

Figure 3. Classification of adult rat ultrasonic vocalizations, including 22-kHz (aversive) and 50-kHz (appetitive) calls. Representative spectrograms illustrate different call types, such as

short and long 22-kHz calls, flat 50-kHz calls, and frequency-modulated (FM) 50-kHz calls. Reprinted from Brudzynski (2013).

3.1. Social-sucrose preference test

The social-sucrose preference task was used in study III to evaluate differences in USV production by rats. The task involved rats freely dividing their time between two actions: consuming sucrose and interacting with an unfamiliar juvenile rat. The positions of each sucrose concentration and the juvenile were fixed in the arms of the X-shaped maze. In the first phase of the experiment, two of the three sucrose concentrations were presented to the subjects in each trial, allowing researchers to confirm through consumption behavior that the rats distinguished between sucrose concentrations. During the second phase, subjects were again allowed to freely explore two arms in each trial, with one arm providing access to a juvenile rat confined in a restrainer and the other offering sucrose consumption.

USVs produced during the second phase were analyzed and correlated with the rats' choices. This task enabled the assignment of relative value to social stimuli as a function of sucrose concentration. Consequently, the study offers an in-depth view of USVs' context-dependent production and their role in reward communication.

4. Overview of principal results

The goal of this thesis is to contribute to the extensive neuroscientific literature and advance our understanding of the mechanisms underlying socialization, the influence of others on individual preferences, and their influences on communication in rats. Substantial evidence supports the translational value of neuroscientific findings from non-human mammals to humans, providing a foundational basis for further exploration (Kalenscher & van Wingerden, 2011; Necka et al., 2015). In study I, we demonstrated the critical role of the NAcSh in modifying prior individual preferences through the integration of social information. Study II investigated the multifaceted effect of OXT in this process. Contrary to our initial expectation, rats adjusted their preferences more significantly when the demonstrator was unfamiliar. Furthermore,

the effects of OXT depended on the dose and the familiarity with the demonstrator. Study III introduced a novel paradigm to assess preferences and vocalization patterns. We first confirmed that rats vocalize more frequently during interactions with juvenile conspecifics than when consuming sucrose water. Moreover, the total number of vocalizations and their subtypes did not fully correlate with behavioral preferences, indicating that USVs reveal additional, otherwise inaccessible information. The general discussion section of this thesis highlights these findings and explores their implications for current neural, psychological, and behavioral theoretical frameworks.

STUDY I

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RESEARCH REPORT

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Lesions of nucleus accumbens shell abolish socially transmitted food preferences

Irina Noguer-Calabús | **Sandra Schäble** | **Tobias Kalenscher**

Comparative Psychology, Institute of Experimental Psychology, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany

Correspondence
Irina Noguer-Calabús, Comparative Psychology, Institute of Experimental Psychology, Heinrich-Heine University Düsseldorf, Universitätsstraße 1, 40225 Düsseldorf, Germany.
Email: noguerca@hhu.de

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Abstract

Rats adapt their food choices to conform to their conspecifics' dietary preferences. The nucleus accumbens shell is a relevant brain region to process reward-related and motivated behaviours and social information. Here, we hypothesize that the integrity of the nucleus accumbens shell is necessary to show socially transmitted food preferences. We made excitotoxic and sham lesions of nucleus accumbens shell in male Long–Evans rats who performed a social transmission of food preference task. In this task, observer rats revealed their original preference for one out of two food options. Afterward, they were exposed to a demonstrator rat who was fed with the observer's originally non-preferred food, and the observer's food choices were sampled again. Sham lesioned observer rats changed their food preferences following interaction with the demonstrator, specifically by increasing the intake of their originally non-preferred food type. This interaction-related change in preference was not found after nucleus accumbens shell lesions. The lesion effects on choice were not the consequence of impaired social or non-social motivation, anxiety or sensory or motor function, suggesting that they reflected a genuine deficit in social reward revaluation. These results highlight the role of nucleus accumbens shell in revaluating food rewards to match a conspecific's preferences.

KEYWORDS
decision-making, motivation, reward revaluation, social behaviour

1 | INTRODUCTION

Rats, as many social species, acquire information from peers in order to make decisions; in consequence, their food choices are based not only on individual experiences but also on conspecifics' feeding behaviour. The socially transmitted food preference (STFP) paradigm is a task that adapts this naturalistic form of social learning to the laboratory settings. In one variant of this paradigm, observer rats choose between two appetitive food items and reveal a preference for one of them. Subsequently, observers interact with conspecifics, called demonstrators, previously fed with the non-preferred food. Upon

Abbreviations: ANOVA, analysis of variance; ITI, intertrial interval; NAc, nucleus accumbens; NAcC, nucleus accumbens core; NAcSh, nucleus accumbens shell; ODI, odour discrimination index; OF, open field; PBS, phosphate-buffered saline; PI, preference index; SEM, standard error of the mean; STFP, socially transmitted food preference.

social interaction, observers have been found to increase their consumption of the food consumed by the demonstrator, thus, overwriting their endogenous food preference (Galef & Whiskin, 2008). Hence, the STFP allows exploring the behavioural and brain mechanisms of a form of socially driven change in preference.

In humans, the alignment to social opinions and preferences has been named *Conformity*, and it has been measured with a variety of stimuli such as music (Zaki et al., 2011) or facial attractiveness (Campbell-Meiklejohn et al., 2010). Several studies have identified the role of the ventral striatum in encoding agreement with the group and stimuli endorsed by others (Wu et al., 2016). In this regard, Nook and Zaki (2015) tested whether group norms shift the food preference of the subjects. They measured the subjects' food ratings before and after the group's rating feedback. The strength of the nucleus accumbens (NAc) activation during consensus between the subject and the group predicted their conformity on food preference. Moreover, group norms changed subjects' internal evaluation of the food; hence, conformity was not a result of public compliance but of a revaluation of the reward.

Revaluation of reward can be observed in non-human animals, too. For example, rats increase their food-seeking behaviour after the upvaluation, driven by a hunger state, of a previously known reward (Wassum et al., 2011). Non-social reward revaluation has been shown to depend on the integrity of NAc (Aitta-Aho et al., 2017; Katsuura & Taha, 2014), in particular NAc shell (NAcSh) subregion (Sweis et al., 2018). In addition, there is some evidence hinting at a potential involvement of NAc in social decision-making and social information processing in rodents in general (De Leonibus et al., 2006; Dölen et al., 2013; Okuyama et al., 2016; Smith et al., 2021). Finally, a recent study identified NAc as the only brain region that showed activity that was selectively correlated with socially motivated helping behaviour (Ben-Ami Bartal et al., 2021). Considering the role of NAcSh in reward revaluation and social behaviour, we asked if the NAcSh is relevant for the adaptive behaviour observed in the STFP paradigm.

To address this question, we trained rats in the STFP paradigm. We compared the strength of the post-versus-pre social interaction preference change between a group of rats with lesions of their NAcSh and sham lesioned rats. We furthermore asked if the socially transmitted change in preference is driven by the devaluation of the preferred food, the upvaluation of the non-preferred food, or both, and if this process is impaired by the NAcSh lesion. Finally, to probe the long-term stability of the post-interaction preference, we evaluated food choices a week after STFP performance. An odour discrimination test and an anxiety test were carried out to control possible confounding effects caused by the surgery.

2 | MATERIALS AND METHODS

2.1 | Subjects

Sixty male Long–Evans rats (*Janvier, France*), 48 observers and 12 demonstrators, were used for this experiment. All animals were about 10–11 weeks old and weighed between 255 and 320 g at the date of surgery. Observer rats were housed in pairs and demonstrators in groups of three until they were all housed individually. The housing room was kept at a constant temperature of 22°C ± 2°C and a humidity of approximately 55% ± 2%, and animals were under an inverted 12:12 light–dark cycle. Before being moved to single housing, rats received standard laboratory rodent food (*Sniff, Germany*) and water ad libitum. During the STFP testing period, rats were food restricted to 85% of their free-feeding body weight, and food rations were given daily after finishing the experimental procedure. All rats were handled for 5 min/day for 2 days prior to the surgery and again after recovery. Six rats did not survive surgery and one was euthanized because it fulfilled standardized criteria for a humane endpoint (OECD, 2000). Three rats were not included in the analysis after applying histology exclusion criteria (see below), and further four rats were excluded because of no food consumption during testing. Consequently, 34 observer rats were included in the final analysis, 16 in the NAcSh lesioned group and 18 in the sham group. All animal procedures were conducted in accordance with the German Welfare Act and were approved by the local authority LANUV (Landesamt für Natur-, Umwelt- und Verbaucherschutz North Rhine-Westphalia, Germany).

2.2 | Surgical procedures

Rats were pseudorandomly assigned to receive either a lesion of the NAcSh or a sham surgery. Prior to surgery, rats received analgesia (5 mg/kg carprofen s.c.). Inhalation was induced with 5% isoflurane until rats lost mobility, and then, isoflurane levels were lowered to 2% to 3% for maintaining anaesthesia. Upon reaching surgical state, rats were fixed in a stereotactic frame using blunt ear bars (David Kopf Instruments, USA). The skull was exposed, two holes were drilled, and bilateral infusions were made using a .3 mm injection needle connected to a microinfusion pump via a polyethylene tubing at the following coordinates relative to bregma: AP .14 cm;

ML ± .08 cm; and DV −.79, −.69 and −.64 cm. Infusions were made using .5 μl of .05 M quinolinic acid (*Sigma Aldrich*) dissolved in .1 M phosphate-buffered saline (PBS) with a pH value of 7.4 for the lesioned animals or PBS for sham animals. The infusion rate was set at .5 μl/min, and the needle remained in place for 1 min allowing liquid diffusion at the injection site. Rats were left to recover for at least 1 week, receiving analgesia (5 mg/kg carprofen s.c.) during the first 2 days after surgery.

2.3 | Open field test

An open field (OF) test was conducted to assess potential differences in locomotion and anxiety between the groups. Rats were placed in the centre of a square arena (50 × 50 cm) and could freely explore it for 10 min while being recorded by a camera from above. Behavioural parameters were assessed by offline analysis using tracking software (Ethovision, Noldus Information Technology, The Netherlands). The time spent in the centre, the entrance frequency and the latency of the first entrance were analysed as measures of anxiety. Time spent in the centre of the OF is understood as a sign of low anxiety, while staying close to the walls displays higher anxiety levels (Prut & Belzung, 2003). In addition, the parameter distance moved was measured to assess potential motor abnormalities induced by the lesion.

2.4 | Odour discrimination task

To assess potential lesion-related differences in odour recognition, an odour discrimination task was conducted the day after the anxiety test in the same OF. The task consisted of a 5-min sample trial, 15-min intertrial interval (ITI) and a 5-min test trial. In the sample trial, two bowls were placed at two corners of the maze. They contained the same odour, either grape or banana-flavoured pellets (*test diet*) diluted with water (1:3 water). The bowls were covered with a lamellar grid preventing the animals from drinking the diluted flavoured pellets; hence, any discrimination between bowls was based on olfactory information only. The location and type of odour were pseudorandomized across subjects. In the test trial, one of the two bowls contained the familiar odour from the sample trial, and the other bowl contained a novel odour. We measured the time rats spent exploring the bowl with the novel odour relative to the one with the familiar odour. The time spent smelling each bowl was manually scored from recorded videos using Solomon Coder (Solomon Coder beta 19.08.02 © András Péter).

2.5 | STFP task

The detailed experimental timeline is shown in Figure 1. Three days before the beginning of the STFP task, observers and demonstrators were housed individually and placed on food restriction. For habituation purposes, 10 grape and 10 banana-flavoured pellets were given to all rats in hanging feeders. The STFP task consists of three phases: pre-interaction testing, interaction and post-interaction testing.

2.5.1 | Pre-interaction testing

On the first day of the testing, observer rats received two weighed cups in their home cage, one containing each food type (grape and banana). The cups were located in hanging feeders and observers were allowed free access for 6 h, after which the cups were removed and weighed. The same procedure was repeated the following 2 days. On completion of pre-interaction testing, observers' individual preferences were calculated by measuring how much of each reward they consumed, quantified as the difference in cup weight before versus after the 6 h consumption period.

2.5.2 | Interaction

On the fourth day of the STFP task, observers and demonstrators were transported to a room adjacent to the interaction room. Demonstrators were fed prior to interaction with the food type that was not preferred by their assigned observers. In order to intensify the corresponding odour, the demonstrator had his back, snout and anal

FIGURE 1 Experimental timeline

area covered with crushed pellets. The interaction took place in the OF and lasted for 20 min. The following mutually exclusive behaviours were analysed for each animal by two independent evaluators using Solomon Coder: partner exploration, genital exploration, social play, mounting, allogrooming, fighting and following.

2.5.3 | Post-interaction testing

Observer rats were placed back in their single cages immediately after the interaction and received two cups, one with each food type. As in the pre-interaction testing, cups were removed and weighed 6 h later. The preference examination was repeated the following day. Afterward, all animals were placed back in group housing.

2.6 | Long-term stability of STFP

To determine the long-term stability of the socially transmitted preference, we conducted another preference test 7 days after completing the STFP task. Again, both food types were given to the observer rats and the amount eaten of each food type was measured 6 h later.

2.7 | Histology

To verify the accuracy of the lesion, rats were deeply anaesthetized with sodium pentobarbital and perfused transcardially with 4% paraformaldehyde in .1 M phosphate buffer. Brains were immediately removed and stored in the fixation solution at a temperature of 5°C. Coronal sections of the NAcSh were cut at a thickness of 50 μm using a vibratome (Leica, Germany) and stained with cresyl violet. Pictures of the NAcSh (Figure 2b) were taken with the microscope Leica DM750 at two magnifications (4× and 10×) and the camera Leica ICC50 HD. Percentage of lesioned areas was calculated using ImageJ (1.53k) software by manually outlining the area with neurotoxic damage. Exclusion criteria were unilateral or misplaced lesions. One rat in the NAcSh lesioned group was excluded due to the wrong location of the lesion, another one due to unilateral lesion and a third one due to a unilateral extension of the lesion to bordering areas.

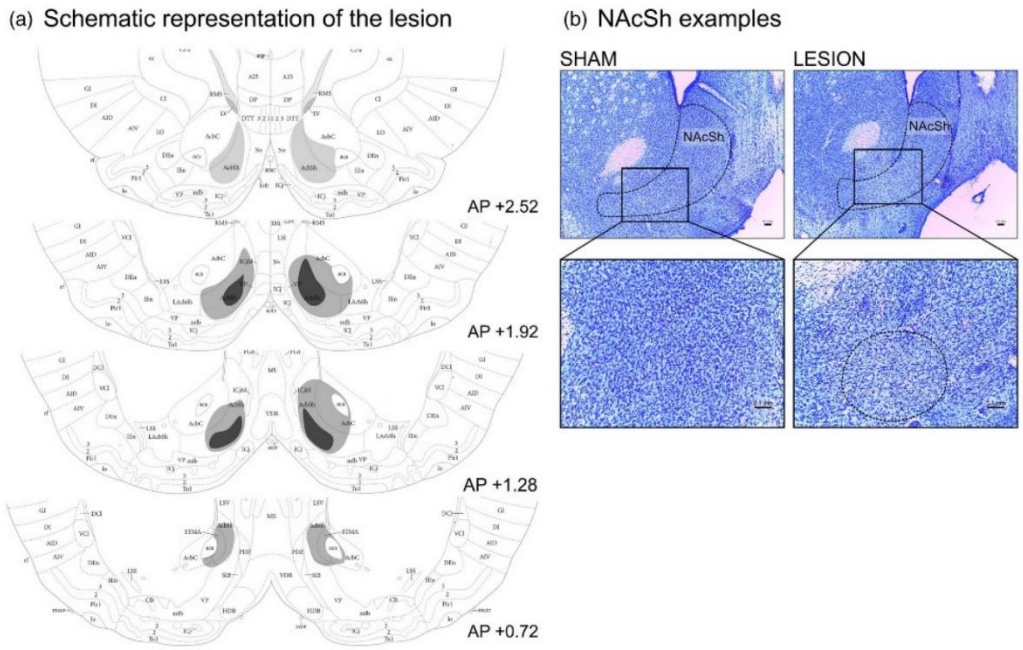

FIGURE 2 Schematic and photomicrograph representation of NacSh lesions. (a) Schematic representation of NAcSh lesion placements from anterior to posterior coordinates. The most overlapping lesions are located in the medial region of the NAcSh and some spread to the core. Light colour: maximum lesion overlap; dark colour: minimum lesion overlap. (b) An example brain slice of sham and NAcSh lesioned group. Pictures are adapted from the atlas of Paxinos and Watson (2006).

2.8 | Data analysis

We expressed the preference magnitude for one food type over another with a preference index (PI). The PI was based on the grams of each food type consumed, and it was computed separately for each day period using the following equation:

$$PI = \frac{(preferred\,(g) - nonpreferred\,(g))}{(preferred\,(g) + nonpreferred\,(g))}.$$

The data were analysed using mixed analyses of variance (ANOVAs; SPSS 27, IBM, USA; R 3.6.3; R Core Team, 2020) with two groups (sham vs. NAcSh lesion) as between-subjects factor and contact (pre-interaction vs. post-interaction) and days (Days 2–5) as within-subjects factor. Original preference (preferred vs. non-preferred) was added as a within-subjects factor to evaluate the effect of each food type on STFP performance. To evaluate the effects of the extensions of the lesions, the difference in the PI before and after the social interaction was correlated with the percentage of lesion extension (averaged between hemispheres). Statistical significance was assumed when $p < .05$. Post hoc analyses were performed with t tests. Correlations were calculated with Pearson's or Kendall's τ_b correlation coefficient as applicable. Benjamini–Hochberg correction was applied to correct for multiple comparisons.

The PI was also calculated after the long-term recall assessment, which was compared with the measurements from Day 3 (before the interaction) and Day 4 (after the interaction) to test stability. Moreover, we also identified the proportion of full preference reversals after the interaction; a full preference reversal was defined as the higher consumption of the originally non-preferred food than the originally preferred food after the interaction. The frequency of reversed preferences was compared between groups on the fourth and the fifth day of the STFP with a Fisher's exact test.

Finally, to assess the social motivation of the rats, we grouped the different interaction subtypes into two variables: the observer interaction time, which includes the subtypes where the observer had an active role (partner and genital exploration, allogrooming and following), and the mutual interaction time where the total amount of time spent interacting was aggregated (partner exploration, genital exploration, social play, mounting, allogrooming, fighting and following). Observer interaction was assessed besides mutual interaction because previous literature has shown that the role of the demonstrator during the interaction has minimum effects on the STFP task (Galef et al., 1983, 1988). To check for differences in social motivation between groups, we performed t test, or Wilcoxon tests, depending on the normality of the distribution of the respective data. Because of technical problems, social interaction data from two NAcSh lesioned rats and one sham lesioned rat were lost and, therefore, not included in the analysis.

For the odour discrimination task, an odour discrimination index (ODI) was computed from the test trial by dividing the total time spent exploring the new odour by the total time spent exploring both odours (new + familiar). We used two-sample t test to determine whether the ODI differed between sham and NAcSh lesioned groups. To analyse anxiety, a multivariate ANOVA was performed with groups as the independent variable and centre-cumulative duration, centre frequency and centre latency as dependent variables.

3 | RESULTS

3.1 | Histology

Histological assessment of lesions was performed by I. N-C. and confirmed by one additional individual (S.S.). The rats in the NAcSh lesioned group had bilaterally a greater number of apoptotic cells or tissue damage in NAcSh than the sham lesioned group. Excitotoxic damage extended rostrocaudally from +2.56 to +.72 mm AP, with average maximal extension between +1.92 and +1.28 mm anterior to bregma and between .6 and 2 mm mediolaterally as defined by Paxinos and Watson (2006). The average percentage of NAcSh lesioned was 20.64% ± 2.29%. However, there was a significant difference in the percentage of NAcSh damaged between hemispheres (Figure S1, Wilcoxon test; $z = 2.43$, $p = .015$), being larger in the left hemisphere (28.03% ± 4.68%) than in the right hemisphere (13.25% ± 2.58%). Lesions occasionally extended unilaterally into the core of the nucleus accumbens (NAcC; $n = 6$; average percentage extension of 26.7% ± 13.83%) but not into other neighbouring regions. Nevertheless, the region that was commonly lesioned in all lesioned rats was NAcSh. Some animals ($n = 8$) in the sham group had small lesions, albeit not confined to NAcC or NAcSh, and the lesions were substantially less pronounced than the ones observed in the NAcSh lesioned group and qualitatively different, that is, less evidence for apoptotic cells. The sham group lesions were probably caused by the needle insertion during surgery. Three animals were excluded from the NAcSh lesioned group following exclusion criteria. A schematic representation of the lesions together with one example image of each observer's group is shown in Figure 2.

3.2 | NAcSh lesions reduce the socially induced preference change after the interaction

In order to compare preferences, we calculated the PI for each observer rat, which reflects how much more preferred than non-preferred food the rat has eaten. To test the hypothesis that the NAcSh lesions impair STFP performance, we ran a mixed ANOVA on the effects of group (sham vs. NAcSh lesion), contact (pre-interaction vs. post-interaction) and day (Days 2–5) on the PI. We found a significant simple main effect of contact ($F_{[1,\ 32]} = 5.955$, $p = .02$), indicating an influence of interaction on the PI. We also found a significant statistical interaction between group and contact ($F_{[1,\ 32]} = 6.798$, $p = .014$). Importantly, we did not find a significant interaction between contact, day and group ($F_{[1,\ 32]} = 1.698$, $p = .202$). Therefore, we average the PI of the days before the interaction (Days 2 and 3) and the days after the interaction (Days 4 and 5) for the following analysis and the graphical representation of the data. A post hoc analysis revealed a decreased post-interaction PI for the sham group ($t_{[17]} = 2.89$, $p = .02$), but not for the NAcSh lesioned group ($t_{[15]} = .064$, $p = .95$), suggesting that the post-interaction change in preference was less pronounced in the lesion than the sham group (Figure 3). We further analysed whether the magnitude of the change in PI was correlated with the extension of the lesion. However, this correlation did not reach significance (Kendall's test; $\tau_b = .217$; $p = .242$; Figure S2). The total amount of food eaten did not differ between groups along the STFP task ($F_{[1,\ 32]} = .812$, $p = .37$; food consumption data in Table S1).

The lack of PI adjustment shown by the NAcSh lesioned group could either be due to an impaired socially transmitted reward revaluation or due to a generally reduced social motivation. To control for the latter possibility, we compared the time the observers spent interacting with the demonstrators between groups. We found that observer interaction time and mutual interaction time were not different between groups (observer interaction, $t_{[22.72]} = .55$, $p = .59$; mutual interaction, $z = -.516$, $p = .606$) indicating that social motivation was unlikely reduced after NAcSh lesion.

The socially transmitted change in preference found in the STFP task may either be the consequence of the upvaluation of the originally non-preferred food type, the devaluation of the originally preferred food type or both. To address this question, and to determine whether NAcSh lesions interfered with reward upvaluation or devaluation, we ran a mixed three-way ANOVA on food consumption (the amount of food consumed) with the within-subjects factors original preference (originally preferred vs. non-preferred food), contact (pre-interaction vs. post-interaction) and the between-subjects factor group (sham vs. NAcSh lesion). Not surprisingly, we found main effects of contact ($F_{[1,\ 32]} = 9.912$, $p = .004$)

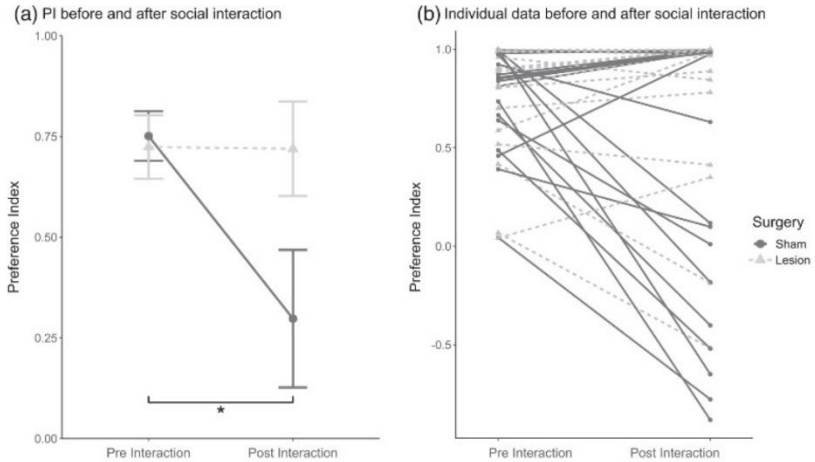

FIGURE 3 Effects of the NAcSh lesion on preference index (PI). Dotted, light grey lines and triangle symbols represent data from the lesioned group, solid, dark grey lines and circle symbols represent data from the sham group. (a) The PI expresses the difference between preferred and non-preferred food eaten relative to the total food eaten before and after the interaction with the demonstrator (mean ± standard error of the mean, SEM). The PI is significantly decreased in sham lesioned, but not in NAcSh lesioned rats, after social interaction. (b) Change in the PI after social interaction for each individual rat. *$p < .05$.

and original preference ($F_{[1,\ 32]} = 58.245$, $p < .001$) on food consumption. Importantly, we also found a significant three-way statistical interaction effect ($F_{[1,\ 32]} = 5.824$, $p = .022$) on food consumption. Breaking down this three-way statistical interaction (Figure 4; individual values in Figure S3), post hoc tests revealed no significant differences in preferred food consumption pre-interaction versus post-interaction in either group of rats (sham, $t_{[17]} = 1.98$, $p = .128$; lesion, $t_{[15]} = -.92$, $p = .495$). By contrast, we did find a significant increase in the amount of non-preferred food consumed pre-interaction versus post-interaction in the sham group ($t_{[17]} = -3.02$, $p = .032$). This increase in amount of non-preferred food consumed was not found in the lesioned animals ($t_{[15]} = -.47$, $p = .644$). This pattern of results suggests that the STFP effect is mainly driven by a socially transmitted upvaluation of the originally non-preferred food type, and to a lesser, statistically insignificant extent, by a devaluation of the originally preferred reward. NAcSh lesions interfered with social upvaluation of reward.

In summary, we found evidence for a socially transmitted change in food preference in the sham group, but not in the NAcSh lesioned group. This effect was mainly driven by the upvaluation of the originally non-preferred food type in a NAcSh-dependent way. These lesion effects on STFP are unlikely due to reduced reinforcer sensitivity, decision-making capacity or general social motivation.

3.3 | NAcSh lesions reduce the frequency of preference reversals

To further characterize the strength of the demonstrators' influence on the observers' food preferences, we compared the proportion of sham versus NAcSh lesioned rats reversing their preferences after interaction (i.e., the preference is considered reversed if a rat consumed more originally non-preferred food than originally preferred food after the interaction); 38.9% (7/18) of the sham lesioned rats reversed their preference completely, while only 6.25% (1/16) of the NAcSh lesioned rats did it on the fifth day (Fisher's exact test; $p = .04$ two sided; Figure 5). Even if not significant, this tendency appeared already on the fourth day, following interaction (Fisher's exact test; $p = .12$ two sided).

3.4 | Post-interaction food preferences are stable across time

To evaluate the stability of the food preference in each group, we measured the observers' food consumption a week after finishing the STFP task. We ran a mixed ANOVA and found a significant simple main effect of time (Day 3 pre-interaction vs. Day 4 post-interaction vs. Day 15 post-interaction) on the PI ($F_{[2,\ 64]} = 5.473$, $p = .006$) and a statistically significant interaction between group and time ($F_{[2,\ 64]} = 3.227$, $p = .046$). A

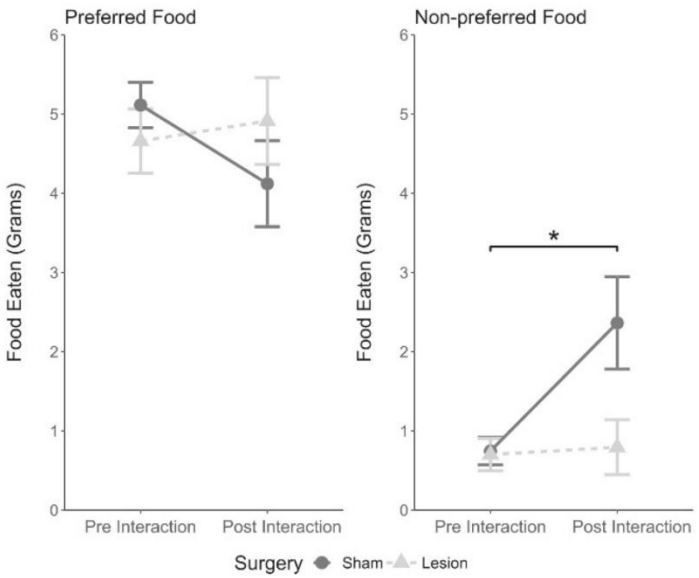

FIGURE 4 Preference change of originally preferred and non-preferred food types. Mean (±SEM) of the originally preferred and non-preferred food consumption in grams before and after the social interaction for each group. The consumption of the preferred food type did not significantly change after interaction in either group. By contrast, sham rats significantly increased their consumption of the non-preferred food type after interaction, which was not observed in NAcSh lesioned rats. *$p < .05$.

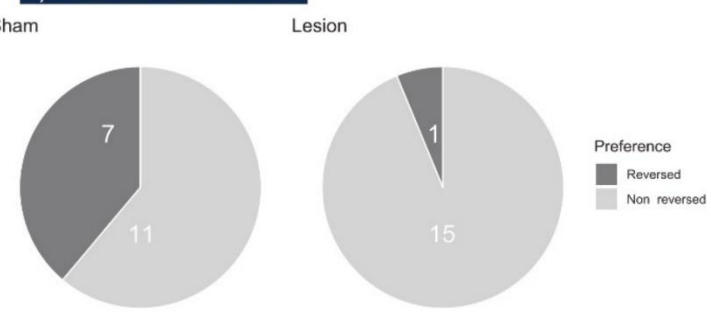

FIGURE 5 Preference reversal. Frequency of full preference reversals on the fifth day (1 day after social interaction). Seven of 18 sham lesioned rats fully reversed their preference, that is, they ate more originally non-preferred than preferred food after the interaction, while only 1 of the 16 NAcSh lesioned rats fully reversed its preference. The frequency of reversed preference was significantly higher in the sham group than in the NAcSh lesioned group.

post hoc analysis revealed that, not surprisingly, the PI of that sham group decreased after the interaction (Day 3 vs. Day 4, $t_{[17]} = 3.23$, $p = .015$). Interestingly, in sham rats, the post-interaction PI on Day 15 was still significantly different from the pre-interaction PI (Day 3 vs. Day 15, $t_{[17]} = 3.2$, $p = .015$), and their preference remained nearly unchanged over time following the interaction (Day 4 vs. Day 15, $t_{[17]} = -.188$, $p = .853$; Figure 6; individual values in Figure S4). Therefore, we conclude that the influence of the demonstrators on the PI of the sham group was long-lasting. By contrast, there were no differences between any of the days in the NAcSh lesioned group (Day 3 vs. Day 4, $t_{[15]} = .273$, $p = .8$; Day 3 vs. Day 15, $t_{[15]} = 1.07$, $p = .6$; Day 4 vs. Day 15, $t_{[15]} = .639$, $p = .8$), revealing temporal stability of food preference and, once again, insensitivity to social influence.

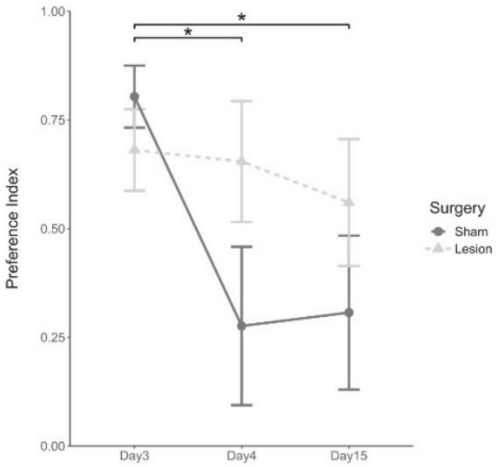

FIGURE 6 Long-term stability of food preferences. The mean of the preference index (±SEM) on Day 3 reflects the original preference pre-interaction, on Day 4 the preference after interaction and on Day 15 the preference 11 days after interaction. *$p < .05$.

3.5 | No lesion effects on odour discrimination

The STFP task requires the rats to be able to distinguish between odours because they have to associate the odour of the food eaten by the demonstrator with its breath. We evaluated whether both sham and NAcSh lesioned rats can discriminate between odours, manifested by exploring the novel odour for a longer time than the familiar one in the odour discrimination task. During the test trial, the ODI for both groups together was higher than chance ($t_{[33]} = 5.722$, $p \leq .001$). In addition, there were no significant differences in the ODI between groups ($t_{[31.8]} = -.682$, $p = .5$; Figure 7e). Thus, both groups were able to recognize odours. Therefore, lesion-related differences in STFP performance were unlikely due to deficits in odour recognition.

3.6 | The NAcSh lesioned group is more anxious than the sham group

Anxiety can modulate sociability and food consumption (Lopes et al., 2012; Shah & Treit, 2003). Therefore, we compared anxiety levels between NAcSh and sham lesioned rats, measured by the time spent in the centre of the test arena, the frequency of entering the arena centre and the latency of the first entrance (see Section 2). The multivariate ANOVA revealed a main effect of group on

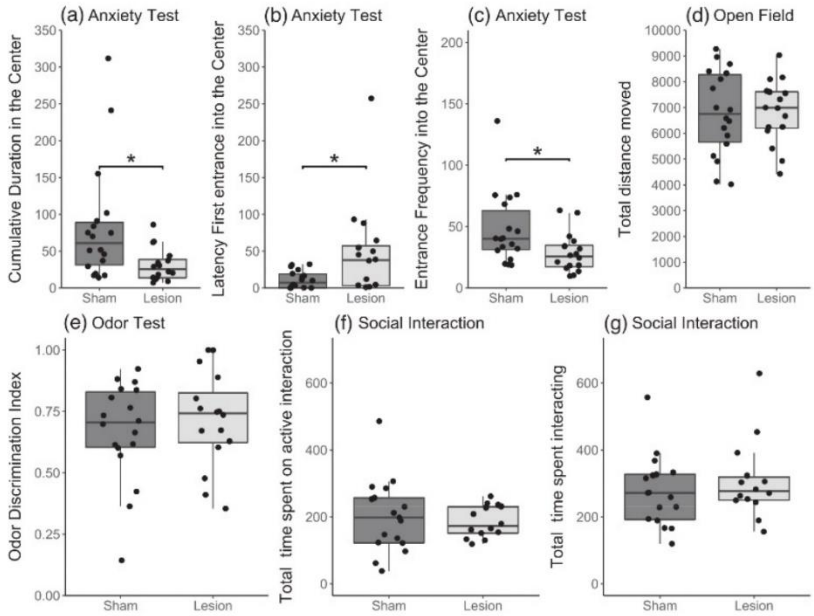

FIGURE 7 Control measures. (a–c) Effect of the NAcSh lesions on anxiety levels. (a) Significant differences in the cumulative duration (s) spent in the centre of the open field (OF) between groups. (b) Significant differences in the latency (s) of the first entrance to the centre of the OF between groups. (c) Significant difference in the frequency of the entrances to the centre of the OF between groups. The three measurements reveal higher anxiety levels for the NAcSh lesioned group than the sham group. (d) Total distance moved. The distance moved (cm) in the OF was not affected by NAcSh lesions. (e) Effect of the NAcSh on the odour discrimination index. Relative time spent investigating the new odour with respect to the total time spent investigating both odours (new and familiar). There were no significant differences between groups in their capacity to differentiate odours. (f,g) Effect of the NAcSh on social interaction during STFP. (f) The total time (s) spent interacting with the demonstrators initiated by the observers was not affected by NAcSh lesions. (g) The total time (s) spent on social interaction independent on the initiator was not affected by NAcSh lesions. $^*p < .05$. Points represent individual values.

anxiety ($F_{[3,\ 30]} = 3.394$, $p = .03$). Post hoc analyses revealed that NAcSh lesioned animals had higher levels of anxiety than sham lesioned rats in each of the three anxiety measurements (centre-cumulative duration, $t_{[32]} = 2.61$, $p = .035$; centre latency first, $t_{[32]} = 2.41$, $p = .035$; and centre frequency, $t_{[32]} = -2.21$, $p = .042$; Figure 7a–c). We also measured locomotion (distance moved) in the OF (Figure 7d) but found no significant differences between groups ($t_{[32]} = -.77$, $p = .939$).

Considering these results, each rat's individual centre-cumulative duration as a measure of anxiety was added as a covariate to the mixed ANOVA we used to analyse the lesion effects on STFP behaviour. Adding anxiety as a covariate did not change the results of the ANOVA reported above on the effects of lesion (sham vs. NAcSh lesion) and contact (pre-interaction vs. post-interaction) on PI (contact and group statistical interaction, $F_{[1,\ 31]} = 5.168$, $p = .03$). The variable anxiety did not significantly explain either the performance differences between groups caused by social interaction in the STFP task (contact and centre-cumulative duration statistical interaction, $F_{[1,\ 31]} = .00014$, $p = .991$). Thus, we conclude that the lesion effects on STFP performance were unlikely due to differences in anxiety levels between sham and NAcSh lesioned rats.

4 | DISCUSSION

Rats modify their food preference in order to conform to a conspecific's preference. In the present study, we hypothesized that the integrity of NAcSh is necessary for adjusting own food preference to match the preferences of a conspecific. To test this hypothesis, we trained NAcSh and sham lesioned rats in a STFP task. Sham lesioned rats changed their food preference after

interacting with a conspecific who was fed with the originally non-preferred food. By contrast, the NAcSh lesioned rats stuck by their original preference, showing no change in food choices after social interaction. We furthermore found that, in sham rats, the STFP effect was driven primarily by an increased demand for the originally non-preferred food item after social interaction and to a lesser extent by a decreased demand for the originally preferred item. NAcSh lesioned rats did neither change their consumption of the originally preferred, nor the non-preferred food post-interaction. In a significant proportion of the sham lesioned rats, the influence of the demonstrator was strong enough to fully reverse their preference, while almost none of the NAcSh lesioned rats showed full preference reversals. We additionally found a long-lasting influence of social interaction on food preference in the sham lesioned group, replicating previous results (Galef & Whiskin, 2003), while, once again, no social long-term effect on food preference was found in the NAcSh lesioned animals. Overall, we provide evidence that NAcSh lesions impair the rats' ability to modify their own food preference to match the preference of a conspecific.

What could be the putative function of NAcSh in STFP? The effects of lesions on STFPs may be the result of a deficit in reward revaluation, that is, in using social information to update reward value representations. Alternatively, it is equally plausible to explain our findings as a general deficit in motivation or decision-making. However, we consider the latter explanation unlikely because the NAcSh lesioned rats showed consistent and stable preferences for one reward over the other, and there was no difference in reward intake and social interaction time between groups. Thus, we have no evidence to assume social or non-social anhedonia in our NAcSh lesioned rats. We, therefore, conclude that the selective deficit in socially transmitted reward revaluation is the more plausible interpretation of our lesion results.

Another explanation of the lesion effects on STFPs is a putative change in anxiety. In line with previous literature (Gebara et al., 2021; Martínez et al., 2002), we report that NAcSh lesioned rats had higher novelty-induced anxiety levels when exposed to an unfamiliar environment than sham lesioned rats. However, we found no differences in social interaction or food consumption between sham and lesioned rats, and adding anxiety as a covariate did not change our results. Hence, the group difference in novelty-induced anxiety did not seem to generalize or transfer to other behavioural domains. Therefore, it is unlikely that differences in anxiety levels between groups explain the lesion effects on STFP performance.

The NAcSh lesion effects on STFP performance could also be due to an impairment in cognitive flexibility instead of the result of an interference with a genuinely social-cognitive process. However, it has been shown that the integrity of the NAcSh is not necessary for reversal learning, a common task used to evaluate cognitive flexibility (Castañé et al., 2010). If anything, the full or partial disruption of the NAcSh seems to facilitate, rather than impair, cognitive flexibility across different paradigms (Gal et al., 2005; Jongen-Rêlo et al., 2002; Milton et al., 2021; Pothuizen et al., 2005; Sala-Bayo et al., 2020; but see Ding et al., 2014). In addition, the experimental designs used to evaluate cognitive flexibility were all based on variations of reinforcement learning paradigms in which animals learn the incentive value of initially neutral stimuli. However, reinforcement learning is probably less relevant for the behavioural change shown in the current study, which presumably does not involve learning or modifying the value of initially neutral stimuli (Galef & Durlach, 1993). In a similar vein, a NAcSh lesion-induced proneness for habit formation might also explain the lesioned rats' tendency to continue choosing the originally preferred reward after social interaction. Habit formation describes the acquisition of action values, independent of the actions' outcomes. However, because the action to obtain one reward or another was nearly identical in our STFP task, lesion-related differences in action values are unlikely to manifest in differences in food choices. In addition, the existing literature points toward the dorsal striatum as the main region relevant for habitual (stimulus–response) behaviour while the ventral striatum, which contains the NAcSh, is more important for goal-directed (action-outcome) behaviours (Belin et al., 2009; Devan et al., 2011; Everitt & Robbins, 2013; O'Tousa & Grahame, 2014). Thus, although we cannot entirely rule out that a lesion-related change in cognitive flexibility or habit formation might account for our findings, we consider these explanations less parsimonious than the social reward revaluation hypothesis presented above.

Thus, in conclusion, we argue that neither social nor non-social anhedonia, novelty-induced anxiety, impairments in cognitive flexibility, non-specific sensory (olfaction) or motor deficits can account for the lesion effects on STFPs. We, therefore, maintain that the most likely explanation is that our findings are the consequence of NAcSh lesion-induced social reward revaluation deficit.

Abundant literature on the STFP paradigm has tested several variations of the original task design to delimit its interpretation. A crucial finding was that the acquisition of the STFP requires exposing the observer rat to the odour together with either a breathing rat or a toy rat moistened with carbon disulphide—a chemical present in rats' breath (Galef et al., 1988). By contrast, the mere exposure to the scent of food alone (Choleris et al., 2011; Galef et al., 1985)

or covering a toy demonstrator does not enhance the preference for such food (Galef & Stein, 1985). Therefore, the change in preference cannot be explained by a recency effect, where the last odour smelled determines the consumption preference. Interestingly, the demonstrator's health state is not relevant (Galef et al., 1983), as observers acquire food preferences even from anaesthetized demonstrators. Although this result seems counterintuitive, it ratifies the breath as the most informative cue among the characteristics of the demonstrator.

The STFP phenomenon is very robust: It is independent of the motivational state of the observer (food deprived or ad libitum), the form of ingesta (liquid or solid), the age of both rats, familiarity and strain when it is assessed unidirectionally (Galef et al., 1984; but see Figueroa et al., 2020). Nevertheless, variability increases when both rats influence each other simultaneously in a more ecological setting. While the major impact on preference is degraded when rats forage in pairs, they make use of the information transmitted by the other in a different manner depending on the context, the partner and individual characteristics (Damphousse et al., 2019). Galef and Whiskin (2004) also showed that the transfer of preferences between conspecifics is stronger in a stable environment than in a variable one. These data indicate that rats, far from acting automatically to the information transmitted by a conspecific, integrate it into a complex decision-making process.

The design of this study comes with some limitations that should be considered. Both groups underwent surgery and were isolated for seven consecutive days in order to conduct the STFP and another 3 days to evaluate the long-term stability of the transferred preference. However, our results are comparable with STFP literature, where those stressors are not present (Galef & Stein, 1985; Galef & Whiskin, 2008). Nevertheless, demonstrators were used for consecutive interactions with different observers (to reduce the number of animals used) understanding that either habituation or sensitization could occur. As the transmission of information seems to be a passive process not dependent on demonstrators' characteristics, as discussed above (Galef et al., 1988), the impact of such order should be limited. Moreover, the order of the observers paired with the demonstrators was randomized between groups.

The NAcSh is a hotspot for many reward-related processes, including hedonic pleasure (Castro & Berridge, 2014) and motivated behaviour (Ito & Hayen, 2011). Berridge and Robinson (1998) proposed that NAc is necessary to attribute incentive salience to reward-associated cues and actions, and their neural representations, boosting their attractiveness and, thus, driving motivation. Several studies have provided evidence supporting their hypothesis (Peciña & Berridge, 2013; Saddoris et al., 2015, 2017; Salamone et al., 1994, 2003; Sclafani et al., 2011; Wyvell & Berridge, 2000). Concretely, NAcSh mediates motivational and affective valence along a rostrocaudal gradient (Reynolds & Berridge, 2002, 2003). Accordingly, NAcSh is a key player in choice revaluation during economic decision-making in mice (Sweis et al., 2018), and in modulating food preferences in a non-social operant conditioning task in rats (Jang et al., 2017; Katsuura & Taha, 2014). NAcSh seems to play a similar role in social tasks, too: Ben-Ami Bartal et al. (2021) have identified the NAcSh as a neural hub for promoting prosocial motivated helping behaviours in rats. We expand on this body of literature by implying NAcSh in socially motivated reward choice behaviour. However, associative processes, valuation and motivation are dissociable mechanisms (Wassum et al., 2009) that our experimental design cannot distinguish. Thus, a conservative interpretation of our results understands the NAcSh as an essential region within the neural circuit encoding the processes involved in the STFP performance and does not assume the localization of the mentioned processes exclusively in the NAcSh. Considering so, we argue that NAcSh integrity is necessary for incorporating social information during the revaluation of food rewards. Consequently, it is plausible that the revaluation deficit seen in the NAcSh lesioned rats is the consequence of their inability to process the incentive salience of the non-preferred food after being associated with a social stimulus, i.e., here, the social interaction. Therefore, we interpret that the change in consumption in the sham group is driven by an upvaluation of the non-preferred food after its salience is increased by the association with the social stimulus.

The neural underpinning of the STFP task is partly known. The orbitofrontal cortex is necessary for STFP acquisition (Ross et al., 2005; but see Smith et al., 2010) as well as the prelimbic cortex (Boix-Trelis et al., 2007; Gold et al., 2011; Portero-Tresserra et al., 2013), the parafascicular nucleus (Quiroz-Padilla et al., 2006) and the basolateral amygdala (Carballo-Márquez et al., 2009; Wang et al., 2006). Indeed, olfactory regions as the anterior olfactory nucleus and the olfactory bulb are crucial for this task acquisition (Wang et al., 2020). Moreover, the perirhinal cortex is required for STFP long-term memory (Feinberg et al., 2011). Still, the most studied memory-related region has been the hippocampus with contradictory results (Alvarez et al., 2001; Burton et al., 2000; Clark et al., 2002; Feinberg et al., 2011; Winocur et al., 2001; but see Thapa et al., 2014). Although the role of the rats' NAcSh in STFP has not been investigated before, our results are in line with a previous study on mice where the activation of the

piriform cortex to the mPFC network targeting the NAc was essential for STFP acquisition and expression (Loureiro et al., 2019). Our demonstration of the role of the NAcSh in this paradigm complements our knowledge of the neural underpinning of the STFP task in rats.

In conclusion, this current study provides evidence that the integrity of rat NAcSh is necessary for STFP. The stability of the original food preference after social interaction observed in the NAcSh lesioned group is not due to a general decrease in social motivation or feeding, nor in locomotion deficits, odour discrimination impairments, nor anxiety. Future research is needed to disentangle the mechanistic processes underlying the STFP and to evaluate which neurotransmitters are involved in the present task and how the NAcSh is connected to the other implicated regions. Our results suggest that NAcSh lesions result in a deficit in socially transmitted reward revaluation and provide novel information about the role of the NAcSh in social behaviour.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ejn.15827.

DATA AVAILABILITY STATEMENT

Raw data supporting the findings presented in the study is openly available in OSF at https://osf.io/p3emb/.

ORCID

Irina Noguer-Calabús https://orcid.org/0000-0003-3507-3907

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SUPPORTING INFORMATION

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STUDY II

Noguer-Calabús, I., Schäble, S., Dören, J., & Kalenscher, T. (2024). Oxytocin effects on socially transmitted food preferences are moderated by familiarity between rats. *Psychopharmacology*, *242*(2), 361–372. https://doi.org/10.1007/s00213-024-06682-x

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ORIGINAL INVESTIGATION

Oxytocin effects on socially transmitted food preferences are moderated by familiarity between rats

Irina Noguer-Calabús[1] · Sandra Schäble[1] · José Dören[1] · Tobias Kalenscher[1]

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Abstract

Rationale In the socially transmitted food preference (STFP) paradigm, rats change their preference for food rewards after socially interacting with a conspecific who has been fed with the originally non-preferred food. Here, we asked if oxytocin (OXT), a neuropeptide known for its role in social affiliation and social behavior, plays a role in STFP. Since OXT's influences on social behavior can be familiarity-dependent, we further asked if OXT effects on STFP are moderated by the familiarity between rats.

Objectives Does OXT modulate rats' socially transmitted food choices in a familiarity-dependent way.

Methods We systemically injected either vehicle, low-dose (0.25 mg/kg) of OXT, or large-dose (1.0 mg/kg) of OXT before social interaction with either a familiar cagemate (in-group) or an unfamiliar conspecific from a different cage (out-group).

Results We found an intergroup bias in STFP: vehicle-treated rats showed larger socially transmitted changes in food preference in the out-group than the in-group condition. OXT modulated STFP in a familiarity-dependent way: OXT prevented the increase in the consumption of the non-preferred food in the out-group, and decreased the consumption of the preferred food in the in-group. These effects were dose-dependent and observed under acute OXT action, but also on the subsequent day when acute OXT effects dissipated, suggesting long-lasting social learning effects of OXT. Additional analyses suggest that the familiarity and dose-dependent effects of OXT on STFP cannot be attributed to OXT's anorexic actions or differences in the duration of the social interactions.

Conclusions OXT modulates STFP in a familiarity-dependent way.

Keywords Familiarity · Food preference · Group bias · Oxytocin · Reward revaluation · Social behavior.

Introduction

What we eat is a daily decision that is influenced by our knowledge of the available resources and our dietary preferences. To make these decisions, we gather relevant information either from our own experience or through social learning. Relying on social information to choose food has proven to be an adaptive foraging strategy in many situations and in several species (Kendal et al. 2005). To operationalize social food learning in animals in a laboratory setup, Galef and Wigmore (1983) established the socially transmitted food preference (STFP) paradigm where one rat (the observer) reveals a preference for a flavored food after interacting with a demonstrator who recently ate it. Years of research using the STFP paradigm have provided solid evidence for socially transmitted food preferences, which occur independent of the observer's energy state (food-deprived or fed ad-libitum) or the demonstrator's characteristics, such as health (poisoned, anesthetized or controls) or age (Galef et al. 1983, 1984; Galef and Wigmore 1983; Galef and Whiskin 2004, 2008a). Aligning food preferences to those of conspecifics is a phenomenon found in many mammals including humans (Nook and Zaki 2015).

Here, we asked what the psychopharmacological mechanism of socially transmitted food preference is. One strong neuromodulator candidate is oxytocin (OXT). OXT is a neuropeptide primarily synthesized in the paraventricular hypothalamic nucleus and the supraoptic nucleus of the hypothalamus that modulates neural activity in many parts

✉ Irina Noguer-Calabús
noguerca@hhu.de

[1] Comparative Psychology, Institute of Experimental Psychology, Heinrich-Heine University Düsseldorf, Universitätsstraße 1, 40225 Düsseldorf, Germany

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of the brain (Ferris et al. 2015; Salvi et al. 2018; Liu et al. 2021). It is prominently involved in social behavior, such as reproduction, social recognition and memory, pair bonding, and prosociality, as well as the regulation of fear, anxiety and food consumption (Jurek and Neumann 2018; Sakamoto et al. 2019; but see Berendzen et al. 2023). OXT can modulate social cognition at different levels. Enhanced OXT release in olfactory circuits increases social exploration and social recognition without interfering with other olfactory-dependent behaviors (Oettl et al. 2016). However, the modulation of social recognition by OXT subcutaneous injections follows an inverted U-shaped dose-response curve. Intermediate doses facilitate social recognition to a greater extent than low or high doses (Popik et al. 1992). In non-human primates, OXT boosts own- and other-regarding preferences (Chang et al. 2012), and in humans, OXT has been shown to promote social cognition and prosocial behavior, too (Jurek and Neumann 2018; Marsh et al. 2021). OXT in mice is also implicated in social learning (Dölen et al. 2013; Choe et al. 2015). For instance, systemic administration of OXT and vasopressin prolonged the memory recall of socially transmitted changes in drink preference (Popik and Van Ree 1993), suggesting OXT is indeed important for at least some cognitive aspects of STFP. However, direct evidence for the effects of OXT on STFP is, so far, elusive (Lindeyer et al. 2013; but see Popik and Van Ree 1993).

In humans, OXT effects on social behavior have been shown to be subject to intergroup-biases: OXT promotes empathy, cooperation, trust and conformity with members of the same social group, but it fosters defensive behaviors and social distancing against members of a competing social group (De Dreu et al. 2010; Scheele et al. 2012; De Dreu and Kret 2016; Strang et al. 2017). Interestingly, in rodents, group affiliation seems to matter for social behavior, too. For instance, rats exhibit intergroup biases in prosociality (Ben-Ami Bartal et al. 2021), and there is evidence, although weak and inconclusive, that STFP also depends on the familiarity, i.e., group affiliation in a wider sense, between the observer and the demonstrator rat (Galef et al. 1984; Galef and Whiskin 2008a; Agee et al. 2019). It is therefore plausible to assume that any putative OXT effect on STFP might depend on the familiarity between demonstrator and observer.

In the current study, we therefore hypothesized that STFP in rats is modulated by OXT action, and that the predicted OXT effects on STFP are dependent on the familiarity between observer and demonstrator rats.

We trained rats in an adapted within-subject variant of the STFP paradigm (Galef and Whiskin 2008b; Jolles et al. 2011; Noguer-Calabús et al. 2022) that allowed us to quantify the individual magnitude in the change of socially transmitted food preference after relative to before social interaction. Briefly, observer rats reveal their original food preferences by choosing between two appetitive, differently flavored food rewards. Subsequently, they interact with a demonstrator rat who has been fed the food that was revealed non-preferred by the observer. After social interaction, we measure the observer rats' food preferences again. Observers typically increase the consumption of the originally non-preferred pellets and/or decrease the consumption of the originally preferred pellets (Galef and Whiskin 2008b; Noguer-Calabús et al. 2022).

We manipulated familiarity, as a proxy of group affiliation, between observers and demonstrators (Ben-Ami Bartal et al. 2014; Agee et al. 2019), as follows: during the social interaction phase of the STFP task, observers were either paired with a familiar cagemate demonstrator (in-group) or with an unfamiliar demonstrator from a different cage (out-group). To evaluate OXT effects on STFP, observers in the in-group and the out-group conditions received one of three treatments: vehicle injections, low-dose OXT, or large-dose OXT, systemically injected prior to social interaction. We measured the observers' revealed food preferences before and immediately after social interaction, hence during acute OXT action, as well as one day later, when the exogenous OXT effects on the brain can be assumed to have faded. The second day of post-interaction preference testing allowed us to test whether OXT facilitates, or hampers, long-term social learning, and to rule out alternative explanations of putative changes in STFP.

Materials and methods

Subjects

We trained and tested 239 observer and 140 demonstrator Long-Evans male rats (Charles River, Germany) for this study, about 9–10 weeks old at arrival and weighing 410 ± 50 g on the injection day. 28 observers met the exclusion criteria (see below) and had to be removed from the analysis, leaving a final sample size of $n = 211$ observers. The temperature in the housing room was maintained at 22ºC ± 2ºC, with humidity set at 55% ± 2%. Subjects were kept under an inverted 12:12 light-dark cycle. Rats were supplied with laboratory rodent food (Sniff, Germany) and water ad libitum except for the STFP testing period when rats were food-restricted to 85% of their free-feeding body weight and fed daily after finishing the experimental procedure. All rats were handled for 5 min/day for 3 days before starting the experiment. All animal procedures were conducted in accordance with the German Welfare Act and were approved by the local authority LANUV (*Landesamt*

für Natur-, Umwelt- und Verbraucherschutz North Rhine-Westphalia, Germany).

Socially transmitted food preference task

Housing and habituation

Three days before the start of the STFP task, all rats underwent a 10-minute habituation session in an open field (50 × 50 × 45 cm, PVC, illumination to 5–15 lx). To this end, cagemates were placed together in the open fields. Upon habituation to the open field, all subjects were henceforth housed individually and were food-restricted. To habituate rats to the feeder setup, for three days, all rats were provided with hanging feeders in their home cages containing 10 grape-flavored and 10 banana-flavored pellets (TestDiet, USA). Then, rats were tested in the STFP task. The STFP protocol involved three stages: individual preference testing (days 1, 2, 3), social interaction (day 4), and post-interaction preference testing (days 4 and 5).

Individual preference testing

On testing day one, observer rats were provided with two weighed cups, each of them containing a different pellet type (grape and banana). These cups were positioned in hanging feeders (pictured in Fig. 1), and observers had unrestricted access for 6 h. Subsequently, the cups were removed and weighed. This process was replicated over the next two days. The observers' consumption was quantified individually and daily as the difference in cup weight before and after the 6-hour testing period. Upon concluding the pre-interaction testing, original individual preferences were determined by how much of each pellet type was consumed on day 3 (see exclusion criteria below).

Social interaction

On the fourth day of the STFP task, both observers and demonstrators were relocated to a room adjacent to the social interaction room. Demonstrators were fed with those pellets that were not revealed preferred on day 3 by their assigned observers. To enhance the corresponding odor, crushed pellets were spread to the demonstrator's back, snout, and anal area. Then, demonstrators and observers were allowed to freely interact in the open field for 15 min. The interaction between the observer and demonstrator was recorded and an evaluator analyzed the time spent by the observer exploring the demonstrator using Solomon Coder (Solomon Coder beta 19.08.02 © András Péter).

Post-interaction preference testing

Following the interaction, observer rats were promptly returned to their individual cages and provided with two cups, each containing one of the two banana- and grape-flavored food types. Similar to the pre-interaction testing, the cups were taken out and weighed after a 6-hour interval. The same preference test was repeated the next day. Subsequently, all animals were reintegrated into prior group housing.

Exclusion criteria

If a rat revealed preferred a particular pellet type on day 3 that was different from the pellet type revealed preferred on days 1 *and* 2, we assumed that this rat's preferences were inconsistent since it was not evidently clear what the truly preferred reward was on day 3. Rats with inconsistent preferences were excluded from further analysis. For example, if a rat preferred grape pellets on days 1 *and* 2, but banana

Fig. 1 Photo example of the individual cage with the metal hanging feeder and two cups containing grape and banana pellets

pellets on day 3, it would be excluded from analysis since we could not tell with certainty if this rat truly preferred banana, or grape. The reason for this exclusion criterion is to make sure that demonstrators were fed with the truly non-preferred food, and to avoid accidentally feeding the demonstrator with actually preferred food.

Familiarity group assignment

We operationalized group affiliation as familiarity between observers and demonstrators (Ben-Ami Bartal et al. 2014). Therefore, there were two familiarity groups: the in-group and the out-group. In the in-group condition, pairs of observers and demonstrators ($n = 100$) were housed together in one cage upon arrival at the animal housing. In the out-group condition, pairs of observers were housed together, but in separate cages from the demonstrators (three demonstrators per cage) to prevent contact before the STFP interaction. The out-group consisted of 111 observers and 40 demonstrators. In general, rats were housed according to this group assignment protocol for 2–3 weeks upon arrival in the animal facility; at the start of the experiment, they were housed individually (see below).

Oxytocin treatment

Within each familiarity group, observers were randomly assigned to one of three treatment groups: the control group (vehicle = saline), the group treated with low-dose OXT (0.25 mg OXT/ml), and the large-dose OXT group (1.0 mg OXT/ml), with an injection volume of 1 ml/kg. All observers received a single intraperitoneal injection immediately before the social interaction phase during the STFP.

Data analysis

We used a mixed analysis of variance (ANOVA; SPSS 27.0.1, IBM, USA; R 4.0.2; R Core Team, 2020, special usage of the ggbreak package for plotting (Xu et al. 2021) with the dependent variable *pellet consumption* (grams eaten), and the within-subject factors *pellet preference* (originally preferred vs. non-preferred pellets), *day* (pre-interaction day 3 vs. post-interaction day 4 vs. post-interaction day 5), and the between-subject factors *familiarity* (in-group vs. out-group) and *treatment* (vehicle vs. low-dose OXT vs. large-dose OXT). Post hoc analyses were performed with two-sided t-tests. Benjamini–Hochberg correction was applied to correct for multiple comparisons.

Occasionally, rats exhibited very strong STFP, resulting in a full preference reversal post- vs. pre-interaction. Preference reversals were defined as higher consumption of the originally non-preferred food than the originally preferred food after the social interaction on day 4. We compared the frequency of full preference reversals between conditions with a Fisher's exact test.

Finally, we measured the time the observer spent socially exploring the demonstrator during the social interaction phase of the STFP. We examined observer behavior exclusively because existing literature indicates minimal effects of the demonstrator's behavior on the observers' STFP performance (Galef and Wigmore 1983; Galef and Whiskin 2008a). To detect differences in social interaction times between groups and conditions, we employed a mixed ANOVA and its corresponding post-hoc two-sided t-tests and corrections for multiple comparisons.

Results

Familiarity modulates STFP in vehicle rats

To evaluate how familiarity modulates STFP in general, i.e., in the absence of OXT effects, we compared the amount of pellets eaten by vehicle observers between days 3 and 4, i.e., before vs. immediately after social interaction, as a function of familiarity (in- vs. out-group) and pellet preference (originally preferred vs. non-preferred pellets; Fig. 2). The mixed ANOVA showed a simple main effect of pellet preference on amount consumed ($F_{[1, 67]} = 136.816$, $p = .000$) and a simple main effect of day ($F_{[1, 67]} = 23.969$, $p = .000$), as well as an interaction effect between pellet preference and day ($F_{[1, 67]} = 21.837$, $p = .000$), suggesting that rats showed STFP. Importantly, we also found a significant interaction effect between pellet preference and familiarity ($F_{[1, 67]} = 8.405$, $p = .005$). The post-hoc tests (all post-hoc tests were corrected for multiple comparisons) indicated that both familiarity groups increased their consumption of the originally non-preferred pellets on day 4 compared to day 3 (in-group: $t_{[30]} = -3.16$, $p = .005$; out-group: $t_{[37]} = -5.53$, $p = .000$), suggesting that STFP was found in both familiarity groups. However, a between-group comparison showed that consumption of the originally non-preferred pellets was higher in the out-group than the in-group on day 4 (in- vs. out-groups: $t_{[66.2]} = -2.19$, $p = .032$), implying stronger STFP in the out-group than the in-group. Consistent with this conclusion, only the out-group decreased the consumption of their originally preferred pellets on day 4 compared to day 3 (out-group: $t_{[37]} = 3.14$, $p = .005$; in-group: $t_{[37]} = 0.48$, $p = .635$) and compared to the in-group (day 4 in- vs. out-groups: $t_{[65.7]} = 3$, $p = .004$). Accordingly, the change in consumption of the originally preferred pellets, but not non-preferred pellets, from day 3 to 4 differed between familiarity groups (difference in originally preferred pellets: $t_{[66.9]} = -2.05$, $p = .044$; originally

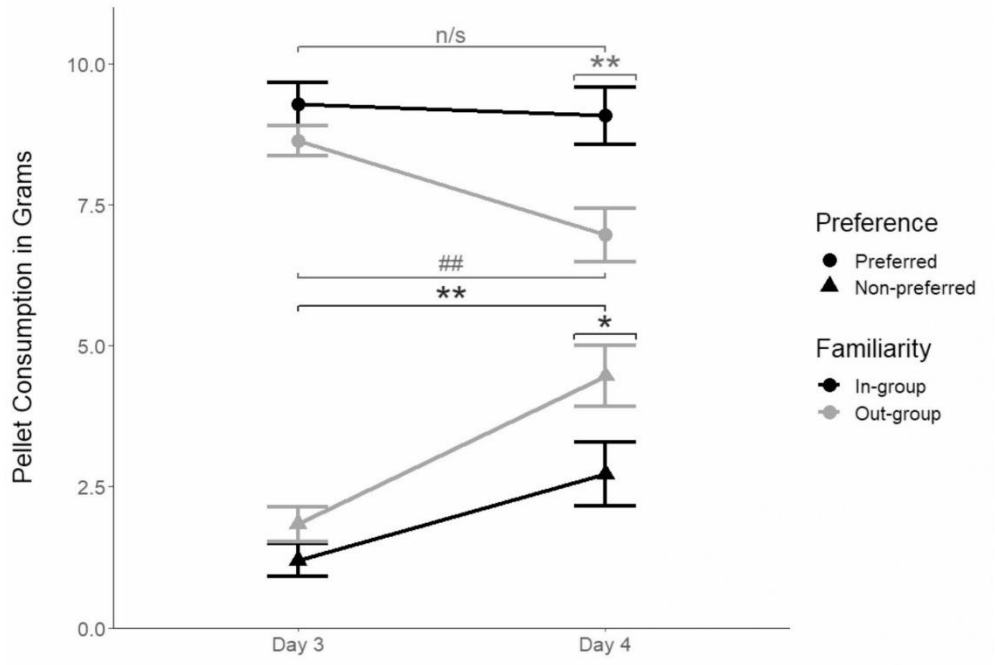

Fig. 2 Vehicle-treated observers' socially transmitted food preferences are modulated by familiarity. Mean (±standard error of the mean; SEM) of the pellets (originally preferred, circle; originally non-preferred, triangle) consumed on days 3 (pre-social interaction) and day 4 (post-social interaction) by observers who interacted with a familiar demonstrator (in-group ($n=31$), black) or an unfamiliar one (out-group ($n=40$), light gray). The change in consumption of the originally non-preferred pellets pre- vs. post-interaction was stronger in the out-group than the in-group, and a change in consumption of the originally preferred pellets was only found in the out-group. * $p<.05$; ** $p<.01$; ## out-group $p<.01$, n/s in-group $p>.05$

non-preferred pellets: $t_{[65.7]}=1.56$, $p=.124$). Hence, both familiarity groups exhibited socially transmitted food preferences, but the effect was significantly more pronounced in the out-group than the in-group (Fig. 2).

Full preference reversal

Rats occasionally exhibited very strong STFP, resulting in a full preference reversal on day 4 vs. day 3. We computed the proportion of vehicle-treated observers who fully reversed their pellet preferences, and compared the proportion of pellet preference reversals between familiarity groups (Fig. 3). In the in-group, only 10% of rats (3/31) fully reversed their pellet preferences, in contrast to the out-group, where 39% of rats (15/38) did so. Hence, consistent with the conclusion of the previous paragraph, these data suggest stronger social transmission of food preferences in the out-group than the in-group condition (Fisher's exact test; $p=.006$, two-sided). Further analyses are available in the supplemental materials, Fig. 1.

Oxytocin effects on social transmission of food preference are modulated by familiarity

To find out if the OXT treatment had an effect on STFP, possibly in a familiarity-dependent way, we ran a four-way mixed ANOVA with pellet preference (originally preferred vs. non-preferred), familiarity (in- vs. out-group), treatment (vehicle vs. low-dose OXT vs. large-dose OXT), and day (days 3 vs. 4 vs. 5) as independent variables on pellet consumption. We found a significant simple main effect of pellet preference ($F_{[1,\ 202]}=440.333$, $p=.000$), a significant simple main effect of treatment ($F_{[2,\ 202]}=9.079$, $p=.000$), and a significant simple main effect of day ($F_{[2,\ 404]}=16.129$, $p=.000$), and a significant four-way interaction between pellet preference, familiarity, treatment and day ($F_{[3.51,\ 354.66]}=3.029$, $p=.023$).

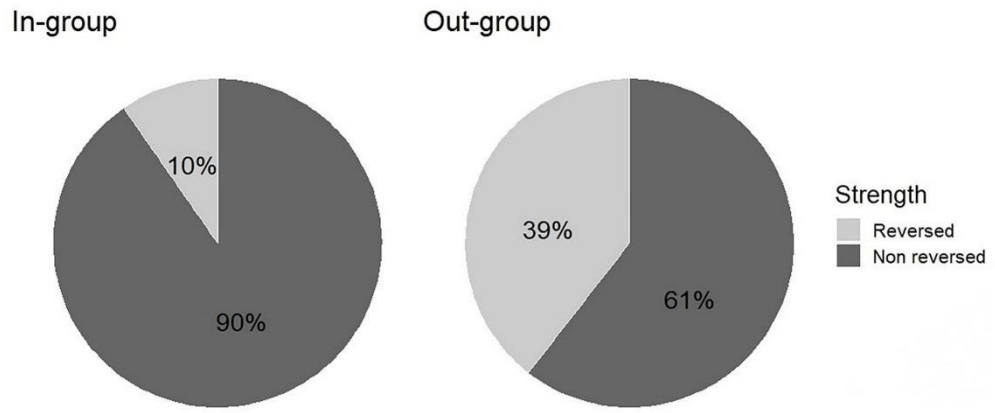

Fig. 3 Frequency of full preference reversals, in percent, after social interaction (day 4 vs. day 3). The frequency of full preference reversals was significantly higher in the out-group than in the in-group

To unpack this complex interaction effect, we ran a suite of post-hoc tests (again, all post-hoc tests were corrected for multiple comparisons). To understand the acute effects of OXT on STFP, we, first, zoomed in on what happened on day 3 vs. day 4 (Fig. 4; individual data plots in Fig. 2 in the supplemental materials). In the in-group (panel A of Fig. 4), we found a significant decrease in consumption of the originally preferred pellets on day 3 vs. day 4 in both OXT groups (low-dose OXT: $t_{[32]}=5.69$, $p=.000$; large-dose OXT: $t_{[35]}=6.69$, $p=.000$), but not in the vehicle group ($t_{[30]}=0.48$, $p=.714$). There was a significant increase in consumption of the originally non-preferred pellets from day 3 to day 4 in all treatment groups (vehicle: $t_{[30]}$ = -3.16, $p=.01$; low-dose OXT: $t_{[31]}$ = -2.93, $p=.013$; large-dose OXT: $t_{[35]}$ = -3.4, $p=.007$), and we found no significant difference in their consumption over days 3 and 4 between treatment groups (vehicle vs. low-dose OXT: $t_{[59.4]}$ = -0.476, $p=.636$; vehicle vs. large-dose OXT: $t_{[40.4]}$ = -1.61, $p=.232$; low-dose OXT vs. large-dose OXT: $t_{[45]}$ = -1.16, $p=.38$). This analysis suggests that, in the in-group, OXT administration led to a stronger decrease in consumption of the originally preferred pellets relative to vehicle administration, but had no marked effect on the consumption of the originally non-preferred pellets.

The picture was different in the out-group (panel B of Fig. 4). Here, we found a significant decrease in consumption of the originally preferred pellets on day 3 vs. day 4 in all treatment groups, including the vehicle group (vehicle: $t_{[37]}=3.14$, $p=.006$; low-dose OXT: $t_{[33]}=3.82$, $p=.002$; large-dose OXT: $t_{[36]}=6.14$, $p=.000$). There was no significant difference in the change in consumption of the originally preferred pellets between any of the treatment groups (vehicle vs. low-dose OXT: $t_{[69.3]}$ = -0.087, $p=.931$; vehicle vs. large-dose OXT: $t_{[66.2]}$ = -0.97, $p=.504$; low-dose OXT vs. large-dose OXT: $t_{[65.5]}$ = -0.969, $p=.504$). By contrast, we found a significant and steep increase in consumption of the originally non-preferred pellets from day 3 to day 4 in the vehicle group ($t_{[37]}$ = -5.53, $p=.000$), but no significant increase in either OXT group (low-dose OXT: $t_{[33]}$ = -2.12, $p=.061$; large-dose OXT: $t_{[36]}$ = -1.7, $p=.136$). Accordingly, vehicle observers in the out-group condition consumed significantly more of the originally non-preferred pellets than the OXT-treated observers (vehicle vs. low-dose OXT: $t_{[63.3]}=3.15$, $p=.024$; vehicle vs. large-dose OXT: $t_{[48.4]}=4.77$, $p=.000$; low-dose OXT vs. large-dose OXT: $t_{[54.3]}=1.74$, $p=.226$). This analysis suggests that in the out-group, OXT had different effects on STFP than in the in-group. In the out-group condition, relative to vehicle administration, OXT dampened the increase in consumption of the originally non-preferred pellets, but it had no marked effect on the consumption of the originally preferred pellets.

Oxytocin has long-term effects on social transmission of food preferences

So far, we presented the results of day 3 (before social interaction) vs. day 4 (immediately after social interaction and immediately after OXT injection, i.e., with acute OXT effects on the rats' system). To understand if OXT had long-term effects on STFP (Fig. 5; individual data plots in Fig. 3 in the supplemental materials), beyond its acute action, we extended our post-hoc analysis to day 5, i.e., one day after OXT or vehicle injection. In the in-group (panel A of Fig. 5), there was no significant difference in originally preferred pellets consumption between day 4 and day 5 in any of the treatment groups (vehicle: $t_{[30]}$ = -0.222, $p=.826$;

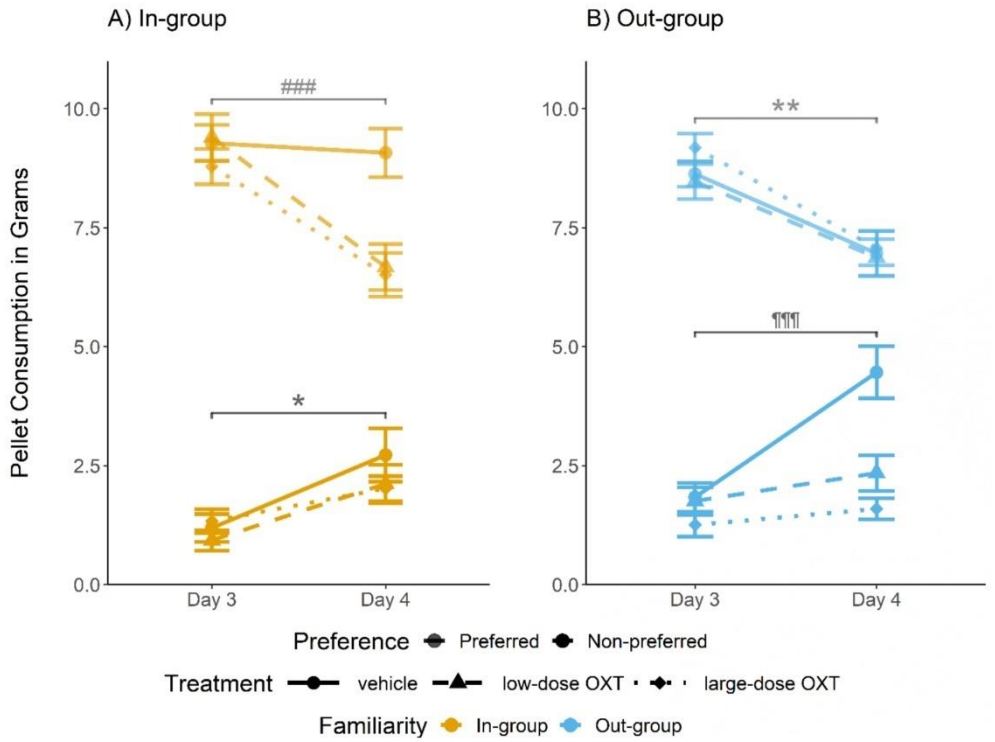

Fig. 4 Acute oxytocin (OXT) and intergroup effects on socially transmitted food preference (STFP). STFP in the in-group (panel A), and the out-group (panel B). In both panels, the pellet consumption (mean ± SEM) of the vehicle group is represented by the solid line and circle symbols, the low-dose OXT group by the dashed line and triangles, and the large-dose OXT group by the dotted line and squared symbols. The originally preferred pellets (upper lines) are indicated in a slightly transparent hue, and the originally non-preferred pellets (lower lines) are in an opaque hue. In the in-group (panel A), rats in all treatment conditions increased their consumption of the originally non-preferred pellets after social interaction on day 4, thus exhibiting STFP. Unlike rats in the vehicle group, rats that received OXT injections prior to social interaction decreased the consumption of the originally preferred pellets. In the out-group (panel B), OXT administration prevented the increased consumption of the originally non-preferred pellets observed in the vehicle group, thus blocking STFP. However, there were no differences between treatment conditions in the consumption of the originally preferred pellets, which decreased between days 3 and 4. * All treatments $p < .05$; ** all treatments $p < .01$; ### OXT-treated groups $p < .001$; ¶¶¶ vehicle group $p < .001$

low-dose OXT: $t_{[32]} = -1.9$, $p = .091$; large-dose OXT: $t_{[35]} = -1.22$, $p = .292$). By contrast, both OXT groups, but not the vehicle group, showed a continued increase in consumption of the originally non-preferred pellets from day 4 to day 5 (vehicle: $t_{[30]} = 1.19$, $p = .292$; low-dose OXT: $t_{[32]} = -2.38$, $p = .043$; large-dose OXT: $t_{[35]} = -2.26$, $p = .045$), even though the amount of originally non-preferred pellets consumed on day 5 did not differ between OXT and vehicle groups (vehicle vs. low-dose OXT: $t_{[57]} = -1.28$, $p = .635$; vehicle vs. large-dose OXT: $t_{[65]} = -1.02$, $p = .635$; low-dose OXT vs. large-dose OXT: $t_{[61.1]} = 0.404$, $p = .843$). Hence, in the in-group, the pattern of effects on STFP observed under acute OXT effects (day 4) persisted, or even increased, on day 5, when the acute OXT effects on the organism can be assumed to have waned.

In the out-group (panel B of Fig. 5), we found a significant increase in consumption of the originally preferred pellets in the large-dose OXT group from day 4 to day 5, but not in the low-dose OXT or vehicle groups (vehicle: $t_{[37]} = 0.944$, $p = .395$; low-dose OXT: $t_{[33]} = 0.366$, $p = .717$; large-dose OXT: $t_{[36]} = -3.35$, $p = .004$). Although we had found significant OXT effects on the consumption of the originally non-preferred pellets on day 4 (see above), this difference disappeared on day 5 for the low-dose OXT (vehicle vs. low-dose OXT: $t_{[70.7]} = 0.565$, $p = .861$) and only remained significant for the large-dose OXT (vehicle vs. large-dose

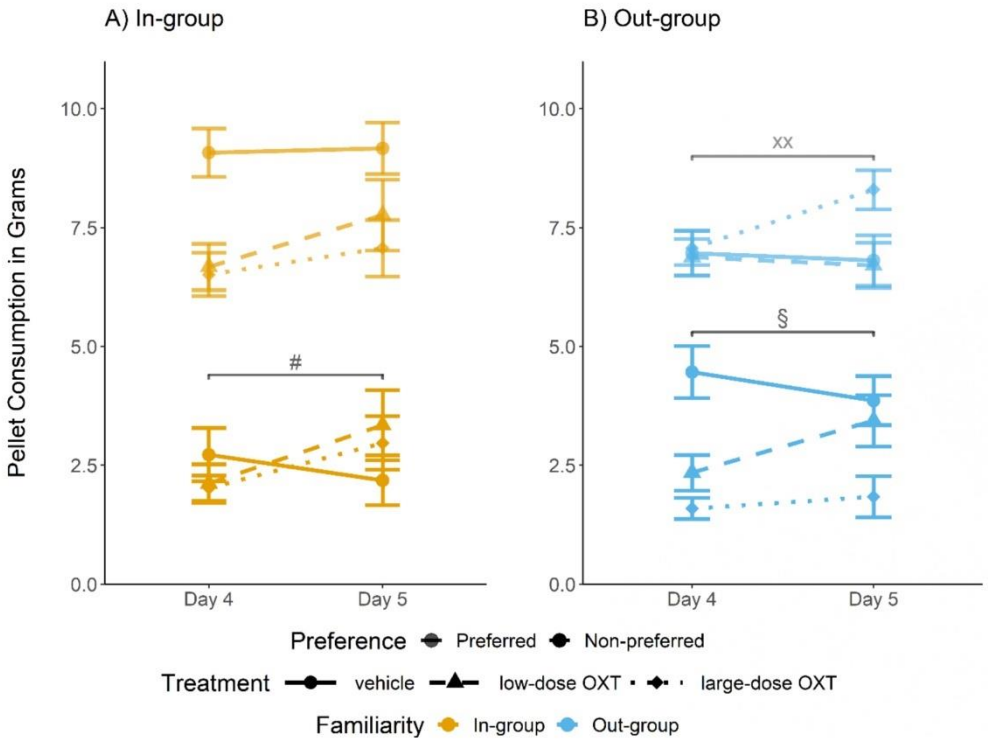

Fig. 5 Long-term oxytocin (OXT) and familiarity effects on socially transmitted food preference (STFP). Line and panel representations are the same as Fig. 4. In the in-group (panel A), rats treated with OXT on day 4 (immediately after social interaction and OXT injection) increased the consumption of the originally non-preferred pellets on day 5 (one day after social interaction and OXT injection) following the previous tendency (from day 3 to day 4). By contrast, the consumption of the vehicle group was relatively constant across days. The consumption of the originally preferred pellets was constant for all treatment conditions. In the out-group (panel B), only the low-dose OXT group increased the consumption of the originally non-preferred pellets from day 4 to day 5. By contrast, the OXT effects on the large-dose OXT group were long-lasting, suggesting that the OXT-related blocking of STFP was stable over time. Regarding the originally preferred pellets, only the large-dose OXT group increased their consumption once acute OXT effects dissipated on day 5; the other treatment conditions remained unchanged. # OXT-treated groups $p < .05$; § low-dose OXT group $p < .05$; XX large-dose OXT group $p < .05$

OXT: $t_{[73.6]} = 3.01$, $p = .024$; low-dose OXT vs. large-dose OXT: $t_{[64.3]} = 2.31$, $p = .086$). In line with this observation, the low-dose OXT group showed an increase in consumption of the originally non-preferred pellets from day 4 to day 5 ($t_{[33]} = -2.45$, $p = .033$), but, the large-dose OXT group continued to show no significant change in consumption of the originally non-preferred pellets from day 4 to 5 ($t_{[36]} = -0.608$, $p = .579$), suggesting that they never acquired STFP. In sum, also in the out-group, we found a complex pattern of results suggestive of the fact that the effects of OXT on STFP outlasted its acute action. Hence, overall, our results suggest that OXT effects on STFP were dependent on familiarity with the demonstrator and reflect long-lasting changes in social learning.

Familiarity-modulated OXT effects on STFP cannot be explained by anorexic effects or social exploration time

Acute OXT action has anorexic effects, especially on palatable food (Olszewski et al. 2010; Herisson et al. 2014). It is therefore possible that the complex pattern of OXT effects on STFP reported here can simply be explained by its anorexic effects. Indeed, we found that OXT injections decreased total pellet consumption (originally preferred and non-preferred pellet types combined) on the day of injections (mixed ANOVA with the factors familiarity, treatment and day; main effect of treatment, $F_{[2, 202]} = 9.079$, $p = .000$; significant simple main effect of day, $F_{[2, 404]} = 16.129$,

$p = .000$, and a significant interaction effect between treatment and day, $F_{[4, 404]} = 21.241$, $p = .000$, Fig. 4 supplemental material). However, these anorexic effects were restricted to day 4, i.e., the day of OXT injection, and did not extend to day 5 (post-hoc test: day 3 vs. day 5; in- low-dose OXT: $t_{[31]} = -1.6$, $p = .165$; in- large-dose OXT: $t_{[35]} = 0.276$, $p = .802$; out- low-dose OXT: $t_{[33]} = 0.252$, $p = .802$; out- large-dose OXT: $t_{[36]} = 1.25$, $p = .284$). In addition, even though we found group-dependent OXT effects on STFP (see analysis above), OXT effects on total pellet consumption did not differ between in-group and out-group ($F_{[1, 202]} = 0.352$, $p = .554$). Our analysis presented above showed that OXT effects on STFP were group-dependent and long-lasting, but OXT effects on total pellet consumption were neither group-dependent, nor long-lasting, suggesting that the reported OXT effects on STFP cannot be straightforwardly explained by its anorexic effects (Table 1 supplemental material; see discussion for further elaboration).

In addition to OXT anorexic effects, OXT and/or familiarity may have modulated the time observers spent interacting with, or socially exploring, the demonstrators. A mixed ANOVA revealed a significant simple main effect of OXT treatment, but not familiarity, on social exploration time (treatment: $F_{[2, 224]} = 7.247$, $p = .000$; familiarity: $F_{[1, 224]} = 1.364$, $p = .244$): rats treated with the large-dose of OXT explored the demonstrators less than the other treatment groups (vehicle vs. low-dose OXT: $t_{[149]} = 0.763$, $p = .447$; vehicle vs. large-dose OXT: $t_{[145]} = 3.55$, $p = .000$; low-dose OXT vs. large-dose OXT: $t_{[148]} = 2.7$, $p = .012$; Fig. 5 supplemental material). Even though the observation that the demonstrator's novelty in the out-group, relative to the in-group, did not lead to a significantly longer duration of partner exploration is somewhat surprising (Oettl et al. 2016), the lack of evidence for a difference in social exploration time suggests that exploration time unlikely explains the familiarity effects on STFP reported above. Likewise, although we did find OXT effects on exploration time, we did not find a significant interaction between OXT and familiarity, suggesting that the complex interaction of OXT and familiarity on STFP cannot be explained by social exploration.

Discussion

In this study, we measured the effects of systemic injections of OXT and the familiarity between observer and demonstrator on STFP. First, our results showed that vehicle rats revealed stronger changes in food preference when encountering an unfamiliar than a familiar demonstrator. Second, we found that systemic OXT administration influenced STFP dependent on whether the demonstrator was familiar or not: when the demonstrator was familiar (in-group), OXT led to a decreased consumption of the originally preferred pellets after social interaction with the demonstrator, but had no effect on the consumption of the originally non-preferred pellets. By contrast, we found opposite effects of OXT on STFP when the demonstrator was unfamiliar (out-group): OXT, relative to vehicle, did not change the consumption of the originally preferred pellets, but, notably, prevented the increase in consumption of the originally non-preferred pellets. These familiarity-dependent OXT effects on STFP could still be found one day later, at least after large OXT doses, when the acute effects of OXT on the organism most likely had waned, suggesting that OXT action during social interaction has long-term effects on STFP. Our results uncover a new mechanism how OXT modulates familiarity-dependent socially transmitted preferences and social reward revaluation.

Previous literature identified an acute anorexic effect after OXT administration in male rats, resulting in less food consumption (Arletti et al. 1989, 1990; Benelli et al. 1991). Our results also show a decrease in total pellet consumption (originally preferred + non-preferred) by the OXT groups. Although anorexic effects might explain our pattern of results, we believe this is not the case. First, if OXT's anorexic effects were the only mechanism, it should reduce consumption of both preferred and non-preferred pellets equally, but we did not find this to be the case (see results above). Second, OXT effects depended on the demonstrator's familiarity – an observation that is also difficult to reconcile with the anorexia hypothesis. Third, and perhaps most importantly, we found that OXT effects on STFP outlasted the acute OXT effects on total pellet consumption, suggesting that OXT action had long-lasting effects on STFP beyond its acute anorexic effects. We, hence, conclude that the results reflect group-dependent OXT effects on social learning, and not merely an OXT-related reduction in hunger or appetite.

Can the observation that vehicle rats showed stronger STFP with unfamiliar than familiar demonstrators be explained by differences in social exploration times? A feasible explanation of this phenomenon in rats is their preference for social novelty. Rats typically interact longer with an unfamiliar individual, which could enhance the chance of olfactory transmission of the demonstrator's food preference via its breath (Galef et al. 1988; Galef and Whiskin 2008a). However, a more recent study could not find support for this explanation, as a more detailed analysis showed that observers spent equal time sniffing the face of their demonstrator or in direct nose contact regardless of familiarity (Agee et al. 2019). In agreement with that, our vehicle rats in both in- and out-group conditions spent equal time sniffing their demonstrator, suggesting that other mechanisms

than merely olfactory recognition or social interaction time accounted for STFP.

So, how can we explain the familiarity- and OXT-dependent changes in consumption of the originally preferred and originally non-preferred pellets? One possibility is that OXT affected the decision weight the observers' placed on the specific kind of social information transmitted by the demonstrator in a familiary-dependent way: in the out-group condition, unlike the control observers, OXT-treated observers simply ignored the food information that was socially transmitted by the demonstrator, and, hence, continued to consume their originally preferred pellets the same way as they did before the social interaction. By contrast, in the in-group condition, OXT-treated observers began to dislike the pellets that were not eaten by the demonstrator, and, consequently, reduced the consumption of those pellets.

However, there are alternative explanations for the complex familiarity- and OXT-dependent effects on STFP that seem equally plausible. For example, one could argue that the information that is transmitted by the demonstrator in STFP would be the palatability of the originally non-preferred reward, but there would be no information transmitted about the originally preferred reward; after all, observer rats smell the scent of the originally non-preferred reward in the demonstrators' breath (Galef et al. 1988), but do not have any social information on the originally preferred pellets. Hence, STFP would mainly manifest as an increase in consumption of the originally non-preferred reward. Since, in vehicle rats, total pellet intake (preferred + non-preferred pellets) usually remains constant after social interaction, the decrease in consumption of the originally preferred reward in STFP would just be the logical, secondary consequence of the increased consumption of the originally non-preferred rewards: if rats eat more of food B after social interaction, they necessarily have to eat less of food A, unless they change their total food intake. According to this view, the difference in consumption of the originally preferred pellets between OXT and vehicle rats in the in-group might just reflect a secondary satiation effect: as mentioned, OXT led to a decreased total amount of pellets eaten on day 4, after the social interaction (see results and supplemental material). OXT-treated rats in the in-group showed STFP much like the vehicle rats, and accordingly ramped up their consumption of the originally non-preferred pellets (Fig. 4), while, at the same time, reducing their overall pellet consumption due to OXT's anorexic action. Hence, the OXT-related decrease in consumption of the originally preferred pellets on day 4 (Fig. 4) may simply reflect satiation effects: [reduced total consumption] minus [increased non-preferred consumption] = [reduced preferred consumption]. Note that this explanation may account for the pattern of results found in the in-group results, but cannot account for our out-group results. Future research needs to disentangle whether the familiarity- and OXT-dependent changes in pellet consumption reported here reflect familiarity-dependent differences in the decision weights attached to social information about the preferred and the non-preferred rewards, or differential satiation effects.

OXT's role in diverse modes of social information processing has become a focus of emerging research, making it a strong candidate for regulating social transmission of food value (Popik and Van Ree 1993; Choleris et al. 2009; but see Lindeyer et al. 2013). A study demonstrated the pivotal role of centrally released OXT in social cue processing, which integrates both odor extraction and social recognition. OXT affected genuine social aspects of social cue processing, as evidenced by the fact that inhibiting OXT signaling in the anterior olfactory nucleus (AON) resulted in compromised social recognition, while object and non-social odor recognition abilities remained unaffected (Oettl et al. 2016). In agreement with the notion that OXT facilitates the olfactory detection of information transmitted by a conspecific, further studies elaborated on that topic. It was shown that OXT signalling in the olfactory sensory cortex is crucial for the association between neutral odors and socially meaningful cues (Choe et al. 2015). Even more strikingly, meeting a conspecific differing in either age or sex activated discrete patterns of OXT neurons in the lateral septum and/or medial amygdala in male rats, hinting at independent subcircuits for certain social modalities (Lukas et al. 2013). While these findings do not explicitly address the different familiarity-dependent OXT effects on STFP in the in- and out-group conditions reported here, it may be reasonable to assume that demonstrators' familiarity, too, activates specialized OXT subcircuits, explaining our observed intergroup differences in flexible social preference revaluation. The differences in STFP between the in-group and out-group conditions might also be suggestive of familiarity effects on the recollection success of social reward revaluation. This familiarity-moderated recollection of reward value might involve hippocampal circuits as they are necessary for STFP (Alvarez et al. 2001; Winocur et al. 2001; Clark et al. 2002; but see Burton et al. 2000; Thapa et al. 2014) though selective OXT effects on GABA action in hippocampus (Maniezzi et al. 2019).

In conclusion, the current study provides evidence that STFP is modulated by OXT in a familiarity and dose-dependent manner. While the socially transmitted changes in food preference were stronger when interacting with strangers, large OXT dosage blocked the integration of social information during reward revaluation. The presented study is in line with the current understanding that OXT can modulate sensitivity to socially significant cues. The interpretation of these cues is affected by contextual elements, particularly

the familiarity of the demonstrator, suggesting that OXT has social effects beyond facilitating prosocial behavior (Anacker and Beery 2013; Olff et al. 2013; Love 2014; Piva and Chang 2018). These results add a layer of complexity to our knowledge of the influence of OXT in social learning. Exploring responsible neuronal areas and their specific dependency requires further investigation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00213-024-06682-x.

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Data availability Raw data supporting the findings presented in the study is openly available in OSF at https://osf.io/sfg3x/.

Declarations

Conflict of interest The authors declare no competing interests.

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STUDY III

Seidisarouei, M., van Gurp, S., Pranic, N. M., Calabus, I. N., van Wingerden, M., & Kalenscher, T. (2021). Distinct Profiles of 50 kHz Vocalizations Differentiate Between Social Versus Non-social Reward Approach and Consumption. *Frontiers in Behavioral Neuroscience*, *15*(Jun), 1–17. https://doi.org/10.3389/fnbeh.2021.693698

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Distinct Profiles of 50 kHz Vocalizations Differentiate Between Social Versus Non-social Reward Approach and Consumption

Mohammad Seidisarouei[1,2], Sander van Gurp[1], Nicole Melisa Pranic[3], Irina Noguer Calabus[2], Marijn van Wingerden[1,4] and Tobias Kalenscher[2]*

[1] Social Rodent Lab, Institute of Experimental Psychology, Heinrich-Heine-University, Düsseldorf, Germany, [2] Comparative Psychology, Institute of Experimental Psychology, Heinrich-Heine-University, Düsseldorf, Germany, [3] Department of Psychology, Cornell University, Ithaca, NY, United States, [4] Department of Cognitive Science and Artificial Intelligence, Tilburg School of Humanities and Digital Sciences, Tilburg University, Tilburg, Netherlands

Social animals tend to possess an elaborate vocal communication repertoire, and rats are no exception. Rats utilize ultrasonic vocalizations (USVs) to communicate information about a wide range of socially relevant cues, as well as information regarding the valence of the behavior and/or surrounding environment. Both quantitative and qualitative acoustic properties of these USVs are thought to communicate context-specific information to conspecifics. Rat USVs have been broadly categorized into 22 and 50 kHz call categories, which can be further classified into subtypes based on their sonographic features. Recent research indicates that the 50 kHz calls and their various subtype profiles may be related to the processing of social and non-social rewards. However, only a handful of studies have investigated USV elicitation in the context of both social and non-social rewards. Here, we employ a novel behavioral paradigm, the social-sucrose preference test, that allowed us to measure rats' vocal responses to both non-social (i.e., 2, 5, and 10% sucrose) and social reward (interact with a Juvenile rat), presented concurrently. We analyzed adult male Long-Evans rats' vocal responses toward social and non-social rewards, with a specific focus on 50 kHz calls and their 14 subtypes. We demonstrate that rats' preference and their vocal responses toward a social reward were both influenced by the concentration of the non-social reward in the maze. In other words, rats showed a trade-off between time spent with non-social or social stimuli along with increasing concentrations of sucrose, and also, we found a clear difference in the emission of flat and frequency-modulated calls in the social and non-social reward zones. Furthermore, we report that the proportion of individual subtypes of 50 kHz calls, as well as the total USV counts, showed variation across different types of rewards as well. Our findings provide a thorough overview of rat vocal responses toward non-social and social rewards and are a clear depiction of the variability in the rat vocalization repertoire, establishing the role of call subtypes as key players driving context-specific vocal responses of rats.

Keywords: ultrasonic vocalizations, social, behavior, reward processing, rats, vocal communications, 50 kHz calls, subtypes

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University of Maryland, United States

Reviewed by:
Anne-Marie Mouly,
INSERM U1028 Centre de Recherche en Neurosciences de Lyon, France
Stefan Brudzynski,
Brock University, Canada

****Correspondence:***
Mohammad Seidisarouei
seidisar@uni-duesseldorf.de

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INTRODUCTION

Rats are social animals (Whishaw and Kolb, 2009) that form relatively large and tightly organized groups. As nocturnal animals, many rodent species rely on complex vocalizations for communication and social coordination. The extent of their vocalization vocabulary depends on their social structure and inter-individual interactions (for a review, see Brudzynski, 2014). Among rodents, rats, in particular, have developed an elaborate system of ultrasonic communication which has been suggested to have adaptive significance by signaling socially relevant information: ultrasonic vocalizations (USVs) emitted by rats have been implied to play a role in warning conspecifics (Litvin et al., 2007; Brudzynski, 2013), as well as acting as indices of rats' affective states (Knutson et al., 2002; Brudzynski, 2013) and social motivation (Mulvihill and Brudzynski, 2018b). Additionally, Himmler et al. (2014) have demonstrated the function of rat USVs in facilitating and maintaining play behavior, pointing to their social communicative value. Thus, it has been suggested that the wide range of calls emitted by rats serve a multitude of context-dependent functions.

The USVs emitted by pups, adolescent and adult rats can be divided to three major sub-groups: (i) 22 kHz alarm calls (Litvin et al., 2007) produced in response to an aversive circumstance (Wöhr and Schwarting, 2013), (ii) 50-kHz USVs that signal appetitive and rewarding states (Panksepp and Burgdorf, 2000) and (iii) 40 kHz vocalizations produced by socially isolated pups (Wöhr et al., 2008). The acoustic features of the 50 kHz calls differ substantially from 22 kHz USVs (Brudzynski and Pniak, 2002; Brudzynski and Holland, 2005; Thompson et al., 2006), allowing distinct and clear-cut classifications. Specifically, 50 kHz USVs have a concise call duration between 30 and 40 ms, a bandwidth of 5–7 kHz, and a peak frequency remaining within 45–55 kHz, although the calls can reach 70 kHz or higher.

The 22 and 50 kHz call categories emitted by rats thus represent general qualitative information regarding the condition of the environment or behavior, but these call categories can be further organized into subtypes of vocalizations (Wright et al., 2010; Himmler et al., 2014; Brudzynski, 2015) that differ in sonographic features. For instance, 50 kHz USVs can be classified into Flat and frequency-modulated (FM) subtypes based on the bandwidth of frequencies they extend over in spectrograms (Burgdorf and Panksepp, 2006; Wöhr et al., 2008). Several lines of evidence demonstrate that rats emit Flat- and FM-50 kHz USVs in different situations, suggesting that these subgroups of 50 kHz USVs may have distinct and disparate communicative roles of behavioral significance. Flat calls, for instance, have been suggested to be involved in (initiating) social contact (Burgdorf et al., 2011) and social coordination (Wöhr and Schwarting, 2008). FM 50 kHz USVs, on the other hand, are more commonly emitted during rewarding situations or high positive emotional arousal (Burgdorf et al., 2011). The FM subgroup of 50 kHz USVs have been further grouped into subtypes based on the extent of their frequency modulation and the shape they assume in the spectrogram (Brudzynski and Zeskind, 2018). In the most comprehensive classification, the 50 kHz USVs were categorized into 14 distinct subtypes (Wright et al., 2010). This categorization, however, is not one without controversy. Coffey et al. (2019), for instance, have recently utilized the DeepSqueak software to classify USVs using unsupervised machine learning techniques into 18 separate clusters instead of 14 subtypes. In addition, the behavioral relevance of these various call subtypes remains largely unknown.

Because of their association with appetitive situations, 50 kHz calls could potentially also be utilized in quantifying the value that individual rats attribute to a reward (Garcia et al., 2015) as well as to the expectation of a reward (Binkley et al., 2014). Calls emitted in the presence of non-social and social rewards have been investigated thoroughly in the literature. Cues for nutritional reward have been shown to elicit 50 kHz responses from rats (Brenes and Schwarting, 2014), and a preference for sweet pellets over regular pellets is associated with an increase in the frequency of 50 kHz vocalizations (Mateus-Pinheiro et al., 2014). Nevertheless, Schwarting et al. (2007) found no difference between the 50 kHz calls produced by food-deprived animals and the ones exposed to ad-libitum feeding, when they were alone in the home cage. In another intricate design, Browning et al. (2011) have demonstrated that rats trained for cocaine and sucrose self-administration showed more 50 kHz calls during the reward self-administration and reinstatement phase (after a period of extinction training), compared to naïve controls who were not rewarded.

Juvenile, adolescent, and adult rats have been shown to emit 50 kHz calls during interactions with their conspecifics, such as rough and tumble play (Knutson et al., 1998) and mating (White et al., 1990). Female rats also produce 50 kHz calls when encountering a social partner (Börner et al., 2016). The calls emitted by adult rats can thus give clues about their social behavior (but see, Manduca et al., 2014). It has been shown that rats emit more 50 kHz calls when exposed to another conspecific (Brudzynski and Pniak, 2002) and display a preference for rats producing more 50 kHz calls (Panksepp et al., 2002). In contrast, rats selectively bred to emit lower rates of 50 kHz calls spent less time with conspecifics in a social interaction test than the randomly bred line (Burgdorf et al., 2009). Similarly, playful experiences are significantly less frequent in pairs of devocalized rats than in their vocalizing counterparts, emphasizing the role of these 50 kHz calls in maintaining play behavior (Himmler et al., 2014).

Łopuch and Popik (2011), Kalenscher (2020), and Kalenscher et al. (2021) have also argued that the cooperative behavior of rats positively correlates with the 50 kHz vocalizations they produce, as 50 kHz USVs may act as social vicarious reward signals (Hernandez-Lallement et al., 2016; Van Gurp et al., 2020; Löbner et al., 2021). Neural processing of USVs has been implicated in the amygdala, with opposing coding schemes for 22 vs. 50 kHz USVs (Parsana et al., 2012), and indeed, lesions of the BLA impair the social approach that is usually observed to 50 kHz USV playback (Wöhr and Schwarting, 2007; Seffer et al., 2014; Schönfeld et al., 2020).

In short, both qualitative and quantitative differences in 50 kHz USV production have been found across a range of social and non-social rewarding situations. Only a handful of studies in the literature, however, have investigated USV production

in the context of concurrent social and non-social rewards. Utilizing selective breeding procedures (Burgdorf et al., 2009), have demonstrated that rats bred to emit higher rates of 50 kHz calls were more likely to prefer a sucrose solution to tap water than randomly bred rats. Willey and Spear (2013) analyzed the calls and approach behavior toward both food-related and social stimuli in rats exposed to varying degrees of social deprivation. The time animals spent investigating the social stimulus within the apparatus positively correlated with the frequency-modulated (FM) calls they emitted. However, these authors did not find a relationship between animals' responses to food stimuli and their USV production. In a novel design, Mulvihill and Brudzynski (2018b) analyzed the USVs produced by male rats separately allowed to freely explore a female, a littermate, as well as two non-social conditions, namely Fruit Loop rewards and 2% ethanol solution. Their results indicated that out of the four groups, only rats exposed to a cycling female produced a higher proportion of calls than the baseline. Mulvihill and Brudzynski (2018b) also demonstrate significant differences between the types of calls made in non-social versus social conditions. Specifically, rats exposed to non-social stimuli produced more flat calls than non-trill FM calls, whereas the non-trill FM subtype dominated the 50 kHz calls in the social contexts.

Thus, in summary, there is growing evidence that 50 kHz USVs, and the 50 kHz subtypes, are related to the subjective experience of social vs. non-social rewards, which could be related to reward processing traits (such as sucrose preferences), to individual communicative traits, or a combination of these factors. If there indeed is a structure to the type of vocalizations emitted in social and non-social situations, akin to a selective "vocabulary" for different behavioral contexts, it should be possible to distinguish these contexts when presented in direct competition, based on the vocalization patterns that are recorded.

To study this question, we employed a novel behavioral paradigm, the social-sucrose preference test. It is conducted on an XCST (X-shape chambered sociability test) maze. The XCST maze is a modified version of a radial arm maze previously utilized by Schönfeld et al. (2020) that can be used to contrast behavioral responses to both a social reward (Juvenile conspecific in an open-bar sociability cage) and varying levels of non-social reward (sucrose solutions) in different arms of the apparatus while recording the USVs emitted by the animals. Thus, we systematically investigated how the occurrence of the 14 subtypes of rat USVs was related to rats' choice behavior in the trade-off between social and non-social rewards.

MATERIALS AND METHODS

Subjects

The experiment was conducted according to the European Union Directive 2010/63/EU for animal experimentation and was approved by the local authority (Landesamt für Natur, Umwelt und Verbraucherschutz North-Rhine Westphalia, Germany). Fifteen male Long-Evans rats (*Charles River, Italy*) in total were obtained in a batch of 12 experimental animals (PND 40, Mw_{eight} = 320 g, at the starting day of the experiment) and 3 Juvenile rats [PND 28, M_{weight} = 290 g, at the starting day of the *Social-Sucrose Preference Test (SSPT)*], serving as social stimulus/reward. Experimental rats were housed in groups of $N = 3$ rats in standard Type IV Macrolon cages under a reversed 12:12 h light-dark cycle. The housing room was kept at a constant temperature of 22°C and a humidity of 60%. Throughout the experiment, all rats received standard laboratory rodent food, *ad libitum*, except for the Sucrose Discrimination Test (SDT) phase in which all experimental animals were limited in their food intake (food per rat per day: 22 g on weekdays and 25 g on weekends).

Behavioral Task Setup

We used an eight-arm radial maze as previously adapted by Schönfeld et al. (2020), detached four arms to arrive at a cross/plus-maze setup (**Figure 1A**). The maze consisted of a central platform (36 cm diameter; so-called neutral zone in our design) and four arms (14 cm wide and 60 cm long) that extended from the central platform in an octagon platform. Each of the four arms was consistently associated with one single reward type: 3 arms with three different levels of a sucrose solution reward (see **Figure 1A**) and one arm with a social stimulus. To circumvent any spatial bias, we divided our subjects into two groups (A and B, per group = 6) with a different allocation of reward positions for each group. Notably, during any test day in the experiment, only 2 out of 4 arms were open at a time to provide a head-to-head preference test between two rewards. On the arm of the maze assigned to the social reward, an unfamiliar Juvenile rat could be placed in a fixed cylindrical restrainer built from metal bars and compact plastic for its floor and ceiling (Height: 25.5 cm, Diameter: 17 cm, Ugo Basile Sociability Cage). The restrainer was fixed on the maze at the end of the Juvenile's arm, and the Juvenile could move around in this restrainer, and social contact through the openings between the bars was possible. On the arms allocated to non-social reward (i.e., different sucrose concentrations 2, 5, and 10%), sucrose solution was provided to the experimental animal in a cube plastic dish (8 × 8 cm) mounted at the end of each arm. Additionally, in order to facilitate spatial learning of the reward conditions in each arm over days, we included sandpapers (17 × 13 cm) in the entrance of each arm that the rats' whiskers touch when entering the arms. The sandpapers had varying grades (Group A:2% [P800], 5% [P400], 10% [P150], and Juvenile [P1200], Group B: 2% [P150], 5% [P1200], 10% [P800], and Juvenile [P400]), following the findings of Guić-Robles et al. (1989) These authors have demonstrated that rats' whiskers can discriminate between sandpapers with 200 and 25 grains/cm^2. To record the ultrasonic vocalizations (USVs), four ultrasonic microphones (Condenser Microphone CM16/CMPA, Avisoft Bioacoustics, Glienecke, Germany) were positioned via a microphone stand to approximately 20 cm on the right side of each reward dish and the restrainer (see **Figure 1A**).

Social-Sucrose Preference Test Design (SSPT)

Behavioral testing on the SSPT included three phases (see **Figure 1B**). In all phases of this study, experimental animals started the trials from the neutral zone facing not toward targeted

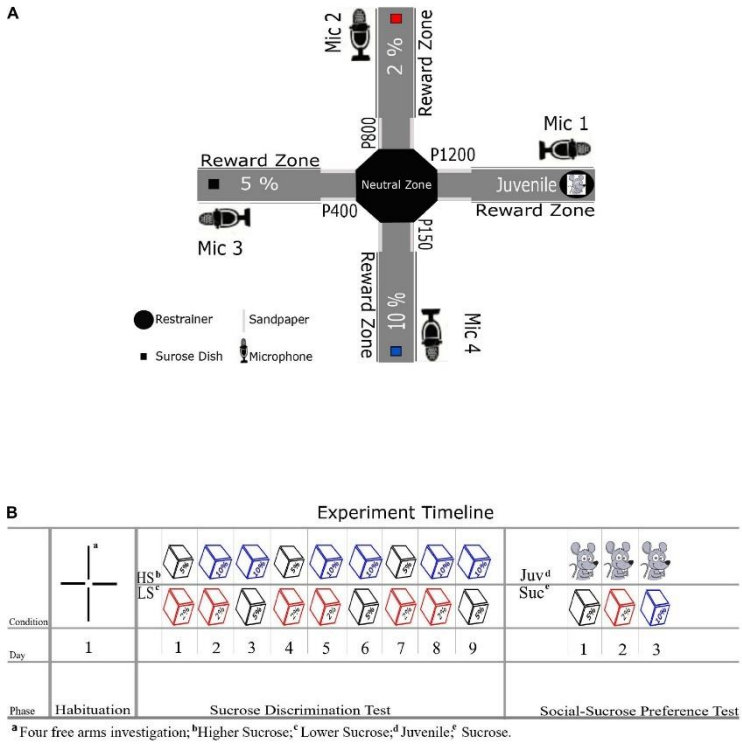

FIGURE 1 | (A) Schematic diagram of XCST setup with non-social reward positions, the restrainer for the juvenile social reward, sandpaper positions, and microphones. Every arm was assigned to a specific reward throughout the experiment. **(B)** Shows the experiment timeline for different phases, days, and conditions—the cubes represent the sucrose in different concentrations.

arms in given condition. In the first habituation phase, all four arms were open and unbaited, and each experimental animal explored the maze for 10 min. This phase aimed to find out whether animals were inherently biased toward selecting one specific reward zone or sandpaper (see **Figures 2A,B**). The second phase of training was the Sucrose Discrimination Test (SDT), which was implemented to verify that the experimental animals could indeed distinguish among the three selected sucrose concentrations (2, 5, and 10%). Food deprived animals were tested on the SDT phase over 9 days in three repetitions of three different conditions. In each condition, only two arms were open, and rats chose to allocate their time between rewards on the maze in the following order of conditions: *2% vs. 5%*, *2% vs. 10%*, and *5% vs. 10%*. Notably, each animal was tested in only one condition each day. Each test trial took 10 min; during this time, experimental animals could move freely in the two open arms and drink up to 20 ml sucrose solution per plastic dish at the end of each arm. Both dishes were filled with fresh sucrose solution for each new trial/experimental animal. After passing the SDT phase (**Figure 2C**), the experiment was continued to the SSPT phase. In this phase, over each trial with a duration of 10 min, the experimental animal could similarly move freely between two open arms: either to explore the arm baited with sucrose, or to investigate the Juvenile rat in the restrainer at the end of the Juvenile arm. Animals were tested once per day in three conditions (Juvenile vs. 2%, Juvenile vs. 5%, and Juvenile vs. 10%) spread out over the three SSPT testing days (see **Figure 1B**). To keep baseline motivation equal for both types of reward (social vs. non-social), food deprivation was stopped after the final SDT test day, and animals were allowed to recover weight over 2 days before starting the SSPT. For the remainder of the experiment, animals were kept *ad libitum*. Rats usually spend more time exploring novel conspecifics than familiar ones

(Smith et al., 2015, 2017), suggesting that the value of social interaction dynamically decreases over days with increasing familiarity with the conspecific. To keep the novelty, and, hence, the value of investigation of the social stimulus similar across testing sessions, three different Juvenile rats were used in all three conditions of SSPT for each experimental animal. The order of the identities of these Juveniles was counterbalanced across experimental animals to exclude identity effects. All USVs from all trials over the two phases (SDT and SSPT) were recorded for the full 10-min trial duration, with the sampling rate set at 250 kHz.

Behavioral Analysis: Video-Tracking

For the recorded videos from all sessions, Ethovision (EthoVision XT version 11.5, Noldus) was used to track the animals' position. Tracking settings were optimized separately for each different phase of the study (Habituation, SDT, SSPT). In the habituation phase, each arm was divided into two zones (Sandpaper zone and Reward zone) to check for any inherent bias for the different reward zones and sandpaper zones. For the SDT and SSPT phases, we used the time that the animals spent in the reward zones (see reward zones; **Figure 1A**). The time spent in the neutral zone was excluded from the analysis.

Ultrasonic Vocalization Recording, Labeling Procedure, and Synchronization

Acoustic analysis of the USVs was executed using the software Avisoft-SASLab Pro (Version 5.2, Avisoft Bioacoustics, Berlin, Germany). Spectrograms were generated with a fast Fourier transform (FFT)-length of 512 points and an overlap of 75% (Flat Top window, 100% frame size). Correspondingly, spectrograms had a frequency resolution of 390 Hz and a time resolution of 0.64 ms. In the setup, we recorded the USVs through 4 microphones, providing a four-channel spectrogram recording. The amplitude of the USVs differed depending on the distance between the animal and the different microphones (**Supplementary Figure A**). The microphone channel that recorded the largest amplitude was selected for labeling for each USV in the spectrograms. This channel differed between the conditions and minutes of the trial. The labeling phase was conducted by two trained, independent scorers who labeled and classified each USV based on its sonographic features (as in Wright et al., 2010). Notably, in the SSPT phases, calls could be emitted by both the experimental animal and the Juvenile social stimulus. In these analyses, we did not attempt to tease apart the source of these vocalizations but instead rely on within-subject comparisons of experimental animals to quantify differences.

The labeling phase consisted of two steps: calibration and final labeling. During the first step, two scorers became familiar (under the supervision of the expert scorers) with sonographic features of each of the 50 kHz USV subtypes (and 22 KHz) according to the classification suggested by Wright et al. (2010; for an overview of the different USV subtypes considered in this study, **Figure 3F**). They initially labeled USVs together to reach a consensus labeling scheme. After this calibration step, they separately labeled the same 400 USVs and, subsequently, compared their labeling match. In total, inter-rater reliability was high (Cohen's kappa = 0.95), such that 94.3% of 50 kHz USV's subtypes were labeled with the same category by both scorers. Due to technical problems, the USV files of the condition *2% vs. 5%* and some animals (1,10,11,12) from the SSPT task were lost. Therefore, for all USV related statistical analyses, we only applied the USVs from 8 animals for both tasks. Thirty-two trials from SDTs' phase, including 2 days (2 and 3) for conditions (*2% vs. 10%* and *5% vs. 10%*), were labeled. For the USVs from the SSPT phase, the recordings from all three test days (N = 24 recordings in total) were labeled. Both scorers tagged half of all USVs from the same conditions (every odd minute of each trial).

USV Call Production Definition and Behavior-USV Synchronization

When labels were assigned in Avisoft, through the self-written code in python, we exported the USV raw data (Avisoft SAS-Lab Pro's output) to generate a time series of vocalization labels with a temporal resolution of 25 Hz, synchronized to the video stream and position data (Ethovision output). Thus, each 0.040 ms sample had a one-hot encoded binary label, corresponding to the presence/absence of each of the 50 kHz subtypes, 22 kHz or background/noise. We first looked at the summed frames spent vocalizing, including all rats, to establish inclusion/exclusion criteria. The 22-kHz USVs accounted for 23.3% of all samples with USVs, counted in ms spent vocalizing. This high proportion of 22 kHz frames is mainly caused by the naturally longer length of a 22 kHz USV compared to the length of a 50 kHz call. As the main goal of this experiment only covers the 50 kHz calls, no further analysis was conducted on the 22 kHz calls. **Figure 3E** shows the inter-individual variation in USV production, warranting a within-subjects approach that includes normalization to correct these inter-individual differences in calculating group contrasts (see below). During the labeling phase, 3.9% of all call frames could not be clearly labeled in any of the 14 categories of 50 kHz subtypes. These USVs with varying sonographic features were called Unclear (Un, and **Supplementary Figure B**) and excluded from USVs within-between analyses. After labeling all 50 kHz USVs, six subtypes (Step-Down, Inverted-U, Step-Up, Multi-Step, Downward Ramp, and Upward Ramp) were excluded because of their small incidence (<2% of all call frames [an arbitrary cut-off]). The selected call subtypes were thus: Trill, Flat, Complex, Composite, short, Flat-Trill-combination, Split, and Trill-with-Jump.

Statistical Analyses

Behavioral Analyses

To rule out any spatial biases for or against some arms over others in the maze, independent of the reward contingencies, we applied independent samples t-tests to check for differences

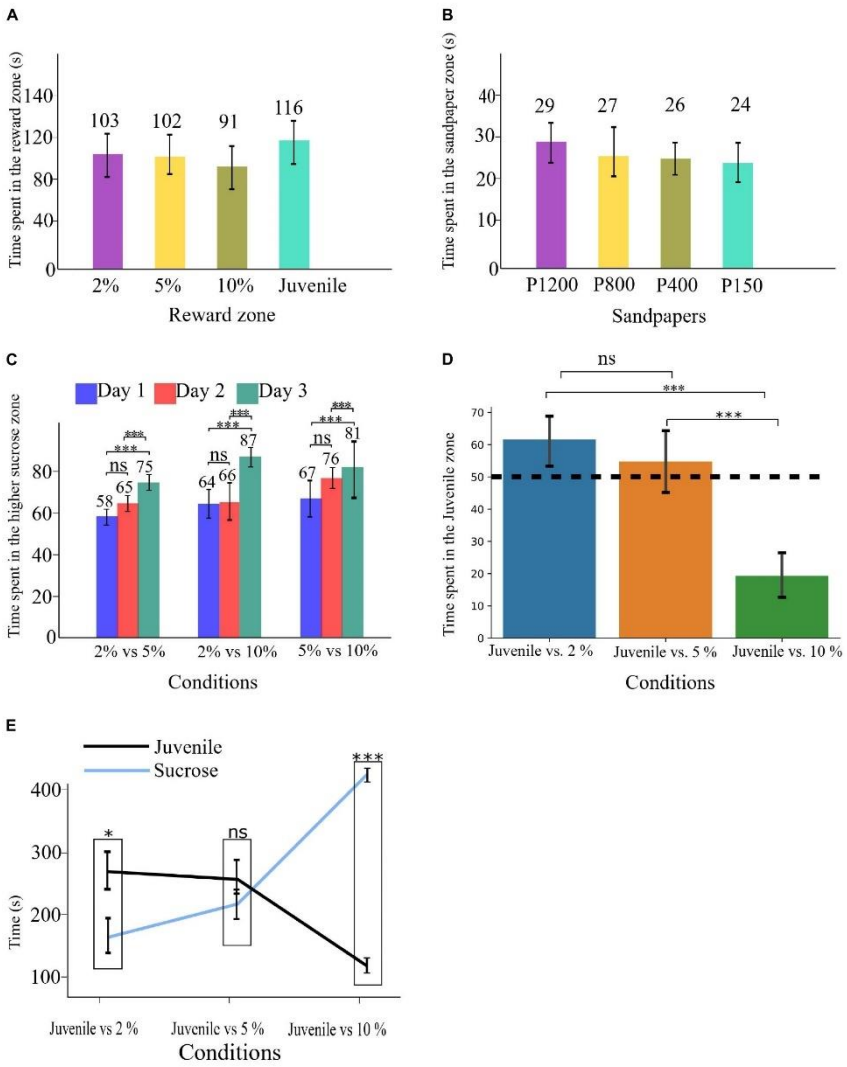

FIGURE 2 | (A) Time spent in each reward zone during the Habituation phase. **(B)** Time spent in each sandpaper zone during the Habituation phase. **(C)** Time spent in the higher sucrose zone in all three conditions of SDT. **(D)** time spent in the Juvenile zone in all three conditions of SSPT, the dashed line shows the 50% point. **(E)** Absolute time spent in each reward zone for all three conditions. All error bars show the standard deviation. $^{*}p < 0.05$, $^{***}p < 0.001$.

in time spent in each reward zone between groups A and B. To check for spatial bias related to any inherent preference for the different reward zones and sandpapers, we performed a repeated-measures ANOVA to assess the effect of sandpaper type and reward zones as independent variables (IVs) on the time animals spent in each reward and sandpaper zone during habituation to the maze, when rewards were not yet introduced. To find out whether rats discriminated between

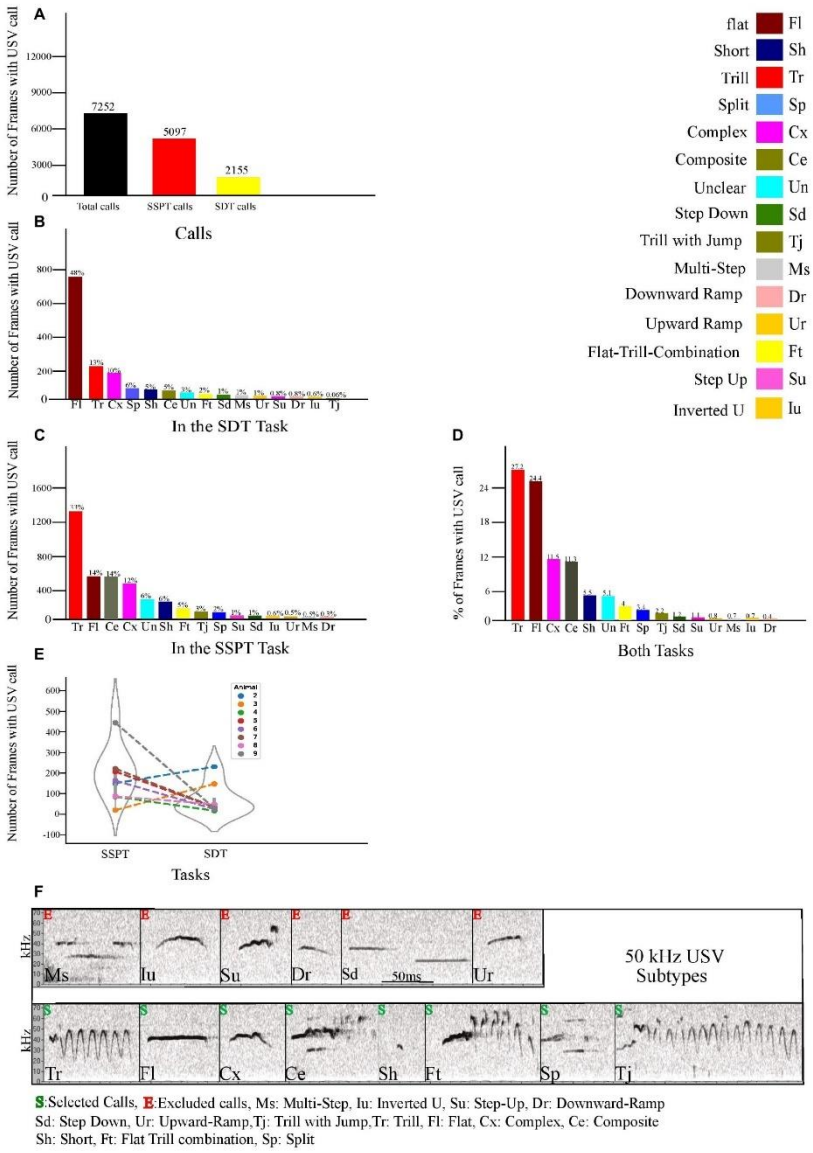

FIGURE 3 | (A) Number of frames animals vocalized in both tasks and separately during each task. **(B)** Number of call frames for each distinct 50 kHz subtype in the SDT task. **(C)** Number of call frames of each 50 kHz subtype in the SSPT. **(D)** Percentage of each subtype vocalized in both tasks. **(E)** Number of call frames per animal per task averaged over all three conditions. **(F)** Examples of the fourteen 50 kHz USV Subtypes (labeled according to Wright et al., 2010). Subtypes are marked with S (selected) or E (excluded, see text).

different sucrose levels in the SDT, first, we calculated the SDT sucrose solution preference score for each day/condition in the SDT as a percentage of time spent with the higher sucrose (**Figure 2C**).

$$\text{SDT sucrose preference score} = \frac{\text{Time spent in high sucrose reward zone}}{\left(\text{Time spent in high sucrose reward zone} + \text{Time spent in low sucrose reward zone}\right)} * 100$$

with these sucrose preference scores, we conducted a two-way repeated-measures ANOVA with the condition (three levels: *2% vs. 5%*, *2% vs. 10%*, and *5% vs. 10%*) and task repetition day (three levels: days 1, 2, 3) as (IVs) and % time spent in the higher sucrose zone as dependent variable (DV) (**Figure 2D**).

Similarly, for the SSPT task, first, we calculated a Juvenile preference score,

$$\textit{SSPT}\text{ Juvenile preference score} = \frac{\text{Time spent in the Social Reward zone}}{\left(\text{Time spent in the Social Reward zone} + \text{Time spent in the Non-social reward zones}\right)} * 100$$

and used this Juvenile preference score to run a repeated-measures ANOVA to detect any differences in Juvenile preference as a function of sucrose concentration (*Juvenile vs. 2%*, *Juvenile vs. 5%*, and *Juvenile vs. 10%*). To find out if animals preferred a particular reward type over the other in each condition, we analyzed their preference by applying a paired samples *t*-test. Finally, regarding the design of the maze, animals could also spend their time in the Neutral zone, as SSPT Juvenile preference score only considered the percentage of the time animals spent in reward zones, in order to know whether animals spent different time for a particular reward (either Social or Non-social) over the three conditions, we conducted a two-way repeated-measures ANOVAs with Conditions and Zone as IVs and absolute time spent per reward zone as DV, and performed *post hoc* paired-sample *t*-tests to compare the absolute time spent between zones per condition. For all statistical analyses, the significance level was $p < 0.05$, and all the *post hoc* tests *p*-values were Bonferroni-corrected for multiple comparisons.

Vocalization Analyses

Our initial analysis focused on a Combined vocalization score (CVS), including all 15 subtypes (Including Un and excluding only the 22 kHz) per session to look for overall differences in vocalization rates between conditions. Here, we first summed up all frames the rats vocalized for each of the 15 subtypes in a certain zone and then divided that score by the time the animal spent in that zone, thus normalizing the vocalization time to the occupation time per zone, creating a normalized vocalization rate. As inter-individual differences resulted in a skewed distribution of normalized vocalization rates, we performed a log transformation on these CVSs to reduced skewness and facilitate visualization. To investigate if the number of vocalizations differed depending on the reward type (social vs. non-social) or sucrose concentration, we applied a two-way repeated-measures ANOVA for each task (SDT and SSPT) separately. Here, we considered the condition with two levels for SDT (conditions: 2 vs. 10% and 5 vs. 10%), three levels for SSPT (*Juvenile vs. 2%*, *Juvenile vs. 5%*, and *Juvenile vs. 10%*), and two levels reward zone (SDT: higher/lower sucrose and SSPT: Juvenile/Sucrose) as IVs, and the log of the CVS of each task as DV.

To zoom in to differences between subtypes, we performed a similar analysis pipeline per subtype: after excluding the 22 kHz, Un, and infrequent call subtypes (excluded calls), for the remaining eight categories, we again normalized the subtype-specific vocalization rate to the spatial occupancy per zone to calculate a subtype vocalization score (SVS). This SVS was thus calculated by summing the number of frames the rat vocalized a specific subtype (1 frame = 0.040 ms) in a given zone and dividing it by the time the animal spent in that zone.

As a within-subjects normalization step, from these SVSs, we calculated a delta SVS score to show the differences in vocalization rate between zones for a given subtype. The delta SVS score was calculated as follows: i) SVS score in the low sucrose zone subtracted from the SVS score in the high sucrose zone for SDT and ii) SVS score in the non-social reward zone subtracted from the SVS score in the social reward zone for SSPT. We used this deltaSVS to compare normalized vocalization rates between subtypes in a given condition (between-subtype analyses) and within a subtype, between conditions (within-subtype analyses).

In the between-subtype analysis, with these dSVS, we ran a Kruskal Wallis test per condition for the SDT and SSPT data, with the subtype as the IV and the dSVS score as DV for each condition.

In the within-subtype analysis, we performed a Wilcoxon Signed-Rank test for the SDT sessions, comparing the vocalization of the given subtype in two conditions [*2% vs. 5%* and *5% vs. 10%*]) and a Friedman test for each subtype across the three SSPT conditions (*Juvenile vs. 2%*, *Juvenile vs. 5%* and *Juvenile vs. 10%*). For all statistical analyses, the significance level was set at $p < 0.05$, and all the *post hoc* tests *p*-values are Bonferroni- corrected for multiple comparisons.

Mixed Linear Model Analyses

To exploit the continuous range of sucrose solutions used in the SSPT, to look for a linear association between vocalizations and sucrose solution, we conducted two mixed linear models, one on total calls (CVS) and one on subtype-specific SVS. Both models entered Animals as random effects, Conditions (2% vs. Juvenile, 5% vs. Juvenile, and 10% vs. Juvenile) as fixed effects, and CVS/dSVS as the dependent variable.

Software

All statistical analyses were carried out using SPSS Statistics (version 24; IBM, United States) and R 3.5.1 (R Core Team, 2018). We applied the following libraries in R: the tidyverse (Wickham, 2017), the haven psycho, the readxl (Wickham et al., 2019b), the tidyr (Wickham et al., 2019a), the tibble (Wickham et al., 2019a), the sjplot (Lüdecke, 2020) the ggstatsplot (Patil, 2018) and the rockchalk (Johnson and Grothendieck, 2019). Moreover, visualizations of some figures (**Figure 2D** and **Supplementary Figure D**) were made using Jupyter Notebook (Kluyver et al., 2016) through the packages matplotlib (Hunter, 2007), pandas (McKinney, 2010), and seaborn (Waskom, 2021). Remaining figures were created by Inkscape (version 0.92.1, Inkscape project, 2020). In order to run the synchronization of USV and Animals' positions we used the packages fileinput (Sinha, 2017) numpy (Harris et al., 2020).

RESULTS

Behavior

A between-group comparison did not find evidence for a difference in spatial/reward preference based on the maze layout for groups A and B (**Supplementary Figures CA,CB**). Similarly, an analysis of the habituation period did not find any evidence for a preference for a specific zone of reward [$F(3, 33) = 1.35$, $p > 0.05$; **Figure 2A**] or sandpaper zone [$F(3, 33) = 1.6$, $p > 0.05$; **Figure 2B**].

SDT. To determine whether experimental animals could indeed discriminate between different sucrose concentrations (i.e., 2, 5, and 10%), we conducted a two-way repeated-measures ANOVA with task condition and task repetition day as within-subject factors and percentage of the higher sucrose reward as DV. We found no significant main effect of task condition, suggesting that animals did not significantly differ in their preference for the sweeter sucrose solution across sessions with different levels of sucrose concentrations. We did observe a significant main effect of day [$F(2, 22) = 15.2$, $p < 0.001$, $\eta_p^2 = 0.581$]. *Post hoc* analysis revealed that animals preferred the higher-percentage sucrose solution significantly more in all conditions on day three ($M = 81.7$, $SE = 2.8$) compared to day two ($M = 69.1$, $SE = 2.7$, $p < 0.05$, $d = 4.6$) and day one ($M = 63.3$, $SE = 2.7$, $p < 0.001$, $d = 6.8$). The data thus showed that animals develop a clearer preference for the sweeter sucrose solution over days (**Figure 2C**), probably as a consequence of learning. There was no significant interaction effect.

SSPT. To assess whether animals expressed a significant preference between social and non-social rewards (with three different sucrose concentrations) in the social-sucrose preference test (SSPT), we conducted a one-way repeated-measures ANOVA on the percentage of time spent with the social reward (Juvenile zone). The results showed that preferences for the Juvenile differed significantly between conditions [$F(2, 22) = 52.2$, $p < 0.001$, $\eta_p^2 = 0.826$]. *Post hoc* tests revealed that the animals' preference for the Juvenile increased significantly from the condition *Juvenile vs. 10%* (juv. pref: $M = 19\%$, $SD = 10\%$) condition to the *Juvenile vs. 5%* (juv. pref: $M = 55\%$, $SD = 15\%$, $p < 0.001$, $d = 12.2$) condition. There was a further but non-significant increase in Juvenile preference when reducing the sucrose concentration to 2%; in this condition, Juvenile preference was also significantly higher than in the *Juvenile vs. 10%* condition (juv. pref: $M = 61\%$, $SD = 13\%$, $p < 0.001$, $d = 9.4$). Three one-sample *t*-tests vs. indifference (50%) showed that animals preferred the social reward in Juvenile vs. 2% [$M = 61.5$, $SD = 13$, $t(11) = 3.06$, $p < 0.05$], were indifferent between Juvenile vs. 5% [$M = 54.7$, $SD = 15$, $t(11) = 1.08$, $p > 0.05$] and preferred the sucrose reward in Juvenile vs. 10% [$M = 80.6$, $SD = 10$, $t(11) = 9.9$, $p < 0.001$]. These results show clearly that animals indeed traded off interacting with a Juvenile to the consumption of sucrose and also that a preference for interacting with the Juvenile when sucrose levels were low (2%) could be reversed when confronted with a more preferred 10% sucrose solution (**Figure 2D**). These between-condition differences could be due to a change in time (%) spent at the sucrose reward, the social reward, or both. To quantify this, we investigated if the absolute time animals spent in each reward zone differed between different conditions. A repeated-measures ANOVA on the absolute time animals spent on social reward showed a significant effect of conditions [$F(2, 22) = 33.2$, $p < 0.001$, $\eta_p^2 = 0.751$]. *Post hoc* tests revealed that the absolute time that animals spent in the Juvenile zone in the condition of *Juvenile vs. 10%* ($M = 97.7$, SD = 55) was significantly less than in the condition *Juvenile vs. 5%* ($M = 250$, $SD = 76$, $p < 0.001$, $d = 2.2$) and the condition *Juvenile vs. 2%* ($M = 259$, $SD = 64$, $p < 0.001$, $d = 2.7$). There was no significant difference between the condition *Juvenile vs. 2%* and *Juvenile vs. 5%*. A second repeated-measures ANOVA on the absolute time animals spent with non-social rewards also showed a significant effect of the condition [$F(2, 22) = 74.7$, $p < 0.001$, $\eta_p^2 = 0.872$]. Here, *post hoc* tests revealed that the absolute time that animals spent in the sucrose zone in the condition *Juvenile vs. 10%* ($M = 408$, $SD = 19$) was significantly more than the condition *Juvenile vs. 5%* ($M = 205$, $SD = 20$, $p < 0.001$, $d = 10.4$) and the condition *Juvenile vs. 2%* ($M = 159$, $SD = 15$, $p < 0.001$, $d = 14.5$). No significant difference was found between the conditions *Juvenile vs. 2%* and *Juvenile vs. 5%*. As a follow-up analysis, a paired sample *t*-test per condition revealed that in Juvenile vs. 2%, the Juvenile side ($M = 259$, $SD = 64$) was significantly ($p < 0.05$, $d = 1.7$) preferred over the sucrose side ($M = 159$, $SD = 53$). In the condition *Juvenile vs. 5%*, animals were indifferent between the reward types (Juvenile: $M = 250$, $SD = 76$; 5% sucrose: $M = 205$, $SD = 71$). In contrast, in the condition *Juvenile vs. 10%*, the sucrose side ($M = 408$, $SD = 67$) was preferred significantly ($p < 0.001$, $d = 5.6$) over the Juvenile ($M = 97$, $SD = 55$) (see **Figure 2E**).

Characterization of USV

As indicated in "Materials and Methods" section, the 50 kHz USVs produced by experimental animals in the SSPT were labeled and further categorized into subtypes. Descriptive statistics were generated for each of the subtypes included in our analyses, along with within-condition and between-condition comparisons. We found that rats emitted vocalizations in a total of $N = 7{,}252$ call frames (290 s, combined SDT, and SSPT, 2.4% of total recorded frames, **Figure 3A**). After exclusion of 22 kHz

calls, based on prevalence, we selected eight subtypes: Trill (Tr), Flat (Fl), Complex (Cx), Trill-with-Jump (Tj), Short (Sh), Flat-Trill-combination (Ft), Split (Sp), and Composite (Ce) for further analysis (**Figure 3F**). Six subtypes (Step-Down, Step-Up, Upward Ramp, Multi-Step, Inverted-U, Downward Ramp) were excluded from analysis due to their limited occurrence (<2% of calling time, **Figure 3D**). From the selected subtypes, Tr (27.2%), Fl (24.4%), Cx (11.5%), and Ce (11.3%) were the most prevalent, while Sh (5.5%), Ft (4%), Sp (3.4%), and Tj (2.2%) were least prevalent in both tasks (**Figure 3D**). Notably, we found Un calls (3% in the SDT task, **Figure 3B**) and (6% in the SSPT task, **Figure 3C**. For more details about Un calls, see section "Materials and Methods").

SDT. In total, throughout the SDT, 2155 call frames were found in which the rats were vocalizing, and after exclusion of 22 kHz calls, from the eight selected subtypes, Fl (48%), Tr (13%), Cx (10%), Sp (6%), Sh (5%), and Ce (5%) were most prevalent while, Ft (2%), and Tj (0.06%), were least prevalent in SDTs' conditions (**Figure 3B**). **SSPT.** In total, in the SSPT, 5097 call frames were found in which the rats were vocalizing, and after exclusion of 22 kHz calls, from these eight selected subtypes, Tr (33%), Fl (14%), Ce (14%), Cx (12%) were most prevalent while Sh (6%), Ft (5%), Tj (3%), and Sp (2%) were least prevalent (**Figure 3C**) in SSPTs' conditions.

Analysis of Total USVs

To determine if the number of frames that the rat vocalized was affected by sucrose concentration or type of rewards in the different conditions, we conducted a two-way repeated-measures ANOVA on the Combined vocalization score (CVS; the number of frames vocalized relative to the time spent in the visited zone, see section "Materials and Methods") with condition and reward zone as factors, separate for SDT and SSPT.

SDT. The SDT analyses found a significant effect of condition on the CVS [$F(1, 7) = 14.9$, $p < 0.01$, $\eta_p^2 = 0.680$]. The main effect showed that the CVS was significantly higher in the condition *2% vs. 10%* ($M = 0.310$, $SE = 0.075$) than in the condition *5% vs. 10%* ($M = 0.128$, $SE = 0.054$; **Figure 4A**). The factor reward zone also had a significant effect on the CVS [$F(1, 7) = 14.3$, $p < 0.01$, $\eta_p^2 = 0.672$; **Figure 4B**]. The main effect showed that the CVS was, surprisingly, higher ($p < 0.01$) in the lower sucrose concentration zone ($M = 0.268$, $SE = 0.065$) compared to the higher sucrose concentration zone ($M = 0.171$, $SE = 0.058$). There was also a significant interaction effect of conditions and reward zones [$F(1, 7) = 5.9$, $p < 0.05$, $\eta_p^2 = 0.459$; **Figures 4C,D**). *Post hoc* comparisons showed that CVS was higher in lower-reward zones only for the condition *2% vs. 10%*. In the zone of lower sucrose concentration ($M = 0.407$, $SE = 0.093$) the animals had a higher CVS ($p < 0.05$) than the condition *5% vs. 10%* ($M = 0.213$, $SE = 0.064$, see **Figures 4A,B**). ***SSPT.*** For the SSPT task, we again performed a two-way within-subjects repeated-measures ANOVA. There was no significant effect of condition (**Figure 4E**), but we found a significant effect of reward type [$F(1, 7) = 13.6$, $p < 0.01$, $\eta_p^2 = 0.658$, **Figure 4F**]. *Post hoc* comparisons showed that the CVS was significantly higher in the Juvenile zone ($M = 0.544$, $SE = 0.075$) than in the sucrose zone ($M = 0.313$, $SE = 0.067$; $p < 0.01$). Furthermore, there was a significant interaction between condition and reward types [$F(2, 14) = 5.1$, $p < 0.05$, $\eta_p^2 = 0.426$, **Figures 4E–I**]. *Post hoc* comparisons showed that animals' CVS in the Juvenile vs. 10% condition was significantly higher ($p < 0.01$) in the Juvenile zone ($M = 0.685$, $SE = 0.121$) compared to the sucrose zone ($M = 0.297$, $SE = 0.064$). No significant differences in CVS between reward zones were found for the Juvenile vs. 2% ($p = 0.06$) and Juvenile vs. 5% conditions ($p = 0.07$).

These results already indicate an interesting finding: while behavioral preferences shifted toward the sucrose reward zone with higher sucrose concentration, the vocalization rate showed the opposite trend, with increasing vocalizations recorded in the juvenile zone with increasing sucrose concentrations. We next investigated whether this pattern was present for specific subtypes and if there were differences between subtypes.

Comparing USV subtypes between and within conditions.

Between-Subtypes Analyses

As one of the main questions of this study, we were interested in finding out if the different sucrose concentrations or different reward types were associated with a different vocalization palette across the 50 kHz USV subtypes. Here, we used the delta Subtype Vocalization Score (dSVS; see section "Materials and Methods"), indexing the relative difference in vocalization rates between reward zones in a given session for these analyses, as it accounts for normalization of inter-individual differences in absolute call rates.

SDT. We conducted a Kruskal Wallis test separately for each condition (*2% vs. 10%* and *5% vs. 10%*) by taking the eight subtypes observed in the SDT as a factor and their dSVS as the dependent variable (DV). We found no significant difference in the dSVS between subtypes for any condition (**Supplementary Figure D**). ***SSPT.*** We similarly conducted a Kruskal Wallis test for each condition (*Juvenile vs. 2%*, *Juvenile vs. 5%*, and *Juvenile vs. 10%*). In the condition *Juvenile vs. 5%*, we found a significant difference [H (7) = 16.6, $p < 0.05$]. *Post hoc* pairwise comparisons showed a significant difference between dSVS of the subtypes Tr (median = 0.3) and Fl (median = −0.04), (Mann-Whitney U-test, $p < 0.01$) and dSVS of subtypes Tr and Sp (median = 0, $p < 0.05$; **Figure 5**).

Within Subtype Analyses

SDT. this analysis was conducted to determine whether dSVS for a given subtype differed between conditions. The Wilcoxon Signed-Rank test results showed that the dSVS score of Tr was lower in condition *2% vs. 10%* (median = −0.4) than in condition *5% vs. 10%* (median = 0), $Z = 2.1$, $p < 0.05$). There was no other significant difference within any subtypes between conditions (**Figure 6**).

Mixed Linear Model Analyses

For the within-subtype analysis of call rates in the SSPT, we exploited the continuous nature of the sucrose concentration in a mixed linear model, estimating the relationship between sucrose concentration (in %) and dSVS with individual animals modeled as random effects. We first modeled the total call rate (all calls combined) using the Combined vocalization score (delta

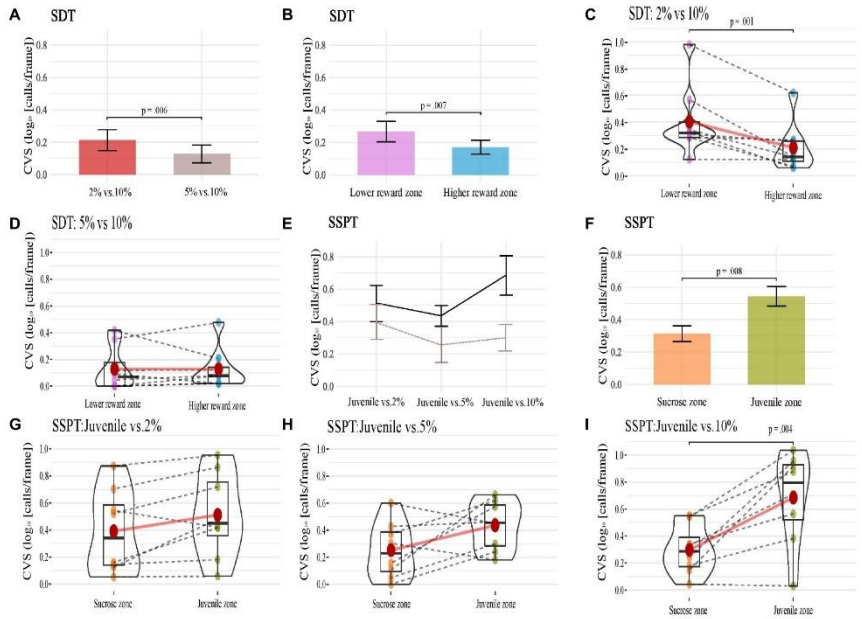

FIGURE 4 | (A–D) Present animals' CVS in conditions **(A)**, reward zones **(B)** of the SDT, and **(C,D)** show each animal's CVS in the two reward zones of the SDT task's two conditions. **(E–I)** show animals' CVS across three conditions (**E**; in both reward zones, black solid line; Juvenile and brown hashed line; non-social), reward zones **(F)** of the SSPT and **(G–I)** show each animal's CVS in the two reward zones of the SSPT task's three conditions. Error bars indicate Standard Error for **(A,B,E,F)**.

CVS; see section "Materials and Methods"). The mixed linear model showed a linear association between the delta CVS and the sucrose level (beta = 0.034, 95% CI [0.01–0.06], $t(15) = 3.27$, $p < 0.01$, R2 fixed effect = 0.208). This suggests that the difference in total vocalization time in the Juvenile over the Sucrose zone significantly *increased* with higher levels of sucrose concentration (see **Figure 7A** and **Supplementary Table 1**). We then modeled the sucrose concentration to delta SVS relationship in linear mixed models separately for each subtype. The models showed a significant association for the subtypes Tr (beta = 0.18, 95% CI [0.05–0.031], $p < 0.05$) and Ce (beta = 0.07, 95% CI [0.01–0.013], $p < 0.05$). This means that, for these two subtypes, the difference in the number of frames vocalized in the Juvenile over the Sucrose zone significantly increased with higher levels of sucrose concentrations (see **Figure 7B** and **Supplementary Tables 2A,B** for more individual model statistics).

DISCUSSION

Communication is essential for social animals, and rats are no exception. Rats utilize vocalizations in the ultrasonic range to communicate with their conspecifics. However, whether these vocalizations differ in response to different rewards when presented together and whether vocalizations quantitatively index reward magnitude remained mostly unexplored.

Here, we presented a paradigm to test preferences for two different reward types head-to-head in distinct spatial locations on a four arm-maze. We simultaneously quantified social vs. non-social reward value through relative reward zone time allocation and reward type preference profiles by estimating slopes over three clearly discriminable (**Figure 2C**) non-social reward values (sucrose concentrations). Rats, indeed, changed their time allocation over reward sites as a function of reward sucrose concentration (**Figure 2E**) and even exhibited preference reversals, switching from preferring social interaction when it competed with 2% sucrose to preferring sucrose consumption when its concentration was upped to 10%. This change in behavioral preference and time allocation could be exploited to estimate the association between different 50 kHz USV subtypes and social vs. non-social reward, controlling for individual differences in overall vocalization rate and variance in time spent at each reward site (**Figure 4**).

We found that, when controlling for occupancy and individual differences in this way, the overall difference in vocalization rate between social and non-social reward sites (dCVS; normalized

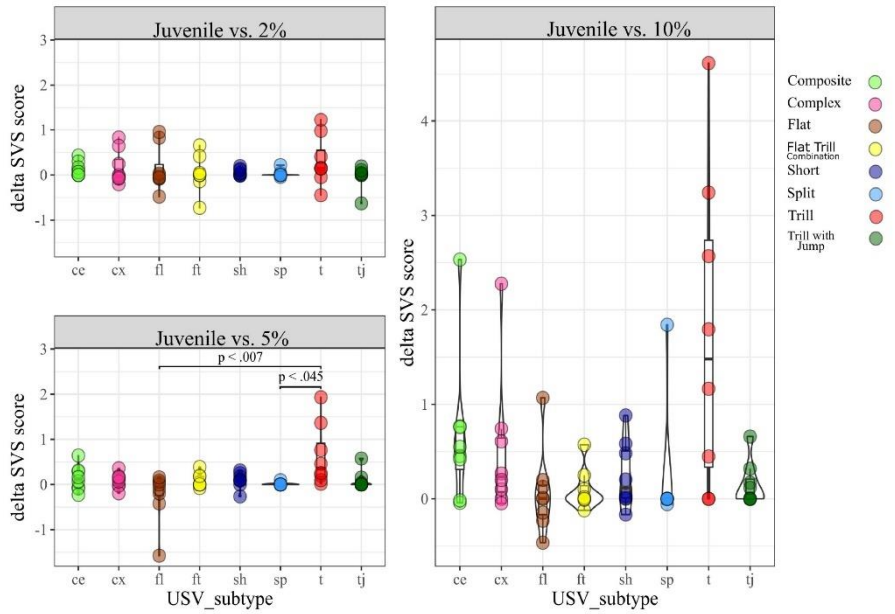

FIGURE 5 | dSVS for each subtype, split by conditions in the SSPT.

vocalization rate social minus non-social) increased from 2 to 5 to 10% sucrose conditions, as estimated with a linear model, suggesting that animals vocalized *more* in the social zone even though the experimental animals spent *less* time in the social side when the alternative was a high-sucrose solution. The vocalization rate was not purely determined by appetitive sucrose consumption either, as witnessed by the dramatic reduction in call rate in the SDT conditions, even though animals exhibited comparable levels of sucrose consumption and behavioral preferences. As several studies already showed, 50 kHz USV calls are emitted during various appetitive states (Brudzynski and Zeskind, 2018), such as sucrose consumption and social play (Browning et al., 2011). Therefore, we hypothesized that, in the SDT task, more calls would be emitted in the *5% vs. 10%* condition than the *2% vs. 10%* condition (overall more sucrose) and that a higher percentage of calls would be scored in the higher sucrose zone in both conditions. Both hypotheses were *rejected*, however, as the rats vocalized significantly *more* in the *2% vs. 10%* condition, controlling for occupancy and more calls we found in the *lower sucrose* zone in both conditions.

These findings, thus, rather support a view of USVs as a context-dependent communicative device aimed perhaps at establishing/inviting social contact compared to the alternative hypothesis that casts USVs as (static) epiphenomena of reward value linked to the consumption of social contact or non-social rewards. Many researchers have pointed to the associations between the various 50 kHz USV subtypes and certain types of overt behavior (Wöhr et al., 2008; Wright et al., 2010; Mulvihill and Brudzynski, 2018a,b). When we zoomed in to the level of the various 50 kHz subtypes, we found that in our experiments, eight subtypes (Tr, Fl, Cx, Tj, Sh, Ft, Sp, and Ce) were vocalized much more prevalently than the other remaining subtypes identified by Wright et al. (Wright et al., 2010). We thus investigated whether the vocalization rate of these subtypes could be used to discriminate between Social and non-social reward-related contexts.

When considering the SDT sessions, the Flat subtype was vocalized at a much higher rate compared to the remaining eight selected subtypes (**Figure 3B**). This parallels the findings of Mulvihill and Brudzynski (2018b), who reported that non-social conditions appeared to induce a greater proportion of flat calls as well as the findings of Wöhr and Schwarting (2013), who found an association of flat 50 kHz USVs and feeding behavior. Likewise, Wright et al. (2010) also found that flat calls were more prevalent in singly-tested rats than pair-tested rats. However, in our hands, the proportion of flat calls across high- and low-reward zones (dSVS) did not differ between flat calls and the other subtypes (**Supplementary Figure D**) or across SDT conditions for flat calls (**Figure 6**), arguing against a direct, parametric association between flat calls and hedonic state.

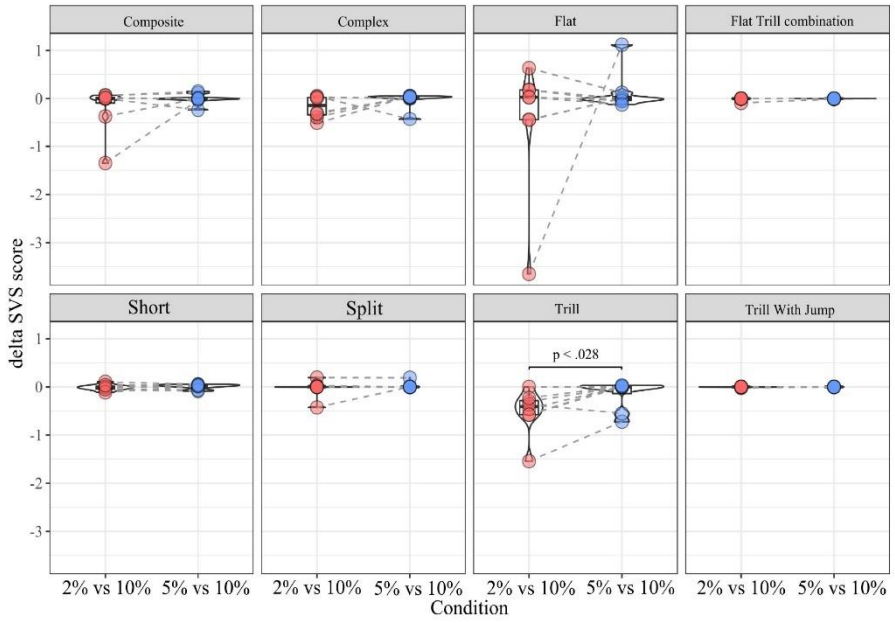

FIGURE 6 | dSVS split by subtype between conditions.

In contrast, similar to the findings of Brudzynski and Pniak (2002) and Wright et al. (2010), demonstrating that animals generally vocalize more in the presence of conspecifics, in the SSPT, our subjects also vocalized more in the social reward zone than the non-social reward zone. Moreover, sucrose levels influenced this effect as conditions with a competing higher concentration of sucrose elicited higher vocalization of 50 kHz USVs in the social zone (**Figure 7A**). This result parallels the results of Mulvihill and Brudzynski (2018a), who demonstrate that social contexts in particular conditions induce call emission more robustly. In particular, the Trill and Composite subtypes drove this effect and were produced at increasing rates in the social zone when animals were deciding between visiting the Juvenile and increasing sucrose (**Figure 7B**). This finding becomes particularly interesting when considering that animals spent more time at the *non-social* zone at higher sucrose concentration conditions (see; **Figures 2E**, **4E**). What could explain this inverse relationship between behavioral preferences and differential USV production? We offer three putative explanations:

(1) The sessions with higher sucrose concentrations induce an overall higher hedonic state that potentiates "chattiness" when the experimental animal visits the Juvenile zone.
(2) The higher sucrose content influences the breath of the experimental animal, which in turn modulates the USV production when the animals are interacting.
(3) With increasing sucrose concentration, the experimental animal shuttle more and faster between reward sites (anecdotal observations). If USV production decays exponentially with interaction time, shorter interactions yield a higher (normalized) call rate.

LIMITATIONS AND FUTURE DIRECTIONS

Adjudicating between these options will require further studies. One important limitation worth mentioning is that we utilized rats raised and tested in laboratory conditions. In a sense, our design is a drastically simplified version of what a rat might encounter in naturalistic settings. Studies such as ours aimed at elucidating the intricate patterns and subtypes of vocalizations in a micro-scale should be consolidated with field studies and naturalistic designs of rodent vocal behavior. Another important limitation of our study is that when the experimental animal was in the juvenile arm, we were unable to determine precisely whether the experimental or juvenile animal was vocalizing. Though several attempts have been made, using triangulation, microphone arrays (Heckman et al., 2017),

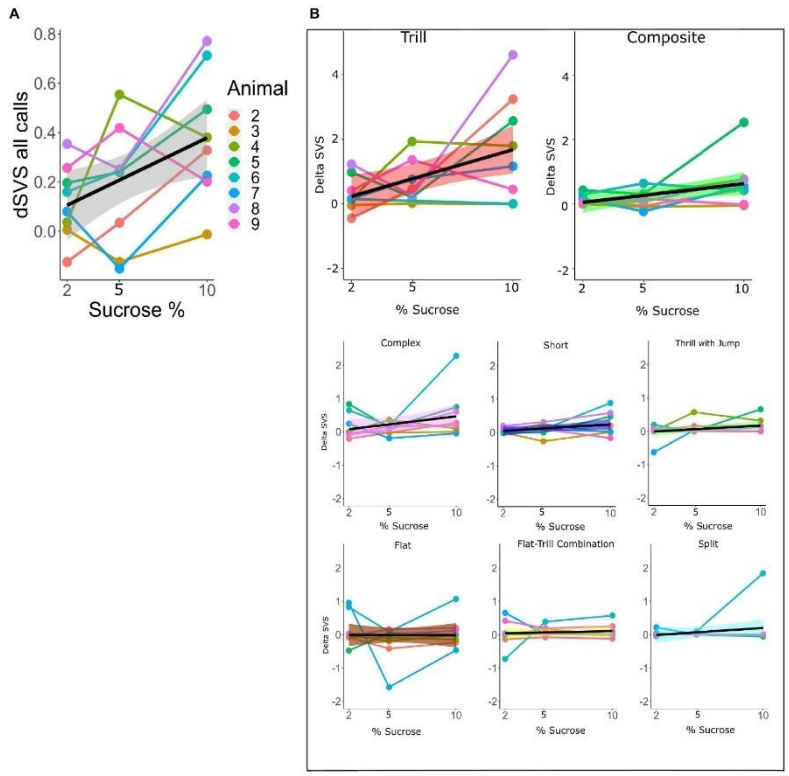

FIGURE 7 | (A) dCVS for all calls across different levels of sucrose in the SSPT. The black line (±standard error of the mean; gray shade) shows the estimated linear relationship between dSVS and sucrose concentrations across all rats. Linked dots represent individual rats, modeled as a random effect. **(B)** Each plot shows the change in dSVS of a certain subtype across three SSPT task conditions. Black lines represent the mean linear trends across all rats and (±standard error of the mean is represented by shade; colored differently for each subtype.). The slopes for Trill and Composite subtypes are significant (**Supplementary Table 2A**).

or onboard wireless EMG recordings of the larynx (Kelm-Nelson et al., 2018) to arrive at precise disambiguation of the USV source, the current setup did not allow this objective to be met in our study. Previous research has shown that, in juvenile rats, a positive correlation between the emission of 50 kHz USV vocalizations and rough-and-tumble play could be found (Knutson et al., 1998; Kisko et al., 2015), and that devocalization in the pair impacts social play (Himmler et al., 2014). In our design, most (but not all) rats increased their total vocalization from SDT to SSPT task (**Figure 3E**). Though we attribute this increase mostly to the addition of the juvenile, we still observed vocalizations with the strongest amplitude on the microphone over the non-social side (data not shown), presumably originating from the experimental animal, arguing against the vocalization originating only from the juveniles. Considering the findings of Wöhr and Schwarting (2007, 2012) that 50-kHz USV constantly gave rise to social approach behavior in juvenile and adult male rats, we interpret our finding of more USVs emitted per second spend investigating the juvenile as a corollary of the juvenile inviting social contact through vocalizations, growing stronger as the experimental animal is spending more time in the non-social zone with increasing sucrose concentration.

Taken together, our study provides a first systematic overview of behavioral preferences and vocalization patterns recorded when rats are choosing between social and non-social rewards. The underlying behavioral and/or genetic traits and the neural correlations regulating the rats' specific preferences are yet to be explored. Recent studies utilizing a combination of cutting edge genetic techniques to pinpoint neural underpinnings of rodent vocal communication (Kisko et al., 2018; Gao et al., 2019; Tschida et al., 2019) have illustrated the value of rodent

models in elucidating the social behavior and pro-social 50-kHz ultrasonic communication as models of psychiatric illness. Our results again highlight the variance in rat vocalizations between individuals and within their repertoire. Not only did the total number of USVs differ depending on the type of and level of reward, but the specific subtypes themselves showed variation between conditions and rewards, and in some cases, were predictive of the level of reward. So what is the ultimate role of the different USV subtypes? We and others propose that these USV subtypes allow rats plasticity in their vocal behavior, enabling flexible communication to respond to the (social) cues from their surroundings appropriately. The conditional probability of one subtype following another is not random (Coffey et al., 2019), suggesting the possibility of syntax, or perhaps even turn-taking in an interacting rodent dyad. Such analyses could be combined with data-driven approaches to USV categorization that include frequency and/or amplitude information and machine learning in addition to expert-based pattern recognition of USV subtypes. Creating synthetic USV sequences that could outperform random sequences in eliciting approach behavior, now used as the gold standard (Seffer et al., 2014), would indicate the importance of subtypes in a USV call structure.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the European Union Directive 2010/63/EU for animal experimentation and was approved by the local authority (Landesamt für Natur, Umwelt und Verbraucherschutz North-Rhine Westphalia, Germany).

AUTHOR CONTRIBUTIONS

MW and MS contributed to the conception and design of the study. MS executed the study, collect the data, did statistical analyses, created the figures, and wrote the manuscript. SG, NP, and IC contributed to writing R script, creating graphs, figures, and tables, and also writing the introduction and discussion. TK and MW contributed to manuscript revision. All authors approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnbeh.2021.693698/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DISCUSSION

The discussion section frames the primary findings of the three studies presented in the thesis within scientific theories that provide additional value to the individual discussions of each paper. The aim is to complement previous discussions without reiterating the main ideas. The STFP paradigm and the novel results obtained from our investigations cover a large part of the section. Subsequently, the section closes with a brief analysis of study III, followed by an integrative overview of all three studies.

1. Socially transmitted food preferences

The STFP subsection focuses on the analysis of behavioral findings from the first two studies. It begins by introducing reward revaluation, a fundamental cognitive mechanism that allows observers to update their preferences. It continues to explore the unresolved questions within the social and non-social learning theories regarding STFP outcomes and their utility in interpreting them. In addition, ecological approaches examine the conditions under which individuals prioritize social over individual information. A particularly influential factor in such decisions is whether the demonstrator is a familiar conspecific or a stranger. Collectively, these elements allow for a critical review of the principal theoretical interpretations of STFP.

1.1. Results overview

The classic version of the STFP paradigm demonstrates that the observer rat consumes more of the socially cued pellet type than the alternative after smelling its odor in the demonstrator rat's breath (Galef et al., 1984, 1988). The adapted version of the STFP paradigm carried out in the first two studies of this thesis, introduces a new phase to the experimental design, building on an earlier adaptation (Galef & Whiskin, 2008a). As an adjustment, the individual pellet preference of each observer is measured first. The demonstrator is then fed the observer's non-preferred pellet type immediately prior to the social interaction. Observer preferences are measured again on the same day of the interaction and the following day. Consequently, the observer is expected to modify its preferences after interacting with the demonstrator.

The results of our two studies using the STFP task showed that social interaction, in control and vehicle groups, led to increased consumption of the originally non-preferred pellets. In study I, the control group exhibited only a decreasing trend in the consumption of the originally preferred pellets. In study II, as said, both vehicle groups showed an increase in the consumption of the originally non-preferred pellets; however, this effect was more pronounced following interaction with an unfamiliar demonstrator than with a familiar one. In contrast, changes in the consumption of the originally preferred pellets were less consistent. Further analysis revealed a significant decrease in the consumption of the originally preferred pellets after interaction with an unfamiliar demonstrator, but not after interaction with a familiar one. The following subsections discuss this first piece of robust behavioral results concerning the control and vehicle groups (neither lesion nor OXT injection) in light of ecological, psychological, and neuroscientific theories.

1.2. Reward revaluation

Subjects assign value to the objects and stimuli they interact with (Bissonette et al., 2013; Kim et al., 2020; Malvaez et al., 2019; Rich & Wallis, 2013; Rudebeck et al., 2013). However, valuation can change over time and varies depending on internal states such as hunger, tiredness, or desire for social interaction, as well as external factors such as availability of food or changing social contacts. Thus, the brain continuously updates the value of stimuli in changing environments and modifies choices (Burke et al., 2014; Dwyer et al., 2017; Huh et al., 2009; Piet et al., 2018; S. R. White et al., 2024). Our results can be described in terms of the decision-making framework as follows: we exposed the observer rat to three unconditioned positive stimuli. These included two types of flavored pellets, which were nutritionally equivalent but differed in flavor, i.e., grape or banana, and one social stimulus matching age and sex (Trezza et al., 2011). The amount of each pellet type consumed by the observer before social interaction allowed us to measure the subjective value assigned to them. The rat's choice – the most consumed pellet type – served as the proxy of its preference (van Wingerden & Kalenscher, 2022). First, revealed individual preferences were exclusively based on their palatability, however, they were updated after interaction with the demonstrator. The decision-making

framework assumes that various sources of information such as taste, smell, and social cues are dynamically integrated into the valuation of a specific stimulus (Heyes, 2012; Levy & Glimcher, 2012; Sheppard et al., 2013). Following such a logic, associating the odor of the originally non-preferred pellet with the demonstrator rat increased the value assigned to that pellet type. Subsequent choice revealed the extent of this change in valuation. In summary, the increased consumption of the originally non-preferred pellets is likely due to the revaluation of this pellet type after integrating social information.

1.3. (Non)-social learning

Can the classic and our adapted versions of the STFP be framed within the theories of conditioned learning – hereafter referred to as non-social learning – or do they display other singularities? To date, there is an ongoing debate on whether social learning follows the same principles as non-social learning (Behrens et al., 2008; Heyes, 2012; Insel & Fernald, 2004; Klein & Platt, 2013; Nielsen et al., 2012). Although, as stressed in the introduction based on neuroscientific data, such a distinction is challenged and these ideas may not be totally mutually exclusive (Reader, 2016).

Learning theories aim to describe behavioral changes prolonged in time after a specific experience (Heyes, 1994; Rescorla, 1988). The simplest form of non-social learning, non-associative learning, occurs through mere exposure to a stimulus, producing a behavioral change that either enhances or reduces the response. The second type, known as Pavlovian conditioning, involves the association of two stimuli leading to the transfer of the unconditioned reward properties to the conditioned reward. The third type named instrumental conditioning, involves an action towards one stimulus being enhanced or reduced by its consequences (Schakner & Blumstein, 2016). Instrumental conditioning, though, will not be further discussed as it does not apply to our results. We must also consider that learning mechanisms are supported by multiple cognitive capacities such as perception, attention, or memory to identify salient stimuli that serve as input for the learning process (Muirz, 1996; N. M. White & McDonald, 2002). When social learning theories build on non-social learning, they identify the social aspect as the catalyst or mediator that drives attention to the object

of learning. In this context, the social stimulus is not the entity being learned about but the input to learn from. In other words, interaction with the object is transferred from the subject to the conspecific, i.e., the social stimulus. Thus, the type of input in the learning process – whether visual, olfactory, social, or otherwise – can provide a useful basis for categorizing different learning processes (Heyes, 1994; Reader, 2016; Zentall, 2006).

Firstly, the non-associative learning theory could be a suitable explanation for classic STFP. According to this theory, the frequency of a response changes with exposure to a stimulus, increasing due to sensitization or reducing due to habituation (Schakner & Blumstein, 2016). Thus, the naïve observer is exposed to the odor of the food eaten by the demonstrator, and thereafter the observer prefers that food type over the alternative. The hypothesis is that this revealed preference following social interaction arises from a social sensitization to the food eaten by the demonstrator. As known, STFP occurs only when the odor is presented with carbon disulfide, but it does not occur when the demonstrator and the odor are presented together without the demonstrator's prior food consumption (Galef & Stein, 1985). Therefore, the input system appears to be highly specialized, relying heavily on this olfactory cue, the carbon disulfide, present in the rat's breath (Choleris et al., 2011; Galef et al., 1988). In a series of experiments conducted by Galef and Durlach (1993), the researchers examined how preexposure to one of two alternative food types influenced the observer's choice after interacting with a demonstrator who had been fed either the preexposed food or a novel food. Contrary to their hypothesis, preexposure to one food type decreased its consumption during the preference testing phase, especially if the alternative food – the novel one – was socially cued. Thus, sensitization based on previous exposure to one of the food options does not occur, and increased consumption of one food type is primarily mediated by social signals.

The classic STFP is generally framed within observational associative learning, where the demonstrator mediates the association between two stimuli, the food odor and his breath, evoking an increased consumption of the cued food. Although interpreted as an example of Pavlovian conditioning, Galef and Durlach did not find several of the phenomena that characterize this type of learning. Specifically, rats that acquire food

preferences socially did not show blocking, overshadowing, or latent inhibition (Galef & Durlach, 1993). Focusing on the adapted version of the STFP employed in this thesis, we would expect to observe latent inhibition if Pavlovian conditioning were the best explanation for this behavior. Theoretically, previous familiarization of the observer rats with the two pellet options should evoke a smaller response or none after social interaction, compared to naïve observers. However, preexposure did not reduce the strength of STFP compared to controls with no prior exposure (Galef et al., 1985; Galef & Durlach, 1993; Galef & Whiskin, 2008a). Galef and colleagues demonstrated that previous familiarization with both food options did not hinder preference acquisition in STFP (Galef et al., 1985; Galef & Durlach, 1993; Galef & Whiskin, 2008a). Furthermore, ill demonstrators should theoretically promote aversive learning towards the consumed food. However, rats acquired STFP from anesthetized demonstrators and even those showing gastrointestinal distress during interaction (Galef et al. 1983; Galef and Whiskin 2000; but see Kuan and Colwill 1997). Observers do not seem to evaluate the suitability of STFP acquisition (Agee et al., 2023), a point that will be further discussed below.

In addition to learning theories, other psychological mechanisms have been proposed to explain classic STFP. Contagion is an innate phenomenon in which two or more animals engage in coordinated behavior that is part of their species' repertoire. Typically, one of the animals initiates the behavior and the others follow or join the first (Thorpe, 1963; Zentall, 2006). By way of example, if a hungry chick is introduced to a box with available food, it will start eating. This is sufficient to trigger the feeding behavior of a satiated chick already in the box (Tolman, 1964). Yet, this thesis focuses on a more complex phenomenon: food preference update following social input. Can this phenomenon be explained without resorting to learning? Does food revaluation intrinsically require learning? So far, STFP can only be partially explained by non-social learning principles, indicating that adaptations of current theories and further investigation are necessary.

1.4. Conformity

When Galef and Whiskin published their adapted version of the STFP, they termed the phenomenon "conformity" (2008a). This choice of terminology was based on the fact that the rats overrode prior individual knowledge with social information (Whiten & Van Schaik, 2007). In their first experiment, rats started developing an aversion towards one diet while gaining experience with the safety of another. Upon interacting with a demonstrator fed the unsafe diet, observers overrode their individual knowledge of diet safety and consumed primarily the demonstrated food. The second experiment induced individual preference for one diet over the other by adding sugar to the first. Upon interacting with a demonstrator fed the less palatable option, observers tended to consume it more than the palatable one. In both experiments, observers appeared to make non-beneficial choices, therefore, the authors concluded that in rat decision-making, social information carries more weight than individual information (Galef & Whiskin, 2008a).

Rat conformity has been investigated in other paradigms as well. Using a Y maze with a white and a black door, rats were trained to follow a demonstrator that used color as a cue to access food at the end of an arm. Therefore, observers learned to follow both the demonstrator and the rewarded color. During testing, observers were divided into three groups: the first had a demonstrator but did not see the colors, the second group only accessed color cues, and the third group had a demonstrator who chose the previously incorrect color cue. Over 60% of the subjects in the third group made incorrect choices by conforming to the current demonstrator, overriding their previous knowledge (Konopasky & Telegdy, 1977). These studies established conformity paradigms in rats to facilitate comparative research with human conformity, as described by Asch (1955), where individuals agree with a group even when the group's answer is incorrect. However, most research on rat conformity has relied on a single demonstrator rather than a group. Nevertheless, some STFP studies have demonstrated that, similar to humans (Asch, 1955), larger groups of demonstrators lead to greater choice homogeneity among observers (Chou & Richerson, 1992; Galef et al., 1990).

To better understand the underlying motivations for conformity across species, it is useful to differentiate between informative and normative conformity, as proposed by Claidière and Whiten (2012). The former relies on social information to enhance performance in individual tasks and the latter to maintain or improve social interactions and status within a group. Galef's experiments are traditionally viewed as examples of informative conformity. According to the authors, informative conformity is composed of distinct characteristics. Firstly, it occurs in uncertain or novel situations (Claidière & Whiten, 2012). Contrary to this statement, a study showed that rats relied more on the demonstrator's information in stable setups where the same food was consistently available, compared to variable environments (Galef & Whiskin, 2004). Still, rats that had been fed unpalatable and energetically poor food, or those having no clear previous information about the safety of the socially paired food, relied more on socially transmitted food information than control rats (Galef et al., 2008). Thus, conformity depends on the degree or the source of uncertainty. Note that, by definition, the adapted version of the STFP used in this thesis is less uncertain than the classic one because observers encounter the same food options repeatedly. Secondly, informative conformity is often related to foraging or dietary choices and results in short-term efficient adaptations (Claidière & Whiten, 2012). In both STFP versions, preference testing occurs immediately after the observer rat detects the food odor from the demonstrator's breath, thus, acquired information is applied right away. This application, however, is not always efficient as discussed above (Galef & Whiskin, 2008a). Thirdly, informative conformity is used in isolation, distinguishing it from normative conformity, which occurs only in social contexts (Claidière & Whiten, 2012). By design, in STFP protocols the demonstrator's information is used in isolation. Finally, informative conformity is not sustained when the demonstrators are unreliable (Claidière & Whiten, 2012). Nonetheless, the acquisition of STFP conformity is not hindered by unreliable or ill demonstrators (Galef et al. 1983; Galef and Whiskin 2000; Agee and Monfils 2018; but see Kuan and Colwill 1997). Consequently, the STFP data partially aligns with the criteria for informative conformity, without meeting all aspects of its definition.

To elaborate on the last point of the informative conformity definition, STFP acquisition from ill demonstrators could be a consequence of the artificial laboratory setup. Several factors support this interpretation. Primarily, rats are neophobic, meaning they temporarily reduce initial intake and overall consumption when encountering novel food (Barnett, 1958; Modlinska et al., 2015). If rats experience intestinal malaise after food consumption they develop a strong aversion to it (Garcia & Koelling, 1966). In nature, food intoxication is relatively rare; however, if toxins are highly lethal, demonstrators who consume the toxic food are unlikely to return to the burrow from the feeding site, preventing social interactions (Noble et al., 2001). Consequently, when interactions with intoxicated conspecifics are rare and rats already show skepticism toward novel foods, it is inefficient for them to evaluate the information source critically. Mistakenly evaluating dietary social information as unsafe could be counterproductive. Considering these factors, there seems to be no evolutionary pressure for rats to develop selectivity based on demonstrator fitness in the context of STFP (Galef, 2012; Noble et al., 2001). However, when observers interact simultaneously with healthy and ill demonstrators who have consumed different diets, they tend to consume less of the diet associated with the ill demonstrator compared to the healthy one (Kuan & Colwill, 1997). This suggests that the availability of alternatives influences the observers' criteria for dietary choices.

1.5. Social familiarity

Conformity theories assume that observer rats learn from familiar conspecifics. This is especially true for normative conformity, where the goal is to maintain status and relationships within the group, but it is also commonly presumed for informative conformity (Claidière & Whiten, 2012). In the OXT study of this thesis (II), we manipulated familiarity as a proxy for group affiliation (Bartal et al., 2014). Demonstrators and observers were either cagemates included in the in-group (familiar conspecifics); or unfamiliar and part of the out-group. Literature suggests that social learning is more likely between familiar conspecifics than unfamiliar ones, simply because they interact more frequently. Additionally, adopting similar behaviors is more beneficial when rats share the same environment (Laland, 2004). Yet previous studies manipulating familiarity in STFP showed no significant effects (Galef et al.,

1984) or a nonsignificant tendency to acquire more information from unfamiliar demonstrators (Agee et al., 2019; Galef & Whiskin, 2008a). Contrary to these findings, our results showed that the vehicle group acquired significantly stronger STFP after interacting with an unfamiliar than a familiar demonstrator. This result challenges the existing hypothesis (Agee et al., 2019; Galef et al., 1984; Galef & Whiskin, 2008b), suggesting that rats attach more value to inputs from unfamiliar conspecifics. The evolutionary reasons for such behavior remain unknown and present an interesting line for future research.

As a synthesis of the points addressed above, general learning theories are necessary but not sufficient to fully account for STFP. Pavlovian learning explains how observers form associations between social cues and food odors, facilitating the revaluation of food rewards. However, ecological perspectives are essential to contextualize these mechanisms, particularly in understanding why individuals rely on social information even when doing so is not optimal or may contradict personal experience. These perspectives also explore the role of familiarity in modulating the strength of social influence. Therefore, a comprehensive understanding of STFP behavior requires an integrated approach that combines internal psychological mechanisms with external ecological and social factors.

2. Neural mechanisms of socially transmitted food preferences

Psychological mechanisms underlying reward revaluation in social contexts are only partially explained by behavioral studies. Therefore, neuroscientific experiments can shed light on this process. The following subsections discuss our findings on the STFP paradigm following neural manipulations. First it examines the effects of the NAcSh lesions followed by the effects of systemic OXT injections.

2.1. Nucleus accumbens shell

While the article provides a comprehensive discussion regarding the role of the NAc in STFP, this section offers further elaboration on some interpretations.

The NAc is an integrative neural hub that, as highlighted in the introduction, translates motivation into action (Goto & Grace, 2005; Kalivas & Nakamura, 1999; Mogenson & Yang, 1991). It is composed of two subregions: the core and the shell. The core primarily contributes to goal-directed actions by evaluating reward efficiency accounting for delays, efforts, and risks (Day et al., 2010; Saddoris et al., 2013). Conversely, the NAcSh encodes the value of reward and plays a critical role in tasks that assess preferences without playing a major role in cost-benefit analyses (Beyene et al., 2010; Ghods-Sharifi & Floresco, 2010; Saddoris et al., 2013, 2017; Stopper & Floresco, 2011). For example, preferences based on variations in reward magnitude depend on the integrity of the NAcSh (Jang et al., 2017; Katsuura & Taha, 2014). Interestingly, NAcSh encodes reward information for longer periods than the core, including post-consumption phases. The authors interpreted this characteristic as the tracking of reward value outcomes (Sackett et al., 2017). More broadly, the NAcSh mediates motivation by attributing incentive salience to rewards. It highlights the hedonic value of rewards (e.g., pleasure) and integrates other motivational factors such as social cues (Amaral et al., 2021; Bassareo et al., 2002; Peciña & Berridge, 2013; Saddoris et al., 2015; Wyvell & Berridge, 2000). Saddoris (2015) proposed a general framework, extending reward processing functions, where the core drives goal-directed actions and the NAcSh inhibits erroneous choices of less favorable options. Thus, the NAc core and NAc shell exert distinct yet complementary roles in optimizing action selection (Floresco, 2015).

Although the STFP paradigm does not dissociate the processes mentioned above and we did not test whether the core is relevant for the STFP, our findings demonstrated that NAcSh-lesioned rats can develop a preference based on taste. However, they failed to update reward values after integration of social information. This is consistent with the idea that the integrity of the NAcSh is not indispensable for the discrimination of primary sensory features of food rewards (e.g., taste); rather, its function is to enhance the salience of rewards associated with the motivational incentive of a social cue (Amaral et al., 2021; Floresco, 2015).

2.2. Oxytocin

OXT is a peptide synthesized in two hypothalamic subregions: the hypothalamic paraventricular and supraoptic nuclei. These brain regions project to several other brain areas and the posterior pituitary gland, where OXT is subsequently released peripherally (Anacker & Beery, 2013; Gimpl & Fahrenholz, 2001). Nevertheless, the functions of OXT are largely determined by the distribution of its receptors, which differs among rodent species (Anacker & Beery, 2013; Insel & Young, 2001). As mentioned in the introduction, a study compared two vole species due to their differences in partner choice: monogamous prairie and non-monogamous montane voles. Activation of OXT receptors in the NAc of prairie voles facilitated partner preference formation. In contrast, the same intervention did not produce equivalent results in montane voles, which had a lower density of OXT receptors in the NAc (H. E. Ross et al., 2009).

OXT has been named the "social peptide" due to its role in regulating sexual and parental behavior, social recognition, and affiliation in rodents while influencing face expression processing and trust in humans (Insel & Shapiro, 1992; Keebaugh et al., 2015; Oettl et al., 2016; Van IJzendoorn & Bakermans-Kranenburg, 2012; but see Leng et al., 2022). Consequently, we hypothesized that OXT would modulate STFP in a familiarity-dependent manner. Summarized, our results indicated that OXT blocked the increase in originally non-preferred consumption by the out-group but not the in-group. The blocking effects were long-lasting in the large-dose OXT out-group. Furthermore, all OXT-treated groups, in both familiarity groups, decreased the consumption of the originally preferred food. However, this effect was not exclusive to OXT treatment, as the vehicle out-group exhibited a decrease in originally preferred food consumption too. Considering the current literature on OXT, can the familiarity-dependent OXT effects on STFP be interpreted as a specialized social mechanism?

Mice with OXT receptor knockouts did not discriminate between familiar and unfamiliar conspecifics (Choleris et al., 2006). However, their social recognition was restored by OXT infusion into the lateral ventricles before the sample phase – first exposure to the conspecific – but not before the recognition phase (Ferguson et al.,

2001). This suggests that OXT is necessary for the acquisition of social recognition. In rats, the effects of OXT were studied at different dosages. Low doses facilitated recognition, whereas high doses impaired it (Popik & Vetulani, 1991). As reviewed by Choleris (2009), OXT infusions into the lateral septum, ventral hippocampus, and medial preoptic area of rats improved social recognition. It is important to note that many of these manipulations exceed natural OXT brain levels, causing artificial effects, a limitation also present in our study. To more precisely characterize the role of OXT in social recognition in rats, Oettl et al. (2016) used optogenetics to activate paraventricular oxytocinergic neurons during social interaction. The stimulated group showed increased social exploration compared to the control. Moreover, the stimulated rats demonstrated social recognition two hours later while controls performed at chance level. The authors proposed that OXT increases peak firing responses and reduces background firing during the sample phase, enhancing the signal-to-noise ratio. This mechanism improves odor recognition accuracy and may also increase the salience of these olfactory stimuli. Interestingly, OXT effects in the olfactory areas were restricted to social odors. These results point toward a specialized OXT subnetwork for encoding social information. Interestingly, OXT receptor density in rodents is high in olfactory areas, whereas in primates, higher densities are found in visual and attentional regions (Freeman & Young, 2016), reinforcing the idea of OXT's role in processing social stimuli.

So far, only elusive preliminary results on the effects of OXT on STFP are available (Lindeyer et al., 2013). Nevertheless, various rodent experiments have explored OXT's effects using conditioned place preference (CPP) and conditioned social preference (CSP) paradigms. In these paradigms, subjects develop a preference for an OXT-associated chamber or an OXT-associated conspecific, respectively. For instance, female mice preferred a conspecific they explored after intranasal OXT administration over one paired with saline, but no preference was developed for a chamber paired with OXT administration (Kosaki & Watanabe, 2016). Similarly, rats did not exhibit CPP following intraperitoneal OXT administration. Strikingly, they did develop CPP when a conspecific was present in the OXT-paired chamber unlike a group administered a different neuropeptide. Thus, OXT enhanced the rewarding

effects of social interaction. Milder yet significant CPP also occurred when an object was placed in the OXT-paired chamber (Ramos et al., 2015). When centrally administered, OXT produced mixed results in CPP depending on the brain region targeted (Baracz et al., 2012; László et al., 2016). Dölen et al. (2013) applied optogenetics and electrophysiology techniques in mice to further explore the influence of OXT on social CPP. The experiments created two distinct chambers: one containing previously socially-paired bedding, and another containing non-paired bedding. The study demonstrated that the coordinated activity of OXT and serotonin in the NAc was necessary to encode the rewarding properties of social interaction and the subsequent development of social CPP.

Due to the systemic administration of OXT in our experiment, its effects could modulate multiple neural circuits and interfere with various psychological mechanisms. Nevertheless, social recognition appeared to be preserved, as the effects of OXT were familiarity-dependent. As discussed, literature suggests a distinct role for OXT in encoding social signals (Choleris et al., 2006; Ferguson et al., 2001; Oettl et al., 2016) within learning neural networks (Dölen et al., 2013). Still, additional research is necessary to determine if the results are due to changes in oxytocinergic pathways that directly affect reward revaluation or indirectly through alterations in the encoding of social information.

Taken together, the NAcSh is a critical brain region for integrating social information in the evaluation of available rewards, while OXT acts as a modulatory factor, particularly in interaction with social familiarity. Future research should further investigate whether OXT within the NAcSh modulates behavior in the STFP paradigm. This modulation may occur directly or indirectly, for instance by influencing the effects of familiarity, environmental stability, or other contextual factors. Neuroscientific investigations are essential to develop more accurate behavioral and psychological models of social learning that account for the plasticity of neural substrates in their dynamic interplay with internal and external influences.

3. Ultrasonic vocalizations

Having discussed the mechanisms underlying STFP, this subsection now focuses on study III, which examines vocal communication as an additional feature of social behavior.

Rats transfer information mostly passively, with no communicative intention. Sometimes, though, they use vocalization to actively inform others. These communications usually signal emotional states, desires, and needs (Brudzynski, 2013; Knutson et al., 2002; Opiol et al., 2015; Wöhr et al., 2015). Rats produce ultrasonic sounds ranging from 20 to 100 kHz that humans cannot perceive. Based on their sonographic features, these USVs are classified into 22 kHz and 50 kHz calls (Brudzynski, 2013). 22 kHz calls are typically emitted in aversive situations, such as in front of danger or threat, but also to signal the satiation of a previously pleasant activity (Burgdorf et al., 2008; Knutson et al., 2002; Kroes et al., 2007; Oliveira & Barros, 2006). Conversely, 50 kHz calls are emitted in positive situations, including playing, sexual behavior, and drug consumption (Burgdorf et al., 2008; Knutson et al., 2002; Pellis et al., 2018).

In the final study (III), we recorded and classified USVs from rats exposed to rewards. The first experimental phase – the sucrose discrimination test (SDT) – consisted of several sessions where rats were given one of three pairs of sucrose water (2% vs. 5%, 2% vs. 10%, or 5% vs. 10% sucrose). In the second experimental phase – the social-sucrose preference test (SSPT) – rats had access to either an unfamiliar juvenile rat or sucrose water at 2%, 5%, or 10% concentrations. Due to the positive nature of the rewards, we focused on 50 kHz calls and subcategorized them into 14 subtypes (Wright et al., 2010) to directly compare vocal profiles based on the type of cue, whether social or non-social. In the SDT, we confirmed that rats discriminated between sucrose concentrations and preferred the highest concentration of each pair. Contrary to our hypothesis, rats vocalized more in the 2% vs. 10% than in the 5% vs. 10% condition. Within sessions, they vocalized more (controlling for time spent per zone) in the lowest sucrose concentration zone. Flat USVs were the most frequent subtype during SDT. In the SSPT, the main behavioral results indicated that rats, as

hypothesized, traded off the value of the sucrose reward for the social reward. Rats preferred social interaction over 2% sucrose, were indifferent between the juvenile rat and 5% sucrose, and switched to prefer 10% sucrose over the juvenile. Vocalizations were predominantly emitted in the social zone. Although rats spent less time near the juvenile when the sucrose concentration was highest (10%), they vocalized more than in the other conditions. Trill and Composite were the most prevalent subtypes in the SSPT phase.

As demonstrated by the summarized results above, the use of USVs depended on the nature of the rewards, whether social or non-social. Our findings align with previous literature showing that social cues are effective natural elicitors of USVs production (Burgdorf et al., 2008; Knutson et al., 2002; Pellis et al., 2018). But is the reward nature the only factor explaining the difference in vocalization rates between social and non-social contexts? We must consider that USV production seems to be mutually exclusive with drinking (Sirotin et al., 2014; Welzl & Bureš, 1977), which reduces the possibility of calling while consuming sucrose water. Additionally, the dietary protocols of each phase could have influenced the differences in vocalization rates. Rats were food deprived during the SDT, which is known to reduce USVs production (Brenes & Schwarting, 2014), but were fed ad-libitum during the SSPT. Despite these considerations, the social component is most likely the primary factor contributing to the difference, while the others amplify it. The subtype distribution across phases is also consistent with the literature (Mulvihill & Brudzynski, 2018; Wöhr & Schwarting, 2013; Wright et al., 2010). Additionally, the high sucrose concentration probably boosted the value of the SSPT session, leading to high USV production in close contact with the juvenile. It is plausible too, from a foraging perspective, that the rat communicated the high value of the sucrose water to the juvenile.

Interestingly, analyses of USVs have provided valuable insights as they do not fully correlate to rats' choices (Brenes & Schwarting, 2014). The discrepancy between time allocation and the amount and type of USVs produced opens a door to explore simultaneous psychological processes that have remained hidden until now.

4. Conclusion

The research presented in this thesis contributes to the extensive literature on reward valuation and social behavior in rats. The discussion of the first two studies highlights that STFP can only be partially explained by associative learning theories. However, general reward processing is still fundamental to the STFP paradigm, as demonstrated by the impaired social transmission in NAcSh-lesioned rats. Furthermore, OXT modulates food preferences transmitted socially in a familiarity-dependent way. Study III provides evidence that USVs produced in social contexts differ from those in non-social situations. However, rats' communication patterns do not directly correlate with their decision-making between social interaction and sucrose rewards. Thus, investigating both simultaneously offers complementary, quantifiable measures to explore underlying psychological and neural processes that were previously inaccessible.

Overall, our results are consistent with a framework that integrates a domain-general reward learning model with socially specialized mechanisms. While associative mechanisms provide a parsimonious explanation, specializations in sensory and cognitive mechanisms may enhance and complement social information processing. These specializations are likely shaped by evolutionary and developmental pressures.

Future research should integrate behavioral, neurological, ecological, and psychological approaches to develop a comprehensive framework. A deeper understanding of rat behavior will facilitate translational research with the potential to improve human lives. Social influence is a phenomenon shared across many mammalian species. Currently, human society is highly interconnected through social networks and influencing preferences has become a profession. Therefore, these dynamics must be studied from multiple perspectives, including those in this thesis.

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AFFIDAVIT

I, Irina Noguer Calabús, declare under oath that I have produced my thesis independently and without any undue assistance by third parties under consideration of the “Principles for the Safeguarding of Good Scientific Practice at Heinrich Heine University Düsseldorf”.

Torroella de Montgrí, 2025 _____

AUTHOR CONTRIBUTIONS

My overall contributions in relation to the other researchers in each of the studies are as follows:

STUDY I

Noguer-Calabús, I., Schäble, S., & Kalenscher, T. (2022). Lesions of nucleus accumbens shell abolish socially transmitted food preferences. *European Journal of Neuroscience*, *65*(10), 1–15. https://doi.org/10.1111/ejn.15827

Overall contribution: 75%. I contributed minor input to the experimental design, collected and analyzed the data, created the figures, wrote the manuscript draft, edited the final version and handled the submission process.

STUDY II

Noguer-Calabús, I., Schäble, S., Dören, J., & Kalenscher, T. (2024). Oxytocin effects on socially transmitted food preferences are moderated by familiarity between rats. *Psychopharmacology*, *242*(2), 361–372. https://doi.org/10.1007/s00213-024-06682-x

Overall contribution: 65%. I analyzed the data, prepared the figures, and wrote the introduction and results sections of the draft. I also edited the final version and handled the submission process.

STUDY III

Seidisarouei, M., van Gurp, S., Pranic, N. M., Calabus, I. N., van Wingerden, M., & Kalenscher, T. (2021). Distinct Profiles of 50 kHz Vocalizations Differentiate Between Social Versus Non-social Reward Approach and Consumption. *Frontiers in Behavioral Neuroscience*, *15*(Jun), 1–17. https://doi.org/10.3389/fnbeh.2021.693698

Overall contribution: 15%. I assisted with data collection, categorized USV subtypes, conducted the analysis, and prepared the corresponding figures.