Vocal expression and neural sensitivity of social reward

Inaugural-Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

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Düsseldorf, November 2022

aus dem Institut für Experimentelle Psychologie

der Heinrich-Heine-Universität Düsseldorf

Gedruckt mit der Genehmigung der

Mathematisch-Naturwissenschaftlichen Fakultät der

Heinrich-Heine-Universität Düsseldorf

Berichterstatter:

1. Prof. Dr. Tobias Kalenscher
2. Dr. Marijn van Wingerden

Tag der mündlichen Prüfung: 01.02.2023

Cover Art

Die größte soziale Belohnung in meinem Leben war die offene und herzliche Umarmung der Menschen in Deutschland, die mich ermutigt hat, weiterzumachen. Danke Deutschland.

(the original photo is from https://www.flickr.com/photos/libertinus/)

Acknowledgments

The time of the doctorate was to me like a canvas on which life splashed all the colors it knew. Sometimes they were bright and warm colors like visiting and working with honorable scientists like **Prof. Dr. Tobias Kalenscher** and **Dr. Marijn van Wingerden**, from whom I learned lifelong lessons, and sometimes dark and gray colors like the sudden death of my father and the impossibility to visit my mother and family all these years.

I would like to thank **Sadaf**, who has been a friend, a family, and a wife to me all the years I have lived in Germany.

If the Ph.D. was a journey, I could happily say I had great fellow travelers.

Dr. Felix Nitsch, with whom we started the Ph.D. together in a small office, and I have always appreciated and used his intelligence, punctiliousness, and good heart.

Dr. Sander van Gurp, my Dutch scientist friend, who, despite his promise, never brought me cheese from the Netherlands**.**

Irina Noguer Calabus, my dear friend, brought the warmth, joie de vivre, and energy of Catalonia to the lab.

Dr. Sandra Schäble, our supportive laboratory manager, always generously shared her extensive knowledge with me.

Dr. Yue Hu, **Dr. Manuela Sellitto**, **Dr. Adam Schweda**, and **Maurice Zech**, with whom I did not have a chance to work, but I learned a lot from them during all of our conversations

My thanks also go to **Valeria Noever**, whose warm welcome is unforgettable for me.

Last but not least, I must kiss my **mother's** hands for her unconditional love and thank my **sister** and **brothers** for all their sacrifices for my progress.

And my last words go to my father: "I love you, **Dad**".

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Zusammenfassung

Ein Leben ohne soziale Kommunikation ist möglich, aber die Erwartung, dass dieses Leben mit psychischem Wohlbefinden einhergeht, scheint unrealistisch. Ein Großteil des Leidensdruck, des Unbehagens und der emotionalen Belastung, unter denen psychiatrische Patienten leiden, ist auf Einschränkungen im Sozialverhalten zurückzuführen. Moduliert wird Sozialverhalten durch verschiedene Belohnungsschaltkreise des Gehirns. Ein Defizit in diesen Belohnungsschaltkreisen führt womöglich zu einer Beeinträchtigung des Sozialverhaltens, und derzeit fehlt es an detaillierter Literatur über Ursachen psychiatrischer Störungen, welche bedeutend durch gestörtes Sozialverhaten gekennzeichnet sind.

Bei Ratten (Rattus norvegicus) ist die 50-kHz-Ultraschallvokalisation (USV) eine stimmliche Manifestation ihres positiven emotionalen und motivationalen Zustands und kann in der Wissenschaft als nicht-invasives Instrument zur Interpretation ihres Verhaltens verwendet werden. In der ersten Studie, die in dieser Dissertation vorgestellt wird, versuchten wir herauszufinden, ob Ratten für differenzierte Belohnungen (soziale und nicht-soziale) unterschiedliche 50-kHz-USVs verwenden. Das Ergebnis bestätigte, dass Ratten je nach Art der Belohnung einen anderen Ruftyp verwenden. In der zweiten Studie wurden die Auswirkungen des Disrupted-in-Schizophrenia-1(DISC1)-Protein-Signalwegs auf die Bewertung sozialer und nicht-sozialer Belohnungen untersucht. Die Ergebnisse zeigten, dass die Motivation für soziale Belohnungen im Vergleich zu nicht-sozialen Belohnungen deutlich reduziert war. Schließlich untersuchten wir unter Berücksichtigung der Ergebnisse der beiden ersten Studien, ob die neuronalen Merkmale (reduzierter Dopaminspiegel im dorsalen Striatum, Hippocampus und in der Amygdala) und Verhaltensphänotypen (reduzierte soziale Motivation) der transgenen DISC1-Ratten auch in ihren 50-kHz-USVs nachgewiesen werden konnten. Diese lies sich durch die Ergebnisse allerdings nicht bestätigen. Zusammenfassend

tragen diese Studien zu einem tieferen Verständnis der 50-kHz-USVs von Ratten und der durch das DISC1-Gen verursachten Beeinträchtigung der sozialen Belohnungsverarbeitung bei.

Summary

Life without social communication is possible, but the expectation that this life will be accompanied by psychological well-being seems unrealistic. Much of the pain, discomfort and emotional distress experienced by psychiatric patients is due to their inability to establish and maintain healthy social relationships. Social relationships are rewarding, and experiencing these rewards requires appropriate social functioning that relies heavily on the brain's reward circuitry. A deficit in the reward circuitry leads to maldevelopment in social functions, and currently, there is a lack of detailed knowledge about psychiatric disorders primarily characterized by impaired social functioning.

The 50-kHz Ultrasonic vocalization (USV) of rats is a vocal manifestation of their positive emotional and motivational state and can be used as a non-invasive tool to interpret their behavior. In the first study presented in this dissertation, we tried to find out whether rats use different 50-kHz USVs for certain rewards (social and non-social). The result confirmed that rats use a different type of call depending on the type of reward. Later, the second study examined the effects of the Disrupted in Schizophrenia 1 (DISC1) protein signaling pathway on the evaluation of social and non-social rewards. The results showed that motivation for social reward was significantly reduced compared to non-social reward. Finally, considering the results of the two first studies, we investigated whether the neural (reduced dopamine level in the dorsal striatum, hippocampus, and amygdala) and behavioral phenotypes (reduced social motivation) of transgenic DISC1 rats could also be detected in their 50-kHz USVs, but the results did not reflect this. The results of these studies contribute to a deeper understanding of rats' 50-kHz USVs and the social reward processing impairment caused by the DISC1 gene.

1. INTRODUCTION

1.1 Reward learning.

Learning occurs in the brain when the expectation and experience do not match. The discrepancy between the expected and experienced outcomes is called the reward prediction error (RPE). The RPE can be positive (when the outcome is better than expected) or negative (when the outcome is worse than our expectation)[1]. The RPE learning process uses dopamine (DA) as a common currency in the brain for communication between DArgic nuclei in the midbrain and its subcortical and cortical targets, such as the striatum and orbitofrontal cortex. In the brain, the value of rewards is under constant scrutiny, and any discrepancy in a learned action-outcome process will be reflected in neural computations through cooperation between multiple brain regions such as the ventral striatum (Vstr), anterior cingulate cortex (ACC), and medial prefrontal cortex (mPFC) [1]. In other words, the brain learns and updates the assignment of reward values through a trial-by-trial process that gives us an adaptive understanding of the environment[2]. In this process, the striatum, in conjunction with prefrontal cortical regions, facilitates updating outcome-based predictions[3], and it is important to note that the amygdala (AMY) and hippocampus (HPC) also contribute to this learning/updating process. Indeed, AMY sends excitatory cue-based signals to the ventral striatum[4], while HPC provides contextual input for prediction-based learning[5].

1.2 Social Reward.

Rewards are beneficial, appetitive, and favorable outcomes of an action that can reinforce the recurrence of the action that depends on it and maintain its strength[6]. For an action to lead to a socially rewarding outcome, it must elicit an appetitive state in the presence (personal or imagined) of one or more conspecifics[7]. Humans live in social communities created through social interactions and relationships, which are social rewards. Social rewards encompass an extensive repertoire of verbal and nonverbal behaviors and emotions, such as the pleasure of a compliment, an affirmation, or the attainment of a good reputation[7,8]. In parallel with social rewards, humans required non-social rewards such as water and food. With respect to these two different types of rewards, there are currently two views in the literature on social reward research[7]. One assumes that humans have evolved and preserved an additional specific mechanism that operates only in the social domain. The other rejects any specificity for social rewards and assumes that all rewards, including social and non-social reward stimuli, share the same neural basis. In conducting the studies presented in this dissertation, we were inspired by the first view.

1.3 The necessity of social reward for social fitness

After years of deliberation since the publication of The Origin of Species (1859)[9], in The Decent of Man (1871)[10], Darwin reaffirmed his belief about sociality in the process of natural selection as the directing force of evolution:

"*Social qualities, the paramount importance of which to the lower animals is disputed by no one, were no doubt acquired by the progenitors of man in a similar manner, namely, through natural selection, aided by inherited habit*" (p. 162).

Of the human race's impetus toward social harmony, Darwin (1871)[10] added:

"These sensations were first developed in order that animals would profit by association, in the same way that the sense of hunger impels us to eat. . . Sympathy . . . will have been increased through natural selection; communities with the most sympathetic members would flourish best, rearing the greatest number of offspring" (pp. 80 - 82).

What Darwin named social instinct and sympathy; Kropotkin (1924)[11] called "mutual aid."

Like Darwin, however, Kropotkin credited the human race's motivation toward social harmony to the processes of natural selection:

"*It is a feeling infinitely wider than love or personal sympathy—an instinct that has been slowly developed among animals and men in the course of an extremely long evolution, and which has taught animals and men alike the force they can borrow from the practice of mutual aid and support, and the joys they can find in social life*" (p. xli).

In order to maintain Darwin's social harmony or Kropotkin's mutual aid, the organism (human being) has to be in constant mutual social exchange with its environment (social community). This social exchange is a key factor inhibiting deviation from natural selection by opening doors that promote social fitness. This social fitness is shaped by the successful formation and maintenance of social relationships[12], which require social skills or abilities that have been established through social rewards[13]. Clearly, a defect in social reward processing cause

disorders that can lead to a lack of social fitness[14]. In this regard, it seems that the mechanism of social reward processing could have been selected and preserved by nature to learn, reward, and sustain social interactions, ultimately providing individuals with crucial fitness benefits. Put another way, humankind has benefited tremendously and achieved high reproduction levels through collective actions such as sharing information and knowledge, collective support, and protection from natural threats and disasters[11]. Therefore, acting as a useful social actor through the brain's proper social functioning is critical to reaping the social benefits.

1.4 Importance of social reward system in daily social exchanges

Social rewards such as a child's happy face as a parent, social acceptance and support from colleagues as an employee, and the company of a friend are the benefits that can result from costly activities such as spending time with the child, engaging in professional duties, and feeling compassion for a friend. Social exchange theory (SET)[15] assumes that these costs and benefits determine our social choices. In other words, the SET perspective states that people calculate the value of a relationship by subtracting the benefits from the costs. If the value is positive, the relationship continues; otherwise, it ends. Although the SET perspective reduces human interactions to a purely rational process derived from economic theory[16], the assumption of cost and benefit calculations for our social relationships is supported by other studies[17,18]. Given the assumptions of the SET perspective, operationalizing these calculations to manage dynamic and beneficial social networks in human life requires a faultless reward system that detects and evaluates social rewards[19].

1.5 Manifestation of social reward deficit in psychological disorders.

Imagine a morning when some people leave home, ignoring their child's goodbye. They choose a longer route to the train station to avoid familiar faces. On the train, they avoid eye contact. In the office, they usually do not engage in conversation, and their colleagues are annoyed by their lack of self-care.

These behaviors can be observed in people who may be diagnosed with major depressive disorder (MDD), schizophrenia (SCZ), or autism spectrum disorder (ASD)[20]. A lack of social motivation (the intensity of the need for social reward) could underlie all the behaviors described above. Social motivation is a powerful drive that controls human behavior[21], and dysfunction of social motivation mechanisms may be a primary deficit in various neuropsychological disorders such as MDD and SCZ[22], and especially in autism[23].

Social anhedonia, the decreased interest in potentially rewarding social activities[24], is one of the main symptoms of neuropsychological disorders[20] that can be explained by the disruption of the social motivation process in the brain[25]. Depending on the particular neuropsychological disorder, social anhedonia can manifest, impact, and resist treatments differently[26]. In MDD, social anhedonia is a key component, and a large body of research suggests that social anhedonia is a trait and can predict both the risk of onset and relapse of MDD[27,28], i.e., social anhedonia is linked to clinical state and improves with recovery[29]. In contrast to MDD, social anhedonia in SCZ appears persistent rather than transient as a negative symptom[29]. In this context, in autism spectrum disorders (ASD), social anhedonia has downstream effects on disorders of social cognition in ASD patients, i.e., deficits in social cognition could result from a decreased interest in social stimuli.

In short, a disturbance in the appraisal of social stimuli leads to social cognitive deficits and decreased social motivation, which manifests differently in different neuropsychological disorders[30,31].

1.6 Tracking the changes in the brain for social rewards

Human decisions are often made under the significant influence of social factors. This influence may have caused exclusive changes in the human reward system, adding social-related functionality to the original reward system of the human brain.[32] (this topic will be discussed in more detail in the general discussion). Indeed, social rewards (facial expressions or social acceptance/approval) can influence our behavior[33], and interestingly neural traces have been found to track these influences in the brain, e.g., the ventral or dorsal part of the ACC (based on the blood oxygen level dependent or BOLD signal) can discriminate whether we are liked by another person or whether the desire to be liked is rejected [34]. When another person's social expectation of being liked is rejected, the BOLD signal in the dorsal ACC (dACC) is elevated, whereas, in the case of acceptance, there is an elevated BOLD in the ventral ACC (vACC). Notably, the person's self-esteem may modulate this response: the lower it is, the higher the BOLD response in the vACC[35]. Another social reward, social recognition (a positive evaluation by others), can trigger a striatal BOLD response. This enhanced striatal BOLD response resembles the enhanced striatal BOLD response elicited by monetary incentives under non-social conditions[36], suggesting that the brain recognizes social acceptance and recognition as rewards.

A look at human history makes it clear that the current state of human advance is primarily the result of cooperation. In this regard, the ventral striatum (VS), mPFC, and ACC, with reciprocal communications using DA, play a role in processing rewards obtained through social cooperation[37]. Indeed, this multi-region communication presumably assigns a high value to cooperation, a process that resulted in a reward.

In order to obtain a reward as an individual through cooperation, we need to establish social interactions characterized by certain expectations towards our partner(s)[38]. These expectations are updated after each action of the partner[39], similar to the trial-by-trial learning process in

non-social situations. As mentioned earlier, the trial-by-trial learning process works through the RPE mechanism, which occurs through communication between different brain regions such as ACC, dorsolateral prefrontal cortex and OFC, using DA[40]. This mechanism should function adequately to have positive social experiences such as self-revelation, emotional support, and trust. All these positive social experiences depend on the ability to cooperate in the long term through reciprocity [17] which depends to a significant extent on a well-functioning reward system in the brain.

In short, social deficits can be caused (partially) by dysfunction of the DArgic system across the brain's reward circuit, and despite the great efforts that have been made to uncover the neural basis of these social deficits, it seems that much remains to be done. This incomplete task should be covered to some extent by studies in animals, such as translational studies, mainly in animals whose developmental processes and well-being are influenced by their social life, such as rats and monkeys.

2. Perspectives and questions of research

In this dissertation, I present three translational studies examining rat ultrasonic vocalization (USV) and the effects of specific disruption of DArgic homeostasis on decision-making in two reward contexts (social and non-social).

Current findings of rat USV have shown that it has excellent potential to be used as a non-invasive tool to better identify rats' emotional states and motivational intentions. However, many exploratory studies are still needed to uncover the potential of this natural tool. In this regard, our first study aimed to determine whether there is a relationship between the type (social and non-social) and magnitude of reward with the 14 different subtypes of the 50-kHz USV.

Considering the increasing importance of studying rewards by type, as differential neural and behavioral sensitivity to social and non-social rewards in psychiatric patients has been found, we used the Transgenic Disrupted in Schizophrenia 1 (tgDISC1) rat model. tgDISC1 is an established rat model that exhibits a full range of behavioral phenotypes, including hypersensitivity to amphetamines, hyperexploratory behavior, and rotarod deficits associated with decreased DA neurotransmission (in the dorsal striatum (DS), AMY, and HPC). In short, our second study focused on whether the DISC1 signaling pathway can partially explain differential sensitivity to reward types.

In our third study, the results of the first two studies and the quantifiable language impairments seen in some psychiatric disorders such as ASD and SCZ prompted us to explore whether tgDISC1s' neural dysfunctions and their reduced interest in a particular type of reward is detectable through different features of the rat 50-kHz USVs.

2.1 Rats USVs: A golden path into the emotional brain.

The life of species, including man, without vocal communication, reminds me of the silent movie era (1880-1930)[41]. I can hardly imagine how difficult it must have been for directors to convey content only through images without sound, and in the same vein, it is even more challenging to imagine how social animals could survive and evolve without vocalizations.

The primary brain system for regulating vocalization has been preserved throughout evolution and is found in the caudate brain of early fish, birds, and mammals[42]. Vocal communication may have originated in mother-infant interactions and was modified in recent evolution and retained in adulthood to interact with members of social groups[43]. Rodents, the most common mammalian group, probably switched from sonic to ultrasonic vocalization in their most recent evolutionary stages for defensive reasons[44]. Indeed, vocal communication is a unique tool; it is not dependent on daylight, visibility, or in some cases, the proximity of the organism. It leaves no trail for a predator, and weather cannot critically alter it [45]. Therefore, as with all mammals that have vocal communication capabilities, it is not surprising that the USV of rats could have played a crucial role in the survival and evolution of their species. Interestingly, the primary neural regulation of USV emissions in rodents, which play a strategic behavioral role, shares some similarities with calling in humans[44].

The early findings on emotional and motivational applications of USV have generated a strong interest in using it as a reliable tool to study social and emotional disorders. In a comprehensive review, Budzynski[45] proposed 22 functions for rats' USV, with roughly the equal distribution for negative and positive emotional states serving rats from birth to death. In this review, Budzynski states, "*The role of vocal communication is situation dependent and changes over a rat's life, from a basic, life-preservation role in infants and the development of social skills in play behavior, to the resolution of social conflicts and the organization of the social group in adults as well as defense against external threats and dangers. Different types of calls in different situations and at different stages of animal life may serve as a qualitative and*

quantitative measure of the functioning of the animal emotional system in physiological and pathological conditions. These basic animal emotional systems are homolog to basic human affective systems—both as to neurophysiological and neurochemical mechanisms—and rat expression of emotional arousal may be used in many preclinical models" (p. 18).

In this context, the high value, importance, and practical functionality of deciphering USVs become even more evident when we know that, according to the European Commission report, almost 62% of animal experiments were performed on rodents in 2019[46]. Therefore, regarding rodent's significant contribution to most animal experiments, more detailed knowledge of this species such as decryption USVs can significantly improve the quality of the corresponding research and scientific findings. Noticeably, the temporal and acoustic characterization of USVs provides far-reaching clinical and practical applications to study the course of development, emotional functions and dysfunctions, and behaviors under the effect of the environment. In other words, USVs can help to uncover the mechanisms that govern the targeted behavior.

There are two categories of USVs: 22 kHz and 50 kHz, each category is strongly associated with aversive or appetitive states in rats, respectively. 22 kHz calls can be triggered by activation of ascending mesolimbic cholinergic system (area in the dark red, figure 1), which originates in the laterodorsal tegmental nucleus and targets anterior hypothalamic-preoptic with cholinergic projections.

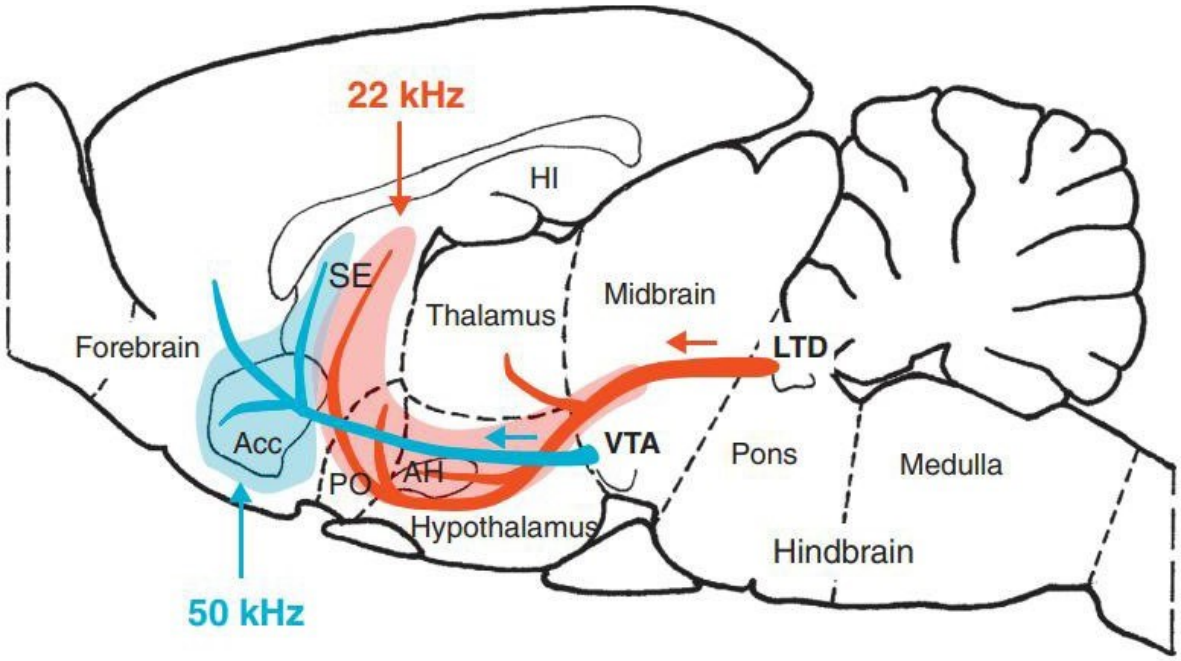

Figure 1. The ascending mesolimbic cholinergic (*dark red*) and mesolimbic dopaminergic (*dark blue*) pathways. Activation of areas shown in *light red* can induce 22-kHz calls, whereas activation of the area in *light blue* can induce 50-kHz calls. Abbreviations: *Acc*, nucleus accumbens; *AH*, anterior hypothalamic area; *HI*, hippocampal formation; *LDT*, laterodorsal tegmental nucleus; *PO*, preoptic area, *SE*; septum; *VTA*, ventral tegmental area [47,48].

As all our three studies had to be conducted in a rewarding context, we excluded 22-kHz calls and focused only on 50-kHz calls, known as happiness calls, emitted by rats in varying positive situations such as playing, mating, and rewards' anticipation or consumption [49–51]. The 50-kHz calls are initiated by activating the ascending mesolimbic DArgic area, which arises from the ventral tegmental area with DArgic projection into the nucleus accumbens, septal nucleus, and parts of the cortex (dark blue area, Figure 1)[52]. The 50 kHz calls comprise a wide range of calls with frequencies from 30 to 70 kHz and different sonographic features, which can be divided into 14 subtypes (Figure 2)[53]. Furthermore, recent studies have shown that some of these subtypes are context-dependent, i.e., rats emit a higher proportion of a specific subtype depending on the social or non-social reward situation. Therefore, the positive emotional valence and context dependence of the

50-kHz USVs' subtypes is a unique characteristic that opens a wide field for studying disorders with emotional, motivational, and social dysfunctions.

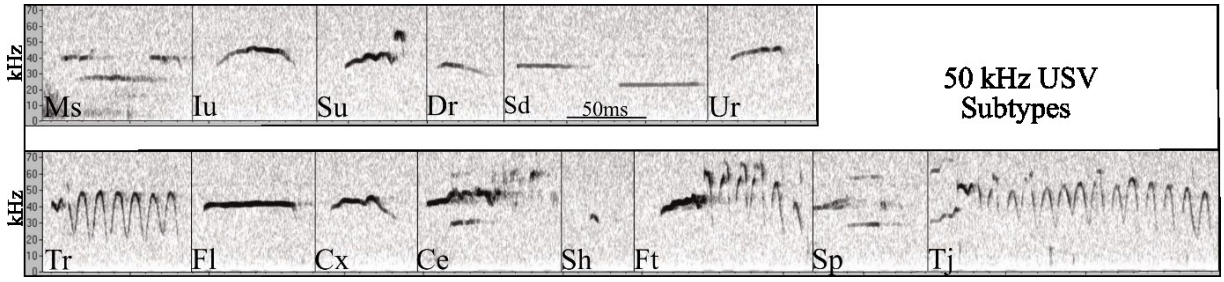

Figure 2. Representative call for every 14 categories of 50-kHz USV. Abbreviations: *SD*; Step Down, *Ur*; Upward-Ramp, *Tj*; Trill with Jump, *Tr*; Trill, *Fl*; Flat, *Cx*; Complex, *Ce*; Composite, *Sh*; Short, *Ft*; Flat-Trill combination, *Sp*: Split, *Ms*: Multi-Step, *Iu*: Inverted U, *Su*: Step-Up, *Dr*: Downward-Ramp.

2.2 Transgenic Disrupted-in-Schizophrenia 1 rat model

Major mental disorders (MMD), including SCZ, MDD, and bipolar disorder, are chronic mental illnesses that can manifest through phenotypes with high variability even within a single clinical diagnosis[54]. This heterogeneity presents a diagnostic barrier to the clear delineation of disorders, compromising the effectiveness of medical and psychological interventions[55]. A practical way out of this diagnostic, therapeutic dilemma could be the exploration of clear biological markers that can be used to delineate the associated phenotypes, which could optimally lead to more efficient therapeutic interventions resulting from a correct diagnosis.

One potential method to delineate subgroups of mental illness patients would be detecting aggregated proteins in the brain that accumulate during MMD due to disturbed protein homeostasis[56]. In this context, in 1990, it was discovered that 33 of 77 family members (in Scotland) available for cytogenetic analysis had a balanced translocation t(1: 11) (q43,q21) and that 16 of these 33 individuals were primarily diagnosed with SCZ, but also with MDD and other emotional disorders[57]. DISC1 was the key gene candidate for this familial mutation.In this regard, in a cumulative work at the institute of neurobiology at Heinrich Heine university, the interactions between neurotransmitter systems, cognitive impairment, and the disease-associated protein DISC1 were investigated in vivo and vitro by generating a trangenic DISC1 (tgDISC1) rat model[56].

The tgDISC1 rat modestly overexpresses the full-length nonmutant human DISC1 protein. The validity of the tgDISC1 rat model was established by comparable protein pathology in the form of DISC1 aggregates and behavioral phenotypes corresponding to alterations in DA neurotransmission, such as amphetamine, hypersensitivity, hyperexploratory behavior, and rotarod deficits. These findings were accompined by changes in the striatal DA system, such as a proportional increase in high-affinity DA D2 ^{high} receptors, elevated DA transporter levels,

enhanced excretion of synaptic dopamine, and reduced total DAcontent (in DS, AMY and HPC)[58–60].

These results suggest a bidirectional link between DISC1 assembly and dopamine homeostasis and highlight a functional role for DISC1 assembly in MMD pathophysiology and the pathogenesis of human DISC1 disorders[56].

The neurocognitive changes of the tgDISC1 rat model, similar to changes seen in MMD patients due to impairment of the dopaminergic system in crucial brain regions for motivational behavior, make this animal model an excellent candidate for further behavioural studies like decision-making in a rewarding context.

2.3 Speech disorder of neuropsychological disorders.

Speech, language, and vocal communication disorders are common features of various neuropsychiatric disorders such as ASD. In fact, less than 50% of individuals diagnosed with ASD are able to control language at the phrase level[61]. Similarly, the deficit in vocal communication in SCZ is one of the three diagnostic positive symptoms[54]. Therefore, investigating these vocal impairments using animal models with neurocognitive deficits similar to those in ASD or SCZ could be an avenue to reach further relevant findings. It should be noted that the advanced level of human speech and its differences from other animals make it very difficult to study vocal impairments using animal models. However, studying vocal impairments in animal models with brain mechanisms similar to those in ASD or SCZ patients may be a starting point for a better understanding of this phenotype.

In this regard, several efforts have been made to study the vocal impairments in different mouse models of ASD, and two good representative ones such as Mecp2 and Fmr1 illustrated a reduction in their USVs, which was related to reduced striatal volume[62,63]. On the other side, except for a few genes, the field of schizophrenia lacks genetic models to study the biology and brain circuitry involved in this disorder compared with ASD[61]. Therefore, a few pharmacological models that produce schizophrenia-like symptoms in rats were used to study the changes in vocalization[64,65]. The results of these studies suggest that USVs may be altered in rodents along with other behaviours and neuroanatomical deficits reminiscent of schizophrenia. Thus, it may be very illuminating to initiate studies utilizing alterations in novel schizophrenia-related genes to assess whether specific aspects of vocalization are affected by altered genes. Furthermore, given the striatal impairments in tgDISC1 rats and the dependence of USV on the rat's dopaminergic system, this animal model could be used to investigate the possible impairment of vocalization associated with the behavioural deficit found in tgDISC1 rats.

3. Studies

In the following section, I will present three articles that cover the experiments conducted to shed more light on the current issues mentioned in the introduction. For clarity, the main question and the title of the work are stated before each study:

3.1 Paper I

The main question of this study was whether rats use specific 50-kHz USV subtypes in conjunction with the type or context of rewards (social and non-social).

The result of the experiment was published in Frontiers in Behavioral Neuroscience under the title: **Distinct Profiles of 50 kHz Vocalizations Differentiate Between Social Versus Non-social Reward Approach and Consumption.**

frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 21 June 2021
doi: 10.3389/fnbeh.2021.693698

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

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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

OPEN ACCESS

Edited by:
Marco Venniro, 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

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Specialty section:
This article was submitted to Motivation and Reward, a section of the journal Frontiers in Behavioral Neuroscience

Received: 11 April 2021
Accepted: 18 May 2021
Published: 21 June 2021

Citation:
Seidisarouei M, van Gurp S, Pranic NM, Calabus IN, van Wingerden M and Kalenscher T (2021) Distinct Profiles of 50 kHz Vocalizations Differentiate Between Social Versus Non-social Reward Approach and Consumption. Front. Behav. Neurosci. 15:693698. doi: 10.3389/fnbeh.2021.693698

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

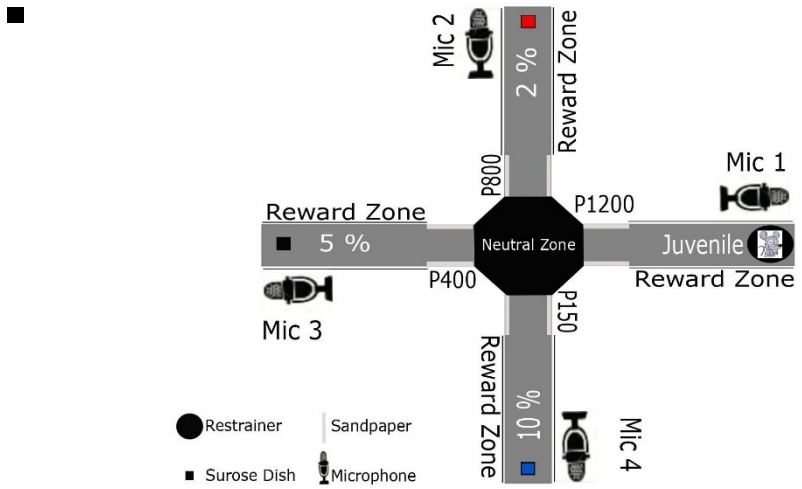
The experiment was conducted according to the European Union Directive 2010/63/EU for animal experimentation and was approved by the local authority (Landesamt für Natur, Umwelt und Verbraucherschutz North-Rhine Westphalia, Germany). Fifteen male Long-Evans rats (*Charles River, Italy*) in total were obtained in a batch of 12 experimental animals (PND40, 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).

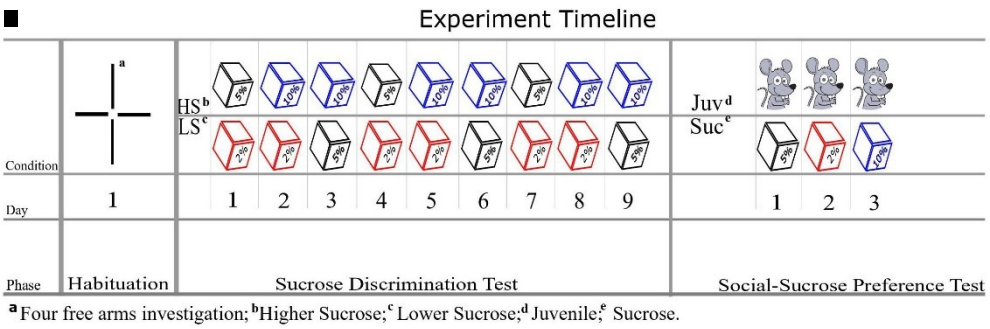
Behavioral Task Setup

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





[a] Four free arms investigation; [b] Higher Sucrose; [c] Lower Sucrose; [d] Juvenile; [e] Sucrose.

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 (AvisoftSAS-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

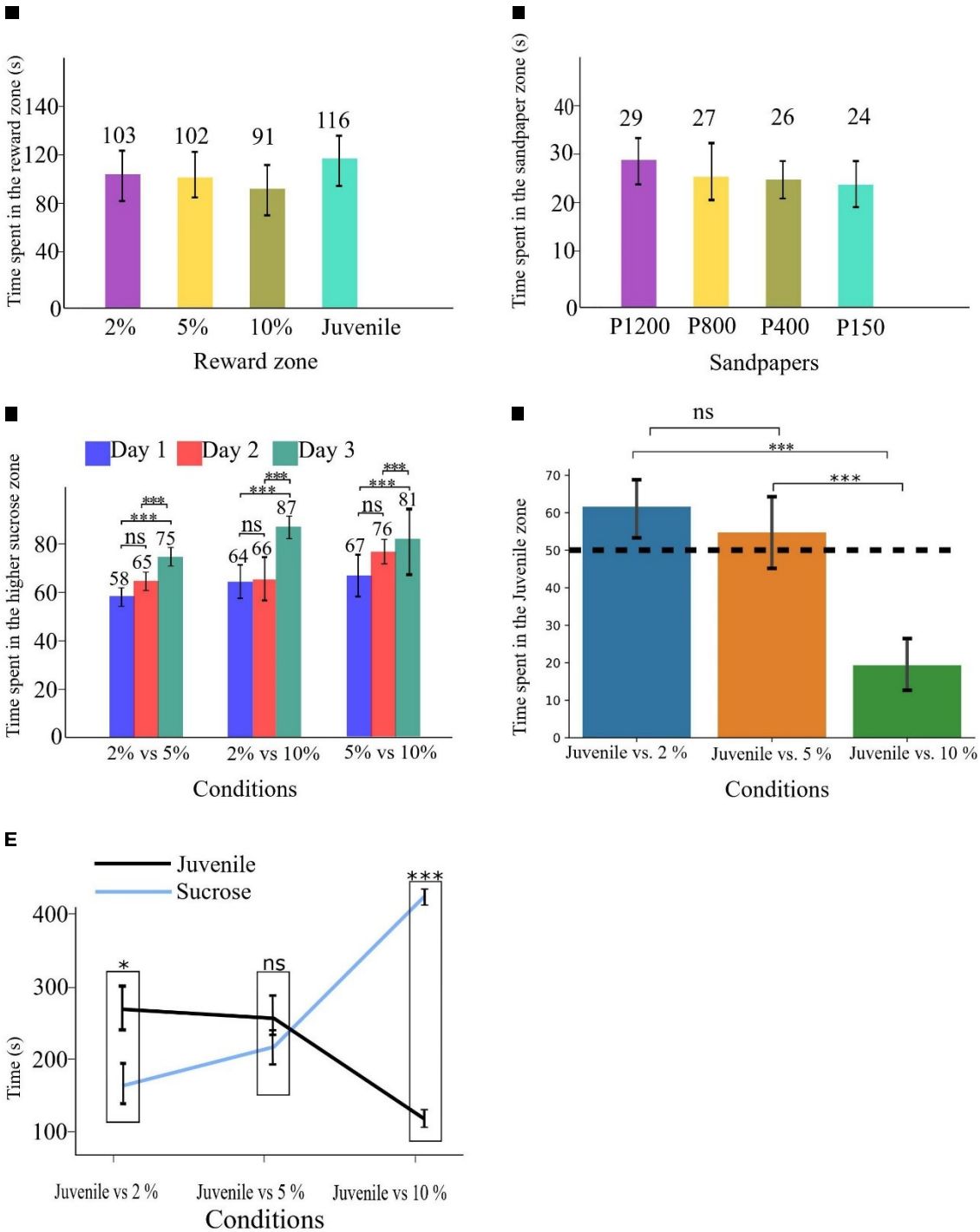
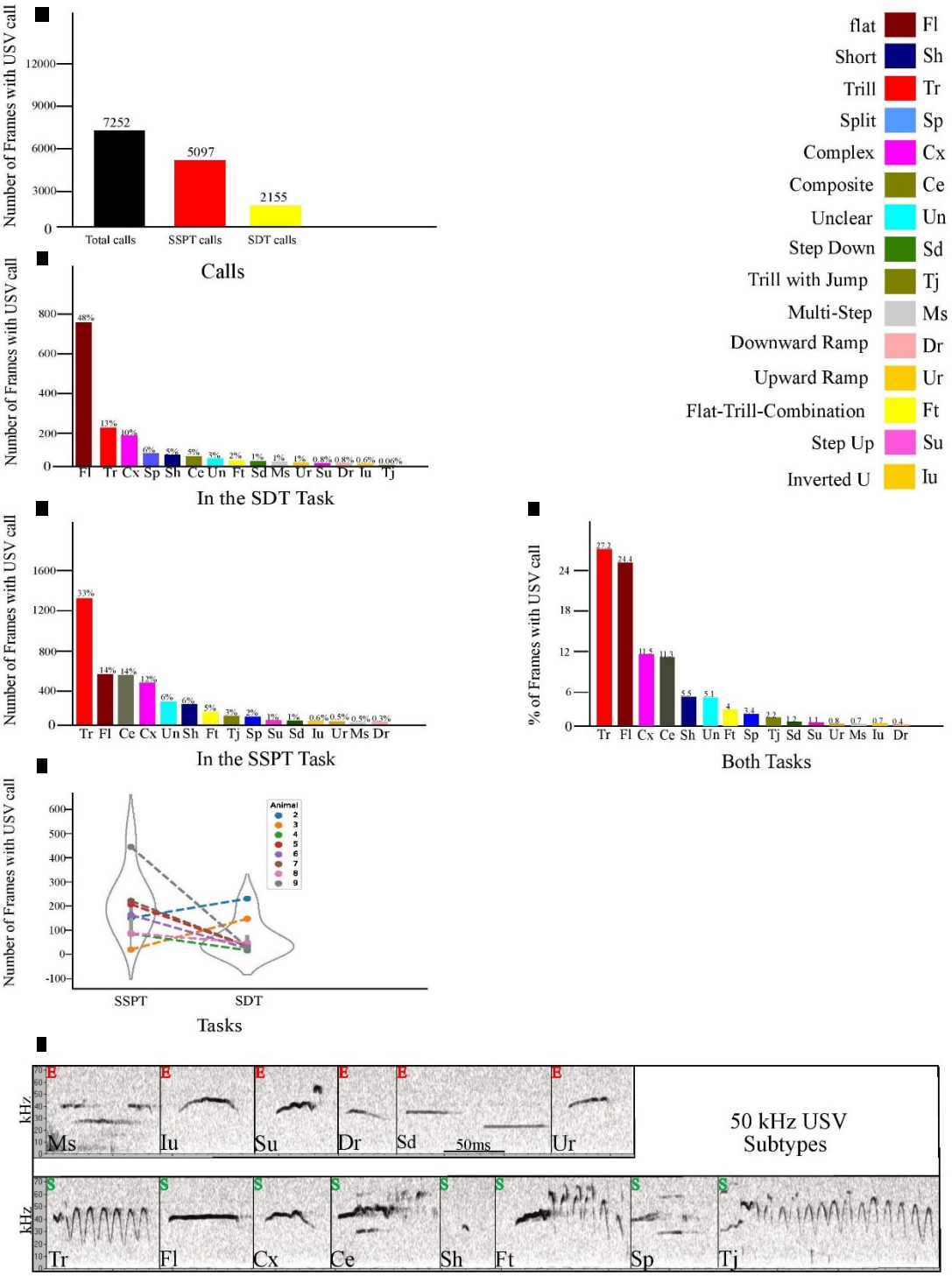


FIGURE 2 | (A) Time spent in each reward zone during the Habituation phase. (B) Time spent in each sandpaper zone during the Habituation phase. (C) Time spent in the higher sucrose zone in all three conditions of SDT. (D) time spent in the Juvenile zone in all three conditions of SSPT, the dashed line 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



S:Selected Calls, E:Excluded calls, Ms: Multi-Step, Iu: Inverted U, Su: Step-Up, Dr: Downward-Ramp
Sd: Step Down, Ur: Upward-Ramp,Tj: Trill with Jump,Tr: Trill, Fl: Flat, Cx: Complex, Ce: Composite
Sh: Short, Ft: Flat Trill combination, Sp: Split

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**).

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

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,

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

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

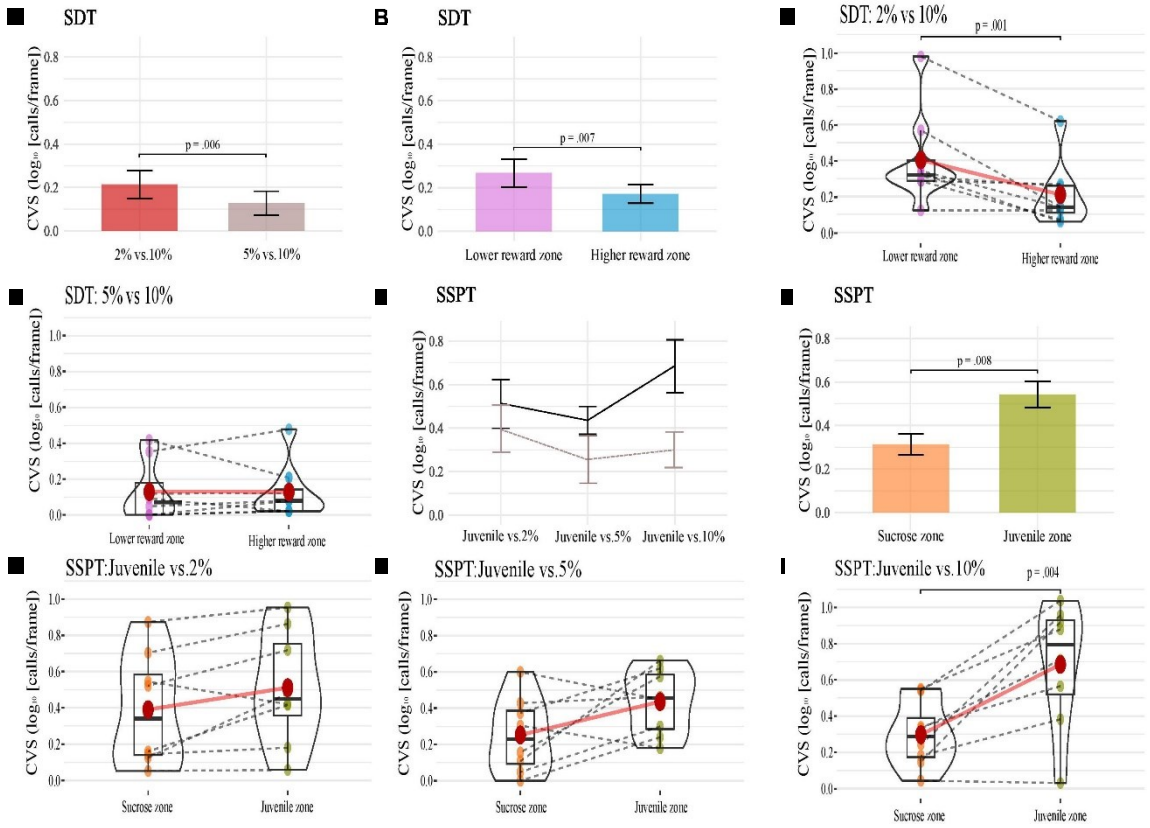


FIGURE 4 | (A–D) Present animals' CVS in conditions (A), reward zones (B) of the SDT, and (C,D) show each animal's CVS in the two reward zones of the SDT task's two conditions. (E–I) show animals' CVS across three conditions (E; in both reward zones, black solid line; Juvenile and brown hashed line; 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

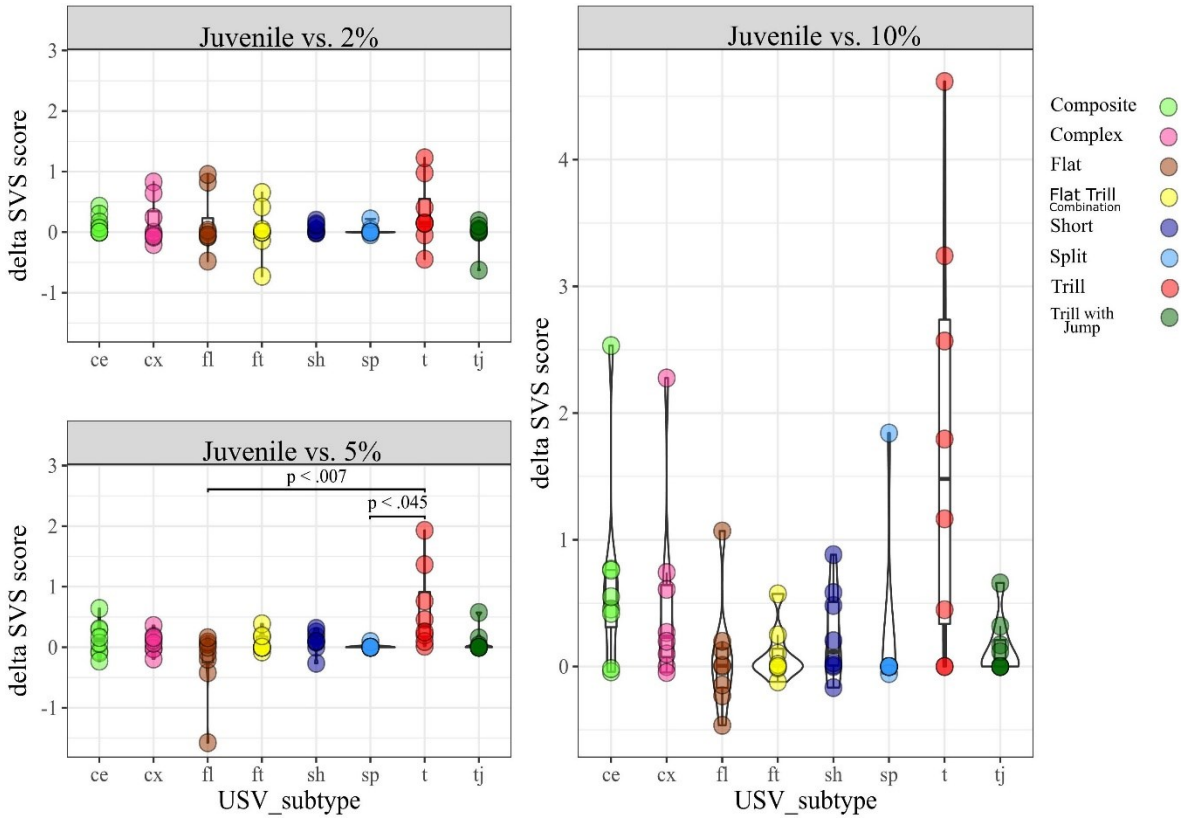


FIGURE 5 | dSVS for each subtype, split by conditions in the SSPT.

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.

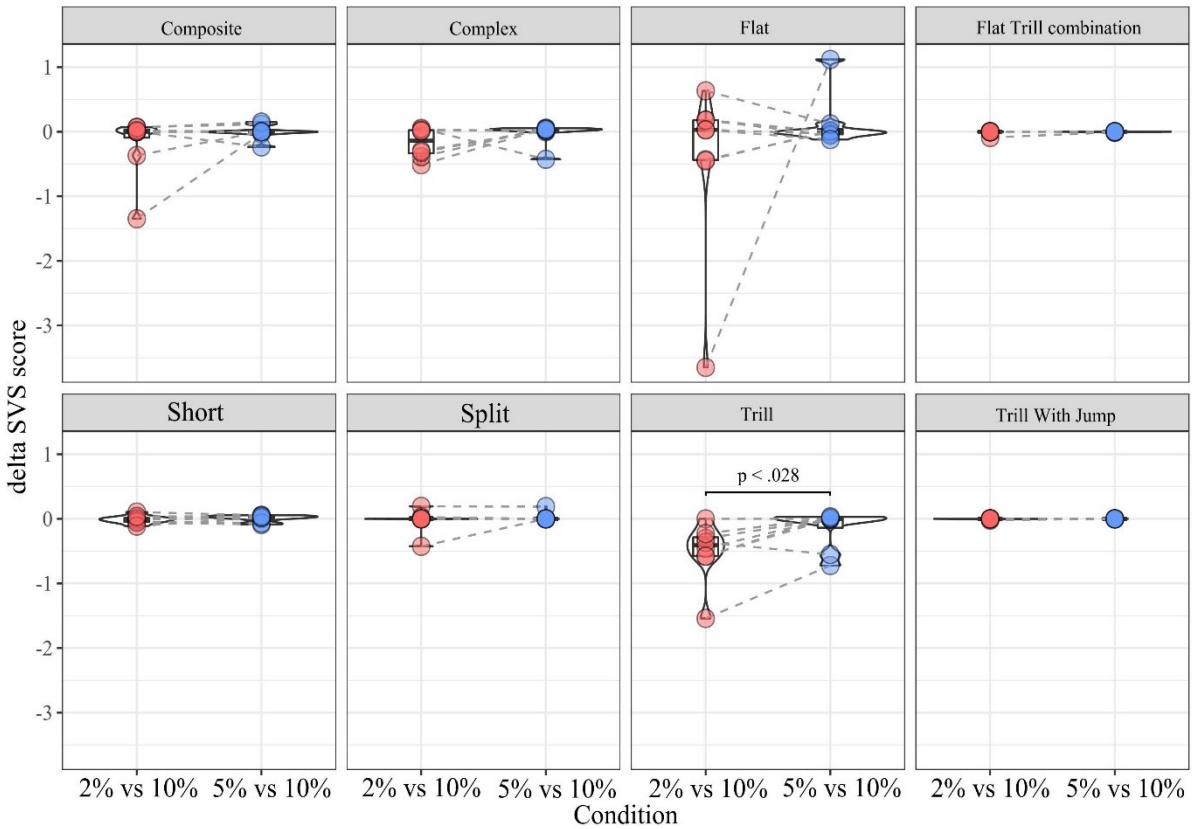


FIGURE 6 | dSVS split by subtype between conditions.

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),

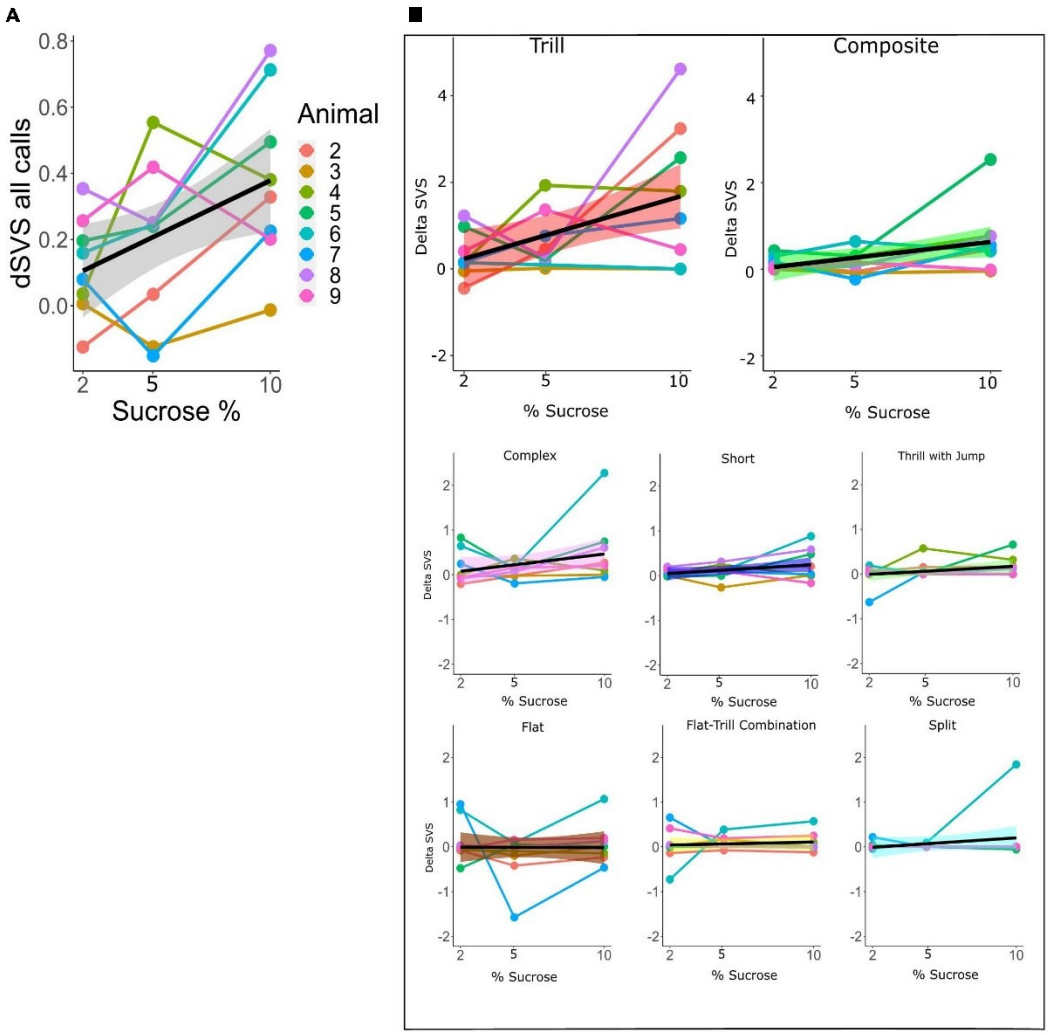


FIGURE 7| (A) dCVS for all calls across different levels of sucrose in the SSPT. The black line (±standard error of the mean; gray shade) shows the estimated linear relationship between dSVS and sucrose concentrations across all rats. Linked dots represent individual rats, modeled as a random effect. (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.

FUNDING

Publication was supported by the open access fund of Heinrich Heine University Düsseldorf-Germany.

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.

3.2 Paper II

The main question of this study was whether DISC1 protein signaling is related to reduced non-social reward processing, social interaction seeking, or both.

The result of the experiment was published in nature, Scientific Reports journal under the title: **Social anhedonia as a Disrupted-in-Schizophrenia 1-dependent phenotype.**

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Social anhedonia as a Disrupted-in-Schizophrenia 1-dependent phenotype

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Deficits in social interaction or social cognition are key phenotypes in a variety of chronic mental diseases, yet, their modeling and molecular dissection are only in their infancy. The Disrupted-in-Schizophrenia 1 (DISC1) signaling pathway is considered to play a role in different psychiatric disorders such as schizophrenia, depression, and biopolar disorders. DISC1 is involved in regulating the dopaminergic neurotransmission in, among others, the mesolimbic reward system. A transgenic rat line tgDISC1 has been introduced as a model system to study behavioral phenotypes associated with abnormal DISC1 signaling pathways. Here, we evaluated the impact of impaired DISC1 signaling on social (social interaction) and non-social (sucrose) reward preferences in the tgDISC1 animal model. In a plus-maze setting, rats chose between the opportunity for social interaction with an unfamiliar juvenile conspecific (social reward) or drinking sweet solutions with variable sucrose concentrations (non-social reward). tgDISC1 rats differed from wild-type rats in their social, but not in their non-social reward preferences. Specifically, DISC1 rats showed a lower interest in interaction with the juvenile conspecific, but did not differ from wild-type rats in their preference for higher sucrose concentrations. These results suggest that disruptions of the DISC1 signaling pathway that is associated with altered dopamine transmission in the brain result in selective deficits in social motivation reminiscent of phenotypes seen in neuropsychiatric illness.

Mental diseases such as schizophrenia, depression, and autism spectrum disorder (ASD) are characterized by strongly altered social cognition, the core feature of processing social information[1,2]. For example, impairments in social cognition, manifested as deficits in recognizing emotions, making contact, inferring thoughts, and responding emotionally to others, are seen in all phases of schizophrenia[3]. Although the interrelationship of dysfunction in social cognition and negative symptoms is still open for further discussion[2], the disrupted social cognitive abilities can be related to withdrawal from social interaction and reduced motivation for engaging in social relationships[4,5]. This reduced motivation for social interaction favors the genesis of co-morbid depression and poor functional or motivational outcomes seen in schizophrenic patients[6,7]. Therefore, given the importance and current masked dimensions of social cognition[2], testing the established schizophrenic animal models in new paradigms seems crucial.

The Disrupted-in-Schizophrenia 1 (DISC1) protein and its signaling pathway play an important role in mental diseases. The DISC1 gene was originally identified in a Scottish family in which a chromosomal translocation directly disrupts the DISC1 gene, leading to several mental disorders including schizophrenia and recurrent major depression[8,9]. Alterations in the DISC1 gene are associated with impairments in brain development in humans, as well as primates and rodents, explicating a possible mechanism for their role in several psychiatric disorders[10–14].

Even though no common genetic variants of DISC1 have been reported to be associated with mental illness[15,16], on a posttranslational level, the DISC1 protein is at the center stage of major signaling pathways relevant for regulating brain functions involved in adaptive behavior[17]. The DISC1 protein's role in neuronal development includes proliferation and migration of the neuronal progenitor cells and synapse formation and maintenance[18], and it acts as a molecular hub that interacts with dopaminergic neurotransmission components

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such as Dopamine (DA) D2 receptors and transporter[19–21]. In this regard, the association between DISC1 and the function of DA, one of the leading candidate neurotransmitters in the pathology of different psychiatric disorders, has been profoundly investigated[22–25]. The findings suggest that DISC1 has a role in the dysregulation of DA functions, such as the increase in the proportion of striatal D_2^{high} receptors[26], an increase of DAT levels in the striatum[27], and a decrease of extracellular DA levels in the nucleus accumbens[28,29].

A novel transgenic rat model (tgDISC1) has been introduced to study the function of the DISC1 protein in disease and normal cognition[21]. The tgDISC1 rat is a model for aberrant DISC1 protein signaling by modestly overexpressing non-mutant human DISC1 leading to DISC1 aggregation and thus representing a subset of sporadic cases with mental illness[30]. Furthermore, studies by different labs[21,31–34] reported a full signature of behavioral phenotypes that included amphetamine supersensitivity, hyper-exploratory behavior, and rotarod deficits associated with reductions in DA neurotransmission of the tgDISC1 rats. Thus, this tgDISC1 rat model could be exploited to investigate behavioral differences, specifically, variation in reward-related behavior caused by altered dopamine homeostasis.

Anhedonia, a consequence of deficits in reward processing, is one of the core symptoms of psychotic disorders. It is described as a lack of motivational ability in experiencing pleasure and reduced response to rewarding objects such as non-social reward (e.g., food) or social reward (i.e., social interaction)[1,34]. Considering that the DAergic system acts as a leading player in the reward learning process, control of motivation[35], and encoding the reward prediction error[36,37], anhedonia might be caused by a dysregulation in DA[38,39].

In addition to general anhedonia, social interaction and cognition have been linked to DA activity[40,41], the abnormal social behaviors in mental disease may stem from the pathological reward and DA processes, too[39,42,43]. However, it is unknown whether general, non-social anhedonia and the social deficits seen in psychiatric disorders stem from the same dopaminergic mechanisms or whether they are the consequence of separate, dissociable processes.

This study exploits the tgDISC1 animal model to address whether abnormal DA homeostasis, previously shown in these animals[21], is linked to reduced non-social reward processing, social interaction seeking, or both. To this end, we compared the choice behavior of tgDISC1 with wild-type rats in a novel paradigm[44] in which they had to choose between the possibility of social interaction with an unfamiliar juvenile conspecific or drinking sweet solutions with different sucrose concentrations.

Methods

Subjects. The experiment was conducted according to the European Union Directive 2010/63/E.U. for animal experimentation, in accordance to all procedure of ARRIVE guideliness and was approved by the local authorities (Landesamt für Natur, Umwelt und Verbraucherschutz North-Rhine Westphalia, Germany). Transgenic DISC1 (tgDISC1) Sprague Dawley rats and their sibling wild-type (WT) littermate controls were bred at the local animal facility (ZETT, Heinrich-Heine University, Düsseldorf, Germany), 36 male Sprague Dawley rats (tgDISC1 = 12, WT = 12, juvenile rats (WT) = 12) in total, consisting of 24 actor rats (PND 57–60, tgDISC1 Mweight = 285 g and WT Mweight = 304 g, at the starting day of the experiment; see supplementary materials 1, Figure S5) and 12 juvenile rats (PND 28, Mweight = 145 g at the starting day of the Social-Sucrose Preference Test (SSPT)), serving as social stimulus rat. Experimental rats were kept in groups of N = 2 for actors and N = 3 for social stimulus rats, in standard Type IV Macrolon cages in a reversed 12:12 h light–dark cycle. The stable room was kept at a constant temperature of 22 °C ± 2 and a humidity of 55% ± 2. Throughout the experiment, all actor rats received standard laboratory rodent food, ad libitum, excepting the Sucrose Discrimination Test (SDT) phase in which all actors were limited in their food intake (food per rat per day: 22 g on weekdays and 25 g on weekends). Notably, group assignments for behavioral testing was randomized within/between-group (tgDISC1 and WT) and within-group for social stimulus rats.

Screening of transgenic animals. Detection of the transgene was performed as previously described 21. In short, biopsies were digested in a buffer containing 100 mM Tris pH 8,5 mM EDTA, 0.2% SDS, 200 mM NaCl and 100 µg/ml Proteinase K and gDNA precipitated with isopropanol and solubilized in water.

For the quantification of transgene load (heterozygous versus homozygous), quantitative PCR with the StepOnePlus Real-Time PCR System and the Platinum SYBR Green qPCR SuperMix-UDG (both Thermo Fisher Scientific, USA) was performed. Primer transgene: forward 5′-CTGATCTCCAGAAGCCCAAA-3′, reverse 5′-CAGGCCTATTCCTTGACAGC-3′; primer housekeeper beta-actin: 5′-GCAACGCGCAGCCACTGTCG-3′, reverse 5′-CCACGCTCCACCCCTCTAC-3′. Quantitative PCR conditions: 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 60 °C for 1 min. The data were processed with the StepOne Software v2.3 (Thermo Fisher Scientific, USA), and transgene expression was normalized to the expression level of the housekeeper. Only transgenic homozygotes (tgDISC1) and negative littermate control animals (WT) were used for the behavioral studies. Heterozygous and female animals were not included in the study as heterozygous tgDISC1 rats only show subtle phenotypes and a lower gene dose might complicate the interpretation of results. We used only male rats to control for sex and because of co-habitation restriction in the colony room. While we acknowledge that this is a limitation to the study, we therefore excluded female tgDISC1 rats from our study. After screening, the heterozygous and social stimulus rats were euthanized using Carbon Dioxide. Female rats were used for different purposes. All actor rats were euthanized with an overdose of the anaesthetic Pentobarbital.

Apparatus and behavioral testing. Rats were trained in an X-shaped chambered sociability test (XCST). The apparatus was a radial maze (eight-arm), reduced to a cross/plus-maze setup by removing four arms (Fig. 1A), as previously described[44]. The maze consisted of a central octagon zone (36 cm diameter, so-called neutral zone) and four arms (60 cm long and 14 cm wide) that extended from the neutral zone. Every arm was

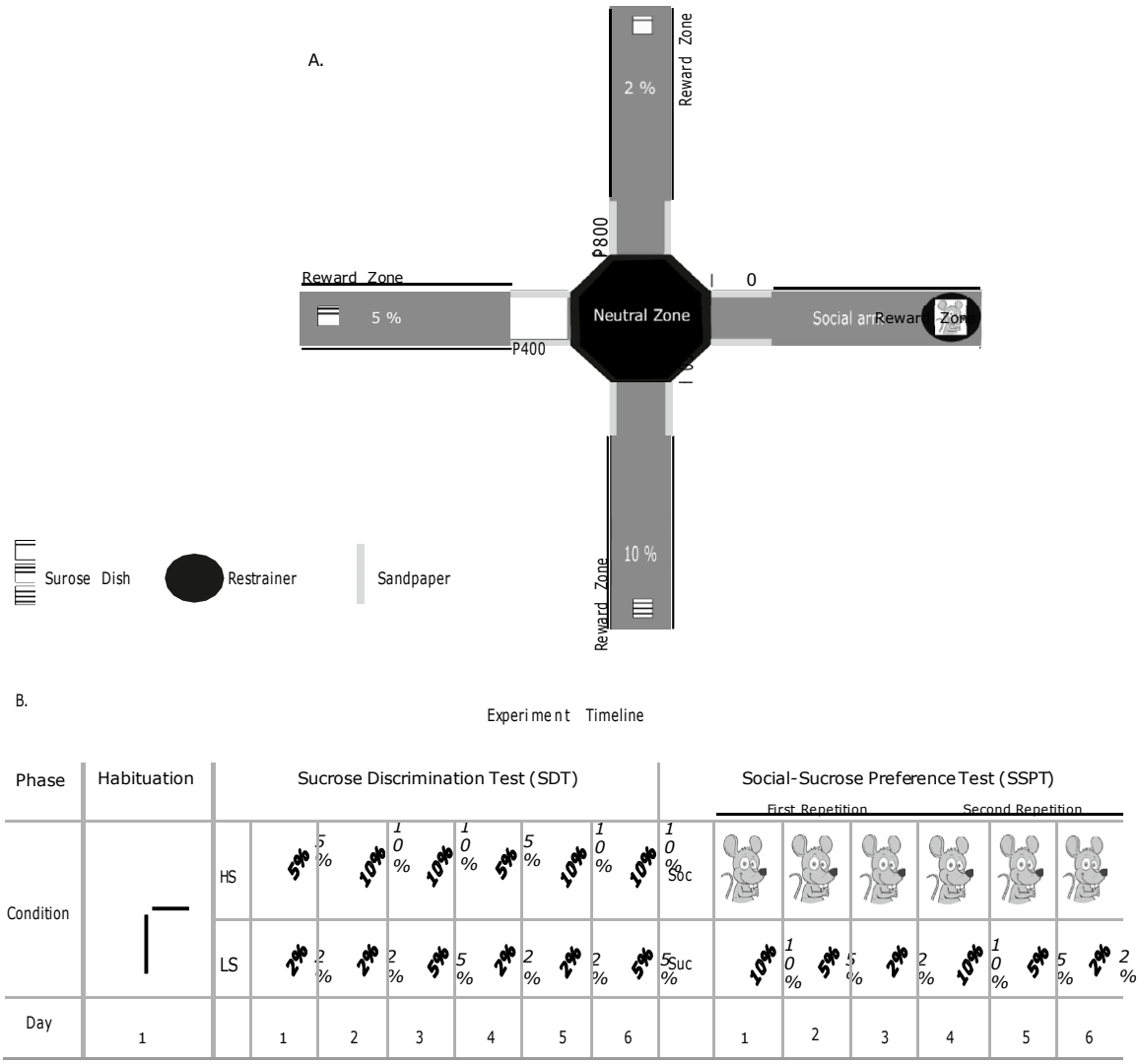

Figure 1. Setup of the study. (**A**) schematic diagram of XCST maze with non-social reward positions, the restrainer for the social reward, and sandpaper positions and grades. (**B**) shows an example of the experiment timeline for different phases, days, and conditions. Habituation: free arm investigation in the habituation phase, Sucrose Discrimination Test (SDT): HS; higher sucrose in a given trial, LS; lower sucrose in a trial, Social-Sucrose Preference Test (SSPT): Soc; social reward, and Suc; sucrose.

consistently associated with one specific reward: 3 arms were assigned to three different concentrations of a sucrose solution reward and one arm with a social stimulus rat (see below for details; Fig. 1A). During all experimental phases, SDT and Social Sucrose Preference Test (SSPT), only 2 out of 4 arms were kept open, depending on the respective task conditions, to provide a direct preference test between two given rewards. One arm was allocated to the social reward (hereinafter as social arm), and an unfamiliar juvenile rat could be placed on this arm in a fixed cylindrical restrainer built from metal bars and compact plastic for its ceiling and floor (Height: 25.5 cm, Diameter: 17 cm, Ugo Basile Sociability Cage). The restrainer was mounted on the maze at the end of the social's arm. The social stimulus rat could move around in the restrainer, and the social/physical contact with the actor rat was possible through the openings between the bars. The sucrose solution was provided to the actor rats in a cube plastic dish (8 × 8 cm) placed at the end of each arm assigned to the non-social reward (i.e., different sucrose concentrations 2%, 5%, and 10%). To facilitate spatial learning of the certain reward in each arm over days, we used sandpaper pieces (17 × 13 cm) attached to the wall at the entrance of each arm that the actor rats would touch with their whiskers when entering the arms. These sandpapers had varying grades (Fig. 1A; 2% sucrose concentration [P800], 5% sucrose concentration [P400], 10% sucrose concentration [P150], and social stimulus [P1200]), following the findings of study[4,5], which has shown that rats, through their whiskers, can differentiate between sandpapers with 200 grains/cm^2 and 25 grains/cm^2. After each trial, the maze was cleaned by using Ethanol solution (70%).

Social sucrose preference test. Behavioral testing in the SSPT was divided into three phases: Habituation, SDT, and SSPT (Fig. 1B). In all phases, actor rats from both groups (tgDISC1 & WT) were tested daily. In the first phase (Habituation), all four arms were kept open without rewards. Each actor rat explored the maze for 8 min. This phase intended to determine whether actor rats were inherently biased towards preferring one given

arm or sandpaper grade. The second phase was the SDT which was designed to verify that the rats can indeed distinguish among the three selected sucrose levels (2%, 5%, and 10%). To overcome a potential novelty-induced hypophagia, all actor rats were served 40 ml sucrose a day before starting the SDT phase. Food-restricted actor rats were tested on the SDT phase over six days. On each day, they could choose between two sucrose level concentrations in two repetitions of three different conditions (2% vs. 5%, 2% vs. 10%, and 5% vs. 10% in a fixed order, Fig. 1B). In each condition, only two arms were open, and actor rats could freely explore the maze to engage in reward consumption according to their preferences. In each trial, actors were placed in the neutral zone facing not toward open arms at the start of each session (one trial per day). Each trial took 8 min; in this time, actors could drink up to 20 ml sucrose solution per plastic dish mounted at the end of each arm. For each new trial and actor, both dishes were filled with fresh sucrose solution. We estimated the time spent in each arm in each condition (see below). After passing the SDT phase, rats were promoted to the SSPT phase. Before starting the SSPT phase, all social stimulus rats were habituated to the experiment room, maze, and restrainer for three days, each day for 8 min. To keep baseline motivations equal for both types of reward (social & non-social), after the final day of the SDT, food restriction was lifted to let actor rats gain weight over two days before starting the SSPT phase. For the remainder of the experiment, all rats had access to food ad libitum. In the SSPT phase, in each trial with a duration of 8 min, the actor rat could freely explore two open arms: the social arm with the unfamiliar social stimulus rat in the restrainer at the end and one of the arms baited with sucrose at the end. There were three conditions in the SSPT (social reward vs. 2%, social reward vs. 5%, and social reward vs. 10%). As in the SDT phase, actor rats were tested only once per day; the order of conditions was pseudo-randomized across days and rats (Fig. 1B). After completing all conditions, actor rats underwent a second round with the same rat-specific order of conditions as during the first round. Hence, the SSPT phase was completed in six days. Again, we recorded the time spent in each arm on each day of testing as the main index of preference.

In comparison to familiar conspecifics, rats usually spend more time exploring unfamiliar conspecifics[46,47]. Hence, if rats always interact with the same conspecific, the value of social interaction will progressively decline over days with increasing familiarity between rats. To counteract such a trend and maintain the novelty and value of social interaction across testing sessions in the SSPT, twelve different social stimulus rats were used. Thus, each actor rat saw a novel social stimulus rat on each day of testing. The actor-to-social stimulus assignment was counterbalanced across actor rats. We opted for juvenile social stimulus rats, instead of older, adult rats, because a decrease in social interactions and avoidance behavior was previously observed between adult rats[48]. By contrast, juvenile rats show social approach behavior and social play that are assumed to reflect predominantly positive interactions[49,50].

Behavioral analysis. Video-Tracking. To track the animals' position, we used Ethovision (EthoVision XT version 11.5, Noldus). For each phase of the study (Habituation, SDT, SSPT), different tracking arenas were designed. For the phase of Habituation, each arm was divided into two zones (Sandpaper and Reward zone). For the SDT and SSPT, we used the time that the actor rats spent in the reward zones (Fig. 1A).

Data analysis. In all analyses, the significance level was set at $p < 0.05$, and all post-hoc tests were Bonferroni-corrected for multiple comparisons. Moreover, the occupancy time for the neutral zone was excluded from all analyses.

Habituation phase. To test for spatial bias related to any inherent preference for the different reward and sandpaper zones, we performed two separate two-way repeated-measured ANOVA. The first one assessed the effect of group (tgDISC1/ WT) and sandpaper type (four types) as independent variables (IVs) on the time actors spent in each sandpaper zones as dependent variable (DV), and the second one measured the effect of group (tgDISC1/ WT) and reward zones (four zones) as (IVs) on the time actors spent in each reward zones as (DV). This data was collected during habituation to the maze when rewards were not yet introduced.

Sucrose Discrimination Performance. To determine whether actor rats discriminated between different sucrose levels in the SDT, we calculated the SDT sucrose solution preference score for each condition and repetition in the SDT as a percentage of time spent in the relatively higher sucrose arm (the arm yielding the higher sucrose concentration on that day; Fig. 1B).

$$\text{Higher sucrose preference score} = \frac{\text{Time(s) spent in higher sucrose zone}}{(\text{Time(s) spent in higher sucrose zone} + \text{Time(s) spent in lower sucrose zone})} * 100$$

By using these sucrose preference scores, we conducted a three-way mixed ANOVA on the higher sucrose zones preference score (DV) with the group as a between-subject factor (tgDISC1 vs. WT), condition (three levels: *2% vs. 5%, 2% vs. 10%,* and *5% vs. 10%)* and test repetition (first vs. second) as within-subject factors.

To control for potential differences in motor activity between tgDISC1 and WT rats, we additionally measured the distance moved (in cm) per day in the entire maze. We ran an independent samples t-test to analyse the group (tgDISC1/ WT) effect on the distance moved (included all repetitions and conditions).

In addition, to determine each group's preference for a higher or lower sucrose reward in a given SDT condition, we also conducted six one-sample t-tests against indifference (50%) on the SDT preference values for higher sucrose at the second repetition.

To explore whether there was a between-group difference in the correlation between *Entrance frequency* and total *duration of stay* (in seconds) in a reward zone, we ran two separate Pearson correlations per group: (1) in

	Sandpaper zone				Reward zone			
	df	f	*p* value	η_p^2	df	f	*p* value	η_p^2
Group	1	.105	.752	.009	1	.034	.857	.003
Zone	1.81	2.34	.125	.176	1.67	2.81	.093	.204
Group*Zone	1.67	.387	.648	.034	3	.483	.697	.042

Table 1. Assessment of inherent bias toward a sandpaper or a reward zone.

the higher sucrose zone. (2) in the lower sucrose zone on the mean respective value of each actor rat across all conditions and repetitions.

Social-Sucrose Preference Analysis. For the SSPT, we calculated a social reward preference score:

$$\text{SSPT social reward preference score} = \frac{\text{Time(s) in the social reward zone}}{(\text{Time(s) in the social reward zone} + \text{Time(s) in the sucrose zone})} * 100$$

To analyse between-group differences in preference between social (social stimulus rat) or non-social rewards (different sucrose concentrations), we ran a three-way mixed ANOVA to assess the effect of group (tgDISC1 vs. WT), repetition (first vs. second), and condition (*social reward vs. 2%, social reward vs. 5%, social reward vs. 10%*) on the social reward preference score.

Additionally, to determine each group's preference for social or non-social reward in a given SSPT condition, we conducted six one-sample t-tests versus indifference (50%) on the SSPT social reward reference scores, averaged per animal across the two experimental repetitions.

Again, to determine if there was a difference in the distance moved (cm) between tgDISC1 and WT actors, we conducted an independent samples t-test.

Similar to the SDT, we explored whether there was a between-group difference in the correlation between *Entrance frequency* and total *duration of stay* (in seconds) in a reward zone. We ran two separate Pearson correlations per group: (1) in the social reward zone. (2) in the sucrose zone on the mean respective value of each actor rat across all conditions and repetitions.

Software. All statistical analyses were carried out using SPSS Statistics (version 24; IBM, USA), and figures were created using Jupyter Notebook[51] through the packages matplotlib[52], pandas[53], ptitprince[54] and seaborn[55]. For improvement of figures, we used Inkscape[56].

Results

Habituation phase. To investigate a potential spatial bias related to any inherent preference for the different reward zones and sandpapers, we executed two distinct repeated measures ANOVAs. These analyses showed no significant bias for either the sandpaper identity or a spatial reward zones location (Table 1).

Sucrose discrimination test. To determine whether actor rats could discriminate between different reward sucrose concentrations (2%, 5%, and 10%), we conducted a three-way mixed ANOVA. Our results did *not* show a significant main effect of group (tgDISC1 vs. WT) on the time spent in the respective higher reward zone ($F(1,22)=0.001$, $p=0.978$, ANOVA, Fig. 2A), but we did find a main effect of condition (2% vs. 5% < 2% vs. 10 and 5% vs. 10%; $F(1.57,34.5)=13.7$, $p \leq 0.001$, ANOVA, Fig. 2B) and repetition ($F(1,22)=29.4$, $p \leq 0.001$, ANOVA, Fig. 2C). Bonferroni-corrected post hoc tests revealed that all rats spent more time in the relatively higher sucrose zone in all three conditions; they spent more time in the 10% zone when the alternative was 5% or 2% sucrose, and they spent more time in the 5% than the 2% zone (Table 2A; Fig. 2B). This suggests that all rats were sensitive to relative differences in sucrose concentrations. The Bonferroni test (Table 2B) also showed that rats in both groups significantly spent more time in the higher sucrose zones on the second compared to the first repetition, reflecting learning of the spatial reward arrangement (Fig. 2C). The analysis did not reveal any significant interaction effect (Table 3A). Overall, these results demonstrate that all rats learned to express a clear preference order from high to low sucrose. It has been suggested that the dysregulation in dopaminergic signaling in tgDISC1 rats goes along with locomotor hyperactivity[21]. Hence, to ensure that there was no systematic difference in locomotion between tgDISC1 and WT rats in our tasks, we compared the total distance moved across all conditions and repetitions between animal groups. However, we found no significant difference in distance moved between tgDISC1 and WT rats ($(t(22)=0.101$, $p=0.921$, (tgDISC; [M = 3536, SE = 125] WT; [M = 3516, SE = 148]), for more details, see Table 2F, G). The post-hoc one-sample t-tests against indifference (50%) revealed that all rats spent more time in the higher sucrose zone than in the lower sucrose zone in all conditions, at the second repetition (see supplementary materials, Table S1). Finally, we computed two Pearson correlations per group to determine whether there was a significant correlation between the *frequency of entrance* and *duration of stay* in each zone. The results did not reveal any significant correlations (Table 2C, Fig. 2D,E). For more information about between-group differences in the duration of stay in the higher and lower sucrose zones, see (supplementary materials, figure S4. A and B).

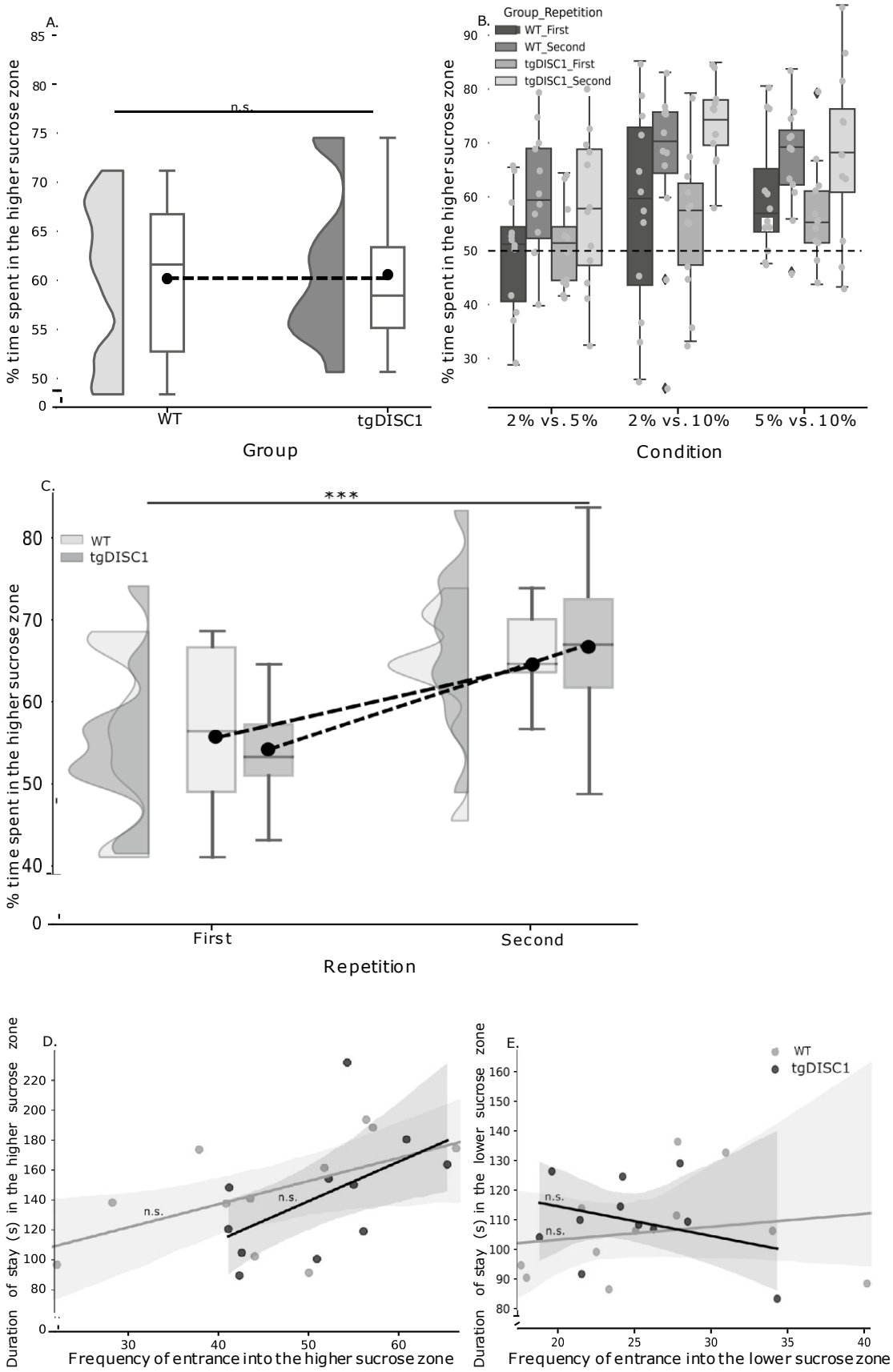
A.
% time spent in the higher sucrose zone
n.s.
WT
tgDISC1
Group
B.
Group_Repetition
WT_First
WT_Second
tgDISC1_First
tgDISC1_Second
% time spent in the higher sucrose zone
2% vs. 5%
2% vs. 10%
5% vs. 10%
Condition
C.
***
WT
tgDISC1
% time spent in the higher sucrose zone
First
Second
Repetition
D.
Duration of stay (s) in the higher sucrose zone
n.s.
n.s.
Frequency of entrance into the higher sucrose zone
E.
WT
tgDISC1
Duration of stay (s) in the lower sucrose zone
n.s.
n.s.
Frequency of entrance into the lower sucrose zone

Figure 2. Sucrose Discrimination Test Results. (**A**) The time spent in higher sucrose zones per group included all conditions and repetitions of SDT. The raincloud and whisker plots show the between-group differences in the distribution of time spent in the higher sucrose zone per group. The dashed line connects each group's mean of time spent (across all conditions and repetitions) in the higher sucrose zone. (**B**) Time spent in the higher reward zone in each condition; The dashed line indicates the indifference point (50%). (**C**) The change of time spent in the higher sucrose, per repetition and group. The raincloud and whisker plots show the change in the distribution of time spent in the higher sucrose zone per repetition and group. The dashed line connects each group's mean of time spent in the higher sucrose zone per repetition. (**D**) Correlation between duration of stay and frequency of entrance in higher sucrose zone. Each data point represents the mean across all conditions and repetitions for one actor rat. The gray zones represent standard error. (**E**) Correlation between duration of stay and frequency of entrance in lower sucrose zone. Each data point represents the mean across all conditions and repetitions for one actor rat. The gray zones represent standard error. Error bars represent the standard error of the mean (SEM). ***$p < .001$, n.s.; not significant. In whisker plots, error bars represent the minimum and maximum of data sets.

Social sucrose preference test. To investigate between-group differences in the times spent in the respective rewards zones in the SSPT, we ran a three-way mixed ANOVA on the social reward preference score. This analysis revealed a significant difference between groups in the social reward preference score ($F(1,22) = 7.3$, $p = 0.013$, ANOVA, Fig. 3A). This result showed that tgDISC1 rats spent a significantly shorter proportion of time (M = 57.5%, SE = 1.0) in the social reward zone than the WT rats (M = 63.6%, SE = 1.3). There was also a significant main effect of sucrose on the time spent in the social reward zone ($F(2,44) = 3.7$, $p = 0.032$, ANOVA, Fig. 3B). Descriptively, WT rats spent more time in the social reward zone when the alternative was a 2% or a 5% sucrose solution than when the alternative was a 10% solution. However, Bonferroni adjusted post-hoc comparisons (Table 2D) did *not* reveal a significant difference in the percent time rats spent in the social reward zone between the conditions. Accordingly, post-hoc one-sample t-tests against indifference (50%) revealed that all rats spent more time in the social reward zone than in the sucrose zone in all conditions (see supplementary materials, Table S2). Next, we also found a significant main effect of repetition on the proportion of time spent in the social reward zone ($F(1,22) = 6.6$, $p = 0.017$, ANOVA, Fig. 3C). Rats spent significantly more time in the social reward zone on the first than on the second repetition (Table 2E). There were no significant interaction effects (Table 3B). To make sure that the difference in preference for the social zone between tgDISC1 and WT rats was not the consequence of a general difference in locomotor activity[21], we, again, compared the total distance moved across all conditions and repetitions between animal groups in the SSPT. However, as in the SDT, we found no significant difference in distance moved between tgDISC1 and WT rats (($t(22) = 0.793$, $p = 0.436$, (tgDISC; [M = 6372, SE = 297] WT; [M = 6041, SE = 293], for more details, see Table 2H, I), suggesting that the tgDISC1 effects on social preference are unlikely the result of altered locomotion behavior. Finally, we ran two Pearson correlations per group to determine whether there was a significant correlation between the *frequency of entrance* and *duration of stay* in each zone (sucrose and social rewards). We found a significant correlation between those variables (Table 2J) in the WT rats in the social rewards zone ($r(12) = 0.954$, $p \leq 0.001$, Fig. 3D), indicating that those WT rats that entered the social zone more often also stayed longer. This relationship could not be found in the tgDISC1 rats in the social rewards zone ($r(12) = -0.432$, $p = 0.161$, see Table 2J and Fig. 3E). For more information about between-group differences in the duration of stay in the social and sucrose reward zones, see (supplementary materials, Figure S4. C&D) Taken together, all rats showed a preference for the social over the sucrose reward in all conditions, but the preference strength, indicated by the percent time interacting with the juvenile, was higher in the WT than the tgDISC1 rats.

Discussion

In this study, we evaluated the effect of aberrant DISC1 signaling on rat behaviour in a paradigm relevant to social cognitive deficits in mental disease: rats could choose between two types of rewards: sweet solutions at variable sucrose concentrations (non-social reward) and the opportunity to interact with a juvenile conspecific (social reward). In our sucrose discrimination task, we found that WT as well as tgDISC1 rats successfully distinguished between sucrose levels and revealed a clear, well-structured preference for higher sucrose concentrations. Hence, we found no evidence to assume an effect of aberrant DISC1 signaling on basic, non-social reward processing. However, when given the choice between drinking sucrose solution or interacting with a conspecific, tgDISC1 rats spent less time with the conspecific than the WT rats, but more time in the non-social reward zones. This might either suggest that, compared to WT rats, tgDISC1 rats had reduced interest in social contact, or that they were lured away from the social interaction zone by the prospect of ingesting more sugar solution in the sucrose zones. However, we consider the latter explanation unlikely since, in the SDT, we found no difference in sucrose preference and sucrose reward-seeking behavior between tgDISC1 and WT rats, suggesting that the reduced time that tgDISC1 rats spent with the conspecific in the SSPT was probably not due to hypersensitivity to sucrose rewards, but the result of genuinely reduced interest in social contact.

By what mechanisms could tgDISC1 rats attach less value to social interaction? It is plausible to assume that this was the result of altered DA signaling in the brain, in particular in the mesolimbic reward system. The reported decreased basal level of DA in striatal samples of tgDISC1 rats was caused by increased D2 receptor and striatal dopamine transporter (DAT) levels, resulting in much faster synaptic DA clearance due to an upregulation of presynaptic DAT[21]. Notably, the upregulation of presynaptic DAT leads to lower net synaptic DA in tgDISC1s. In mice[57], it is shown that an oxytocin-dependent DAergic projection from the VTA to the NAcc Shell region

A	Condition	Mean(%)	Sd.Error	Conditions	p value	Group Mean (%)	Group-Std.Error
SDT: Time in higher sucrose zone	2% vs 5%	54.5	1.76	2% vs 10%	.001	WT = 54.8	WT = 2.54
				5% vs 10%	.000	tgDISC1 = 54.2	tgDISC1 = 2.4
	2% vs 10%	63.7	2.59	2% vs 5%	.001	WT = 62.7	WT = 3.63
				5% vs 10%	1.000	tgDISC1 = 64.7	tgDISC1 = 2.8
	5% vs 10%	63.2	1.98	2% vs 5%	.000	WT = 63.7	WT = 2.26
				2% vs 10%	1.000	tgDISC1 = 62.7	tgDISC1 = 2.8
B	**Repetition**	**Mean(%)**	**Sd.Error**	**Repetition**	**p value**	**Group Mean(%)**	**Group-Std.Error**
SDT: Time in higher sucrose zone	First	55.4	2.04	Second	.000	WT = 56.2	WT = 2.4
						tgDISC1 = 54.5	tgDISC1 = 1.7
	Second	65.6	2.04			WT = 64.6	WT = 2.15
						tgDISC1 = 66.5	tgDISC1 = 2.4
C	**Group**	**Zone**					
Correlation: between duration of stay and frequency of entrance in SDT	WT	Higher	Lower				
		Pearson'r. 557 *p* value .075	Pearson'r. 557 *p* value .595				
	tgDisc1	Pearson'r. 532 *p* value .092	Pearson'r. 321 *p* value .335				
D	**Condition**	**Condition-Mean(%)**	**Condition-Sd.Error**	**Conditions**	**p value**	**Group Mean(%)**	**Group-Std.Error**
SSPT: Time in social reward zone	Social reward vs. 2%	62.5	1.27	Social reward vs. 5%	1.000	WT = 66.2	WT = 1.26
				Social reward vs. 10%	.135	tgDISC1 = 58.9	tgDISC1 = 1.9
	Social reward vs. 5%	61.9	1.4	Social reward vs. 2%	1.000	WT = 66.0	WT = 1.9
				Social reward vs. 10%	.116	tgDISC1 = 57.8	tgDISC1 = 1.9
	Social reward vs. 10%	57.3	2.1	Social reward vs. 2%	.135	WT = 58.7	WT = 3.0
				Social reward vs. 5%	.116	tgDISC1 = 55.9	tgDISC1 = 1.7
E	**Repetition**	**Mean(%)**	**Sd.Error**	**Repetition**	**p value**	**Group Mean(%)**	**Group-Std.Error**
SSPT: Time in social reward zone	First	62.4	1.17	Second	.017	WT = 64.3	WT = 1.5
						tgDISC1 = 60.6	tgDISC1 = 1.7
	Second	58.7	1.48			WT = 63.0	WT = 2.1
						tgDISC1 = 54.4	tgDISC1 = 1.1
F	**Condition**	**Condition-Mean(cm)**	**Condition-Sd.Error**	**Conditions**	**p value**	**Group Mean (cm)**	**Group-Std.Error**
SDT: distance moved	2% vs 5%	3855	108	2% vs 10%	.059	WT = 3767	WT = 140
				5% vs 10%	.000	tgDISC1 = 3943	tgDISC1 = 138
	2% vs 10%	3503	108	2% vs 5%	.059	WT = 3551	WT = 169
				5% vs 10%	.232	tgDISC1 = 3455	tgDISC1 = 144
	5% vs 10%	3275	123	2% vs 5%	.000	WT = 3333	WT = 180
				2% vs 10%	.232	tgDISC1 = 3216	tgDISC1 = 129
G	**Repetition**	**Mean(cm)**	**Sd.Error**	**Repetition**	**p value**	**Group Mean(cm)**	**Group-Std.Error**
SDT: distance moved	First	3676	108	Second	.035	WT = 3537	WT = 94
						tgDISC1 = 3814	tgDISC1 = 91
	Second	3412	105			WT = 3563	WT = 214
						tgDISC1 = 3262	tgDISC1 = 133
H	**Condition**	**Mean(cm)**	**Sd.Error**	**Conditions**	**p value**	**Group Mean (cm)**	**Group-Std.Error**
SSPT: distance moved	Social reward vs. 2%	5433	293	Social reward vs. 5%	.008	WT = 5364	WT = 305
				Social reward vs. 10%	.051	tgDISC1 = 5502	tgDISC1 = 316
	Social reward vs. 5%	6645	334	Social reward vs. 2%	.008	WT = 6648	WT = 485
				Social reward vs. 10%	1.000	tgDISC1 = 6641	tgDISC1 = 390
	Social reward vs. 10%	6541	276	Social reward vs. 2%	.051	WT = 6263	WT = 309
				Social reward vs. 5%	1.000	tgDISC1 = 6109	tgDISC1 = 286
I	**Repetition**	**Mean(cm)**	**Sd.Error**	**Repetition**	**p value**	**Group Mean(cm)**	**Group-Std.Error**
SSPT: distance moved	First	6040	175	Second	.065	WT = 5658	WT = 171
						tgDISC1 = 6421	tgDISC1 = 289
	Second	6373	277			WT = 6525	WT = 404
						tgDISC1 = 6423	tgDISC1 = 292

J	Group	Zone					
Correlation: between duration of stay and frequency of entrance in SSPT	WT	Social reward	Sucrose				
		Pearson'r. 951 *p* value .000	Pearson'r. 573 *p* value .052				
	tgDisc1	Pearson'r. 432 *p* value .335	Pearson'r. 334 *p* value .289				

Table 2. The results of the pairwise comparisons (Bonferroni) for within-subject factors (Condition/Repetition) in both tasks (SDT/SSPT) on IVs (Time in reward zone/Distance moved) respectively and the result of Pearson correlations in both tasks (SDT/SSPT).

SDT phase (Time in higher sucrose zone)	f-value	*p* value	η^2_p
A			
Group*Repetition	(1,22) = .956	.339	.042
Group*condition	(2,44) = .711	.344	.015
Condition*Repetition	(2,44) = .577	.566	.026
Group*condition*Repetition	(2,44) = 1.83	.175	.076
SSP T phase (Time in social reward zone)	**f-value**	***p* value**	η^2_p
B			
Group*Repetition	(1,22) = 2.88	.104	.116
Group*condition	(2,44) = .956	.392	.042
Condition*Repetition	(2,44) = .063	.906	.003
Group*condition*Repetition (2,44) = 1.09	.344	.047	

Table 3. The results of three-way mixed ANOVA (not-significant interaction effects) in both tasks (SDT/SSPT).

is necessary and sufficient to support real-time social conditioned place preference, strongly suggesting that an altered DA turnover in the NAcc could interfere with social interaction preferences.

Developmentally, in terms of neuro development, the previous results on the modification (increase in binding) of D2 receptor density by the change in the social environment (social isolation)[58], the role of DAT levels in the regulation of social behaviors (by DAT knockout of mice)[59], and the highlighted interplay of regular social contact and striatal function[60], all suggest that striatal DA signaling is critical for proper social interactions. Behaviorally, in terms of behavior, the tgDISC1s rats' reduced motivation to seek out juvenile conspecifics interaction opportunities aligns with previous studies with neuropsychiatric patients who revealed similar dissociations between social and non-social reward processing[61–63]. For example, patients with schizophrenia may experience impairment and disconnection between several components of social motivation required for interactions with positive social outcomes. Likewise, the result of an investigation[64,65] found the selective anhedonia (diminished enjoyment) only for social and not non-social reward in children with ASD. A more recent study[64] reported a decreased reward prediction error signaling (a critical component of reward-based learning) in frontal brain regions only for social reward in patients with ASD, in line with insensitivity to social rewards found for this group[66].

Another study[67] also reported a finding pointing to the distinctiveness of social and non-social information processing in schizophrenia and suggested that individuals with schizophrenia may show a selective impairment in processing social stimuli. Likewise, in depression, an association between elevated depressive symptoms and decreased approach to social reward (social feedback) was reported; however, in the same study, the results showed a higher effort by individuals with elevated depression for food rewards[68].

In summary, our results demonstrate that the tgDISC1 rat features deficits in social interaction and thus is a possible model for this phenotype relevant in schizophrenia or other mental diseases. The here presented social deficits of the tgDISC1 rat align well with the goals of the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) that has included social cognition as a major category for biology-based monitoring of clinical trials[69].

Limitations and future directions. Rats use different sensory inputs (auditory, olfactory, and visual) in their social interactions. However, it is thought that the most significant rewarding aspect of social interactions for rats is thigmotactic stimulation. In addition, providing a sufficiently large spatial area for social interaction plays an important role in reward experience as well[70,71]. In our design, however, rats could only interact through steel bars which potentially decreases the subjectively rewarding experience of the social interactions. Therefore, in future studies, improving the design in a way that facilitates social interactions is recommended.

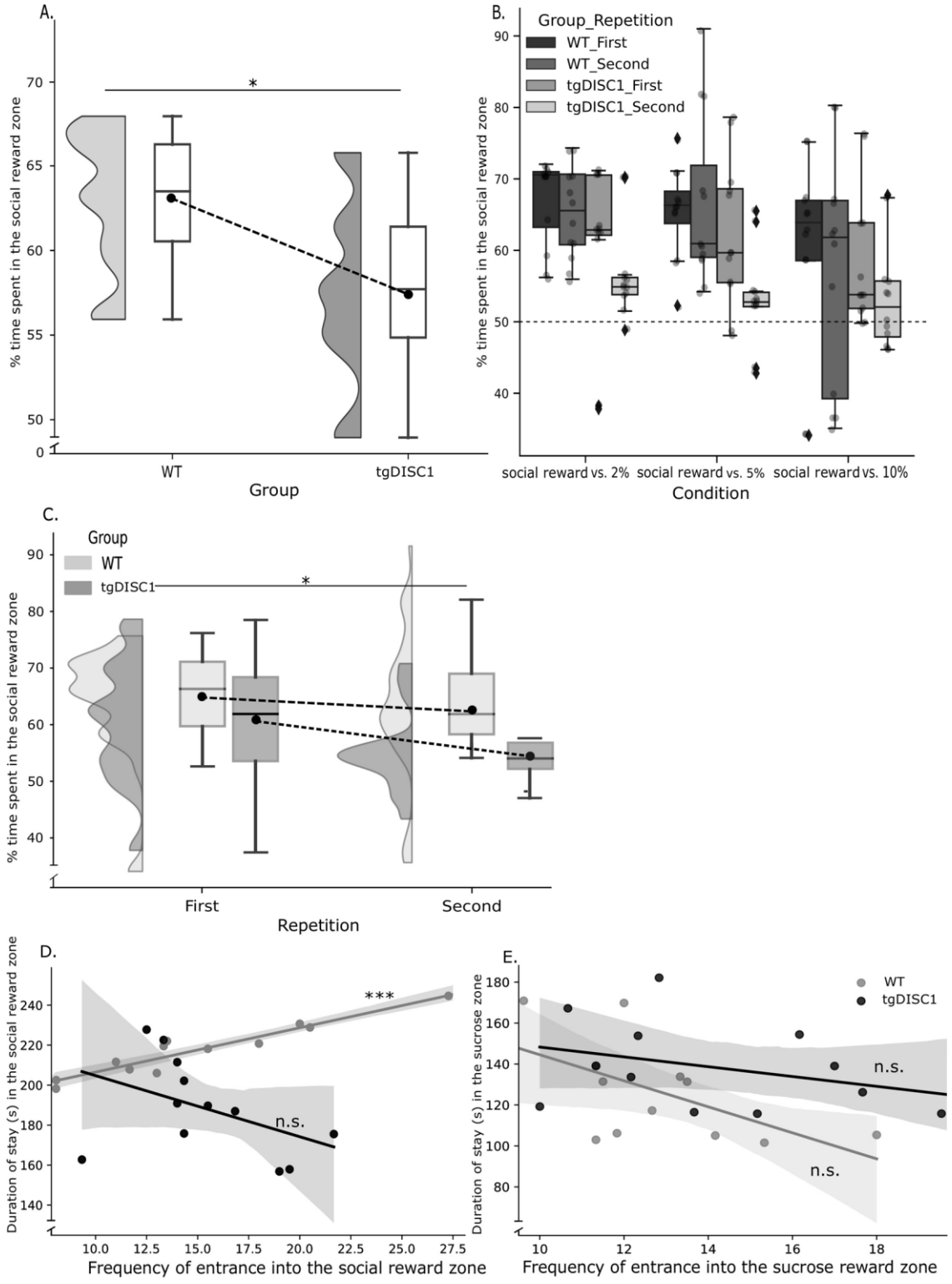

Figure 3. Social Sucrose Preferences Results. (**A**) The time spent in the social reward zone per group included all conditions and repetitions of SSPT. The raincloud and whisker plots show the between-group differences in the distribution of time spent in the social reward zone per group. The dashed line connects each group's mean of time spent (including all conditions and repetitions) in the social reward zone. (**B**) Time spent in the social reward zone. The dashed line indicates the indifference point (50%). (**C**) The change of time spent in the social reward zone per repetition and group. The raincloud and whisker plots show the change in the distribution of time spent in the social reward zone per repetition and group. The dashed line connects each group's mean of time spent in the social reward zone per repetition. (**D**) Correlation between duration of stay and frequency of entrance in social reward zone, per group. Each data point represents the mean of all conditions and repetitions for one actor rat. The gray zones represent standard error (**E**) Correlation between duration of stay and frequency of entrance in sucrose zone, per group. Each data point represents the mean of all conditions and repetitions for one actor rat. The gray zones represent standard error. Error bars represent the SEM. $*p < .05$, $**p < .01$, $***p < .001$, n.s.; not significant. In whisker plots, error bars represent the minimum and maximum of data set.

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Furthermore, we did not test the olfactory performance of the animals, which is a key factor in social behavior[72]. Considering that dopaminergic transmission plays an important role in the olfactory circuit[73], it might be informative to investigate the difference of olfactory performance between tgDISC1 and WT in future studies.

It has been pointed out that despite activation of the same brain area (ventromedial prefrontal cortex) by both types of rewards (social/non-social), certain areas, such as the amygdala, are more specifically involved in social reward and social cognition[69]. Alongside other study[74], and we recently demonstrated that amygdala lesions reduce prosocial behavior in rats[75]. This possible regional specificity[76] might open up possibilities for local DA transmission reinstatement with the aim of rescuing the DISC1 impairment in social reward processing shown here.

In addition, to design this study we relied on the neuronal findings of study[21] which was performed only with male tgDISC1 rats, thus, we did not use female rats, which should also be considered in future studies.

Last but not least, rats communicate through ultrasonic vocalizations (USV) by employing certain call types that are tuned towards social and non-social conditions[44]. Therefore, in future studies, in addition to the neuronal investigations, recording and analysing USVs in a similar design could shed more light on differences in the subjective affective state of the rats.

Taken together, the results of this study align with previously found associations between DISC1 and neuronal/behavioural impairments and differences in social vs. non-social reward processing in patients with various psychiatric disorders, suggesting that the tgDISC1 animal model is sensitive to capture the altered social reward processing seen in psychiatric illness, qualifying it as a potential standard for understanding the neural and psychopharmacological basis of abnormal social behavior in mental diseases.

Data availability

The datasets collected and analysed for this study are available from the corresponding author on reasonable request.

Received: 8 February 2022; Accepted: 1 June 2022
Published online: 17 June 2022

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Author contributions

MS: conducted the experiment, collected the data, analyzed, created graphs and tables, and wrote the manuscript. SS: contributed to data analysis, writing, and revising the manuscript. MW: designed the experiment, contrib- uted to data analysis and revised the manuscript. ST: performed the entire process of breeding the transgenic animals and collaborated in the discussion in addition to revising the manuscript. CK: revised the manuscript. TK: revised the manuscript.

Funding

Open Access funding enabled and organized by ProjektDEAL.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/ 10.1038/s41598-022-14102-3.

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3.3 Paper III

The main question in this study was whether dysregulation of the DA system and social anhedonia, as a neural/behavioral phenotype of tgDISC1 rats, could be tracked through their 50-kHz USVs.
The result of this study was published in the Brain Behaviour journal under the title: **50-kHz ultrasonic vocalizations do not signal social anhedonia in transgenic DISC1 rats.**

Abstract

Patients diagnosed with neuropsychiatric disorders, such as autism and schizophrenia, suffer from disorganized speech. The disrupted-in-schizophrenia 1 (DISC1) protein pathway is considered a risk factor for the development of several psychiatric disorders and plays an important role in the dysregulation of dopamine (DA), which in turn plays an important role in the regulation of ultrasonic vocalizations (USVs) in rats. Moreover, the DISC1 protein pathway has been identified as a cause of social anhedonia, that is, a decrease in the drive for social interactions. USVs transmit specific affective information to other rats, with 50-kHz calls indicating a positive affective state in rats. Dysregulation of the dopaminergic system impacts the qualitative and quantitative features of USVs, such as duration, peak frequency, and the call rate. In this study, we thus used a well-established transgenic DISC1 (tgDISC1) rat line to investigate whether the neural (decreased DA levels in the dorsal striatum, amygdala, and hippocampus (HPC)) and behavioral (social anhedonia) features of tgDISC1 rats could be manifested through the modulation of their 50-kHz USVs. Analyses of three features (call rate, duration, and peak frequency) of all 50-kHz revealed no significant differences between groups, suggesting decreased DA levels in the dorsal striatum and amygdala, and HPC may affect social interaction but leave 50-kHz USV production intact.

1 INTRODUCTION

The disrupted-in-schizophrenia 1 (DISC1) gene was initially identified in a Scottish family with an unusually high prevalence of mental disorders, including schizophrenia, and the disruption was due to a balanced translocation of the chromosome (1:11) (q43, q21) (Millar et al., **2001**). The DISC1 protein signaling pathway has been linked to multiple deficits in brain development both in humans and animals, which may lead to schizophrenia, bipolar disorder, recurrent major depression, and other neuropsychiatric disorders in humans, as well as phenotypical alterations reminiscent of human psychiatric disorders in animals (Austin et al., **2003**; Clapcote et al., **2007**; Hashimoto et al., **2006**; Kirsty Millar et al., **2000**; Shokouhifar et al., **2019**). Recently, several studies have shown that the neural dysregulation caused by DISC1 impairs the Dopamine (DA) system by increasing the affinity of DA-D2 receptors and increasing the removal of DA from the synaptic cleft because of translocation of the DA transporter, resulting in decreasing DA levels in the dorsal striatum, amygdala, and hippocampus (HPC, Hennah & Porteous, **2009**; Ripke et al., **2014**; Trossbach et al., **2016**; Wang et al., **2017**). In a recent study (Seidisarouei et al., **2022**), we compared the choice behavior of transgenic DISC1 (tgDISC1) rats (Klein & Platt, **2013**; Seidisarouei et al., **2022**; Wang et al., **2022**) with that of wild-type (WT) control rats in a novel reward paradigm in which animals could choose between two types of reinforcers, an opportunity for social interaction (social reward) versus consumption of sucrose solution (nonsocial reward). tgDISC1 rats showed a significantly reduced interest in social interaction but a similar preference for sucrose consumption, compared to WT rats. In other words, tgDISC1 rats spent significantly less time interacting with a juvenile conspecific, which may resemble social anhedonia, that is, the decreased interest in potentially rewarding social activities (Chapman et al., **1976**), seen in patients with depression or schizophrenia (American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders [DSM-IV], **2012**). This social anhedonia in tgDISC1 rats, most likely caused by DA dysregulation, may also manifest in rat vocal communication, as DA also plays an important role in the processing and production of rat 50-kHz USVs (Burgdorf & Knutson, **2001**). In support of this idea, it has been reported that rats with reduced social motivation vocalized fewer 50-kHz USVs (Riaz et al., **2015**) and rats that selectively bred to low levels of 50-kHz USVs showed significant changes in their social interactions (Harmon et al., **2008**). Presumably, a deficit in USV expression and perception might interrupt the natural back-and-forth of social communication, thereby reducing social interest or motivation.

In recent years, a body of convergent studies demonstrated the implication and importance of USVs by rats in representing their emotional and motivational states.

In terms of the frequency at which USVs are emitted, they can broadly be categorized as 22 and 50 kHz. The USVs of these two distinct families (22 and 50 kHz) signal aversive and appetitive qualitative information, respectively, about the rats' affective states that may be triggered by either social or nonsocial stimuli that possess affective valence. Rats emit 22-kHz USVs under aversive conditions such as fear, pain, and danger (Sadananda et al., **2008**; Wöhr & Schwarting, **2010**), whereas they emit 50-kHz calls in response to or anticipation of appetitive stimuli, such as playing, social interaction, eating, mating, and administration of drugs with rewarding properties (Bialy et al., **2000**; Brudzynski & Pniak, **2002**; Mulvihill & Brudzynski, **2018**; Simola & Granon, **2019**). Accordingly, playback of prerecorded appetitive 50-kHz USVs induces social approach behavior that paves the way for social interactions, supporting the idea that 50-kHz USVs serve as social contact calls to (re)establish or maintain contact between rats (Kalenscher et al., **2020**; Wöhr & Schwarting, **2012**). Because of these properties, USVs are believed to have substantial adaptive value for the survival and well-being of rats as a social species (Wöhr & Schwarting, **2013**).

To better understand and more clearly interpret rat USV, it is fundamental to uncover its neural basis. In this regard, studies demonstrated a fundamental role of dopaminergic neurotransmission in USV production. For example, the DA agonist apomorphine (by acute systemic injection) can promote 50-kHz calls (Williams & Undieh, **2010**), and the D2/D3 agonist quinpirole (by intra-NcAcc administration) modulates USV production (Brudzynski et al., **2012**). Conversely, DA receptor antagonists prevented the expected emission of 50-kHz USVs by various rewards (natural and artificial), such as systemic cocaine (Williams & Undieh, **2010**), intracerebral amphetamine (AMPH) (Thompson et al., **2006**), tickling, electrical brain stimulation (Burgdorf et al., **2007**), and mating contexts (Bialy et al., **2010**; Ciucci et al., **2007**). Furthermore, DA agonists or antagonists cause not only changes in quantity but also the quality of 50-kHz USVs. For example, haloperidol is a D2 receptor antagonist, reduces the bandwidth, amplitude, and complexity of 50-kHz calls, similar to the effects of a unilateral infusion of 6-hydroxydopamine (6-OHDA, Ciucci et al., **2007, 2009**). In addition to the decreased call rate and altered call profile, antagonism of D1 and D2 receptors alone or combined altered several features of 50-kHz calls, such as duration, amplitude, and latency to call (Ringel et al., **2013**; Wright et al., **2013**).

Thus, 50-kHz USVs are associated with appetitive social and nonsocial situations and DA. We can exploit this property to quantify the expected and experienced value that rats attribute to a reward, including social contact (Heyse et al., **2015**; Knutson et al., **1998, 1999**). Therefore, here, we investigated whether changes in patterns of 50-kHz USVs emission accompany the social anhedonia expressed in tgDISC1 rats. To this end, we analyzed different quantitative and qualitative characteristics of the 50-kHz USVs, such as call rate, duration, and peak frequency, in rats performing a social decision task in which they chose between social and nonsocial rewards.

2 MATERIALS AND METHODS

Subjects

The animal experiment was permitted by the local authorities (Landesamt für Natur, Umwelt und Verbraucherschutz North Rhine-Westphalia, Germany) and conducted according to the European Union Directive 2010/63/E.U. The findings that, compared to WT rats, tgDISC1 rats are less motivated to socially interact with juvenile conspecifics, but have comparable preferences for sucrose rewards, have been published before (Seidisarouei et al., **2022**), based on the current sample of animals. tgDISC1 Sprague Dawley rats and their sibling WT littermate controls were bred at the local animal facility (ZETT, Heinrich-Heine University, Düsseldorf, Germany). In total, we used 36 male Sprague Dawley rats for our study. The rats were divided into three groups: (1) tgDISC1 group (n = 12 rats), weighing m = 285 g and aged 57–60 days at the beginning of the Social-Sucrose Preference Test (SSPT), serving as the actor rats; (2) WT group (n = 12 rats), weighing m = 304 g and aged 57–60 days at the beginning of the SSPT, serving as the actor rats; and (3) a juvenile WT group (n = 12 rats), weighing m = 145 g and aged 28–30 days, serving as the social stimuli. The tgDISC1 rats were bred through the identical method introduced by Trossbach et al. (**2016**). Experimental rats were kept in groups of n = 2 for actors and n = 3 for social stimulus rats in standard Type IV Makrolon cages in a reversed 12:12 h light–dark cycle. The stable room was kept constantly at a temperature of 22°C±2 and a humidity of 55% ±2. All actor rats received standard laboratory rodent food, ad libitum.

Behavioral task

The USV data analyzed in this study were recorded from the SSPT (see later). SSPT was the final phase of a behavioral study published recently (Seidisarouei et al., **2022**), and the so-called X-shaped chambered sociability test (XCST, see Figure **1A**, Seidisarouei et al., **2021**). The XCST task is designed to detect differences in preference for two types of rewards, social reward (interaction with the social stimulus rat) and nonsocial reward (consumption of liquid rewards with either 2%, 5%, or 10% sucrose concentration). The XCST consists of three phases: Habituation, Sucrose Discrimination Test (SDT), and SSPT. The habituation phase aimed to determine whether animals have an inherited bias for, or against, any of the features used in setup or apparatus, such as a side-bias (Figure **1A**). In the second phase, SDT, the goal was to determine whether animals can discriminate between the three sucrose concentrations (2%, 5%, and 10%) used as nonsocial rewards. In the SSPT, animals chose simultaneously between nonsocial reward and social reward. To this end, rats were trained in a 4-arm plus maze in which sandpapers of different gradations marked the entrance of the arms. Each arm was baited with one of the three sucrose rewards, or the social stimulus rat, with the arm-reward contingency randomized across rats (for details, see Seidisarouei et al., **2021**). The SSPT comprised three choice conditions, social reward versus 2% sucrose, social reward versus 5% sucrose, and social reward versus 10% sucrose (Figure **1A**). On each testing day, two of the four arms in the XCST maze were open, and the other two were closed. At the beginning of each test, rats were placed in the center of the maze, and they could choose to explore both open arms for 8 min, yielding either the social reward or one of the three sucrose rewards, depending on the task condition. All rats underwent two repetitions of all three choice conditions. The order of conditions was pseudo-randomized across repetitions and rats (Figure **1B**).

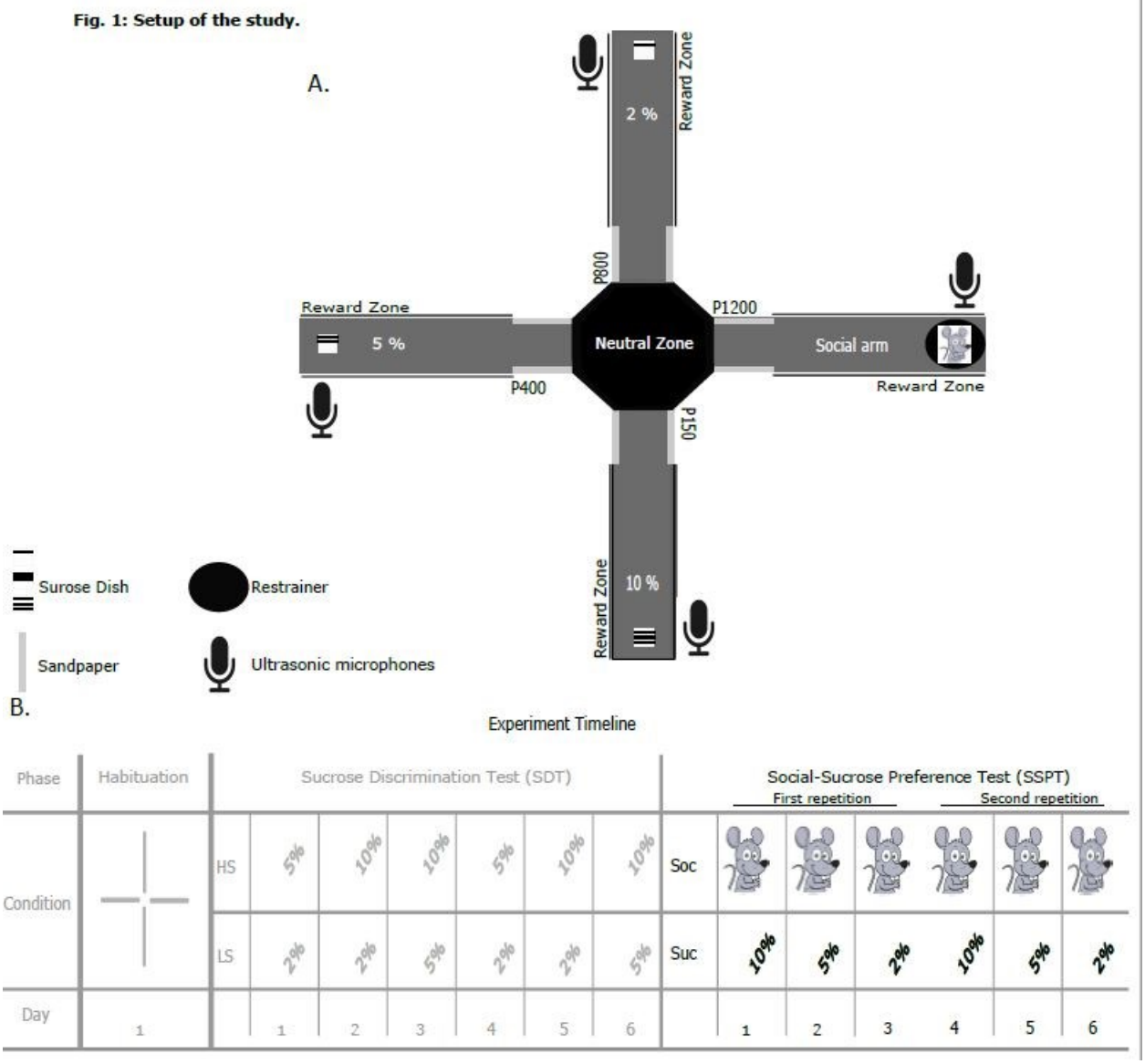

Figure 1

Design of the X-shaped chambered sociability test (XCST). (A) Schematic diagram of the XCST maze showing the positions for the nonsocial reward, microphones, the restrainer for the social reward, and the positions and gradations of the sandpaper (P150, P400, P800, and P1200). Part (B) shows an example of the schedule of the experiment for different phases, days, and conditions. Habituation: examination of the free arm in the habituation phase, sucrose discrimination test (SDT): HS; higher sucrose in a given trial, LS; lower sucrose in a given trial, social-sucrose preference test (SSPT): Soc; social reward, and Suc; sucrose. We show all details of the experiment for the sake of completion, but the grayed-out parts of the table refer to task phases reported elsewhere (Seidisarouei et al., 2022); here, we only report data obtained from the phases in the black part of the table.

As previously shown, the value of social interactions declined over time with increasing familiarity between actor and social stimulus rats (Smith et al., **2015**, **2017**). To prevent this effect from affecting USV production, 12 different social stimulus rats were used to maintain the novelty and value of social interaction across testing sessions in the SSPT. In addition, the social stimulus rats' assignment was counterbalanced across actor rats.

3 BEHAVIORAL ANALYSES

Video-tracking

We used EthoVision (XT version 11.5, Noldus) to track the animals' positions. The arena setting of the SSPT phase was designed to track the animals in reward zones (Figure **1A**).

4 USVS RECORDING, ANALYSIS, AND LABELING PROCEDURE

Recording

In order to record USVs, four ultrasonic microphones (condenser microphone CM16/CMPA, Avisoft Bioacoustics, Glienecke, Germany) were positioned by a microphone stand at a distance of approximately 20 cm on the right side above each reward dish, and also to perform acoustic analysis of USVs, we used the Avisoft-SASLab Pro (Version 5.2, Avisoft Bioacoustics, Berlin, Germany). In Avisoft-SASLab Pro, the spectrograms with a frequency resolution of 390 Hz and a time resolution of 0.64 ms were created by a fast Fourier transformation with a length of 512 points and an overlap of 75% (flat top window, 100% frame size).

Labeling

A trained scorer identified the calls and assigned them either to a 22-kHz (frequency <30 kHz) or a 50-kHz (frequency >30 kHz) category. In total, the calls of 144 trials (24 actors × 3 conditions × 2 repetitions) in the SSPT had to be recorded, but due to technical issues, we lost USVs of 33 trials in different conditions of SSPT (juvenile vs. 2%; WT = 2, tgDISC1 = 3, juvenile vs. 5%; WT = 5, tgDISC1 = 8, Juvenile vs. 10%; WT = 8, tgDISC1 = 7). In addition to 50-kHz calls, rats also vocalized 22-kHz USVs; however, because the main focus of this study was 50-kHz calls, we did not include 22-kHz calls in our analysis.

USV localization

We generated a USV position map that shows where in the maze the individual USV events were emitted, as explained as follows. To reach a time series of vocalization labels with a temporal resolution of 25 Hz, we exported the USV raw data (Avisoft SAS-Lab Pro's output) and synchronized them to the video stream of rat positions within the maze (Ethovision output). Notably, in behavioral tracking and analyses, only the time spent in reward zones was measured; therefore, we only identified, labeled, and analyzed the 50-kHz USVs emitted in both reward zones (social and nonsocial).

Software

All statistical analyses ran using SPSS Statistics (version 24; IBM, USA), and figures were created by Jupyter Notebook (Kluyver et al., **2016**) through the packages matplotlib (Hunter, **2007**), pandas (McKinney, **2010**), ptitprince (Allen et al., **2019**), and seaborn (Waskom, **2021**). To edit the figures, we used Inkscape (Inkscape, **2020**).

Acoustic feature analysis

In order to detect between-group differences, we examined three features of all USVs: call rate (number of calls vocalized per animal in each reward zone/time (s) animal spent in each specific reward zone), duration (s), and frequency (kHz) by conducting three separate two-way ANOVAs with group (tgDISC1 and WT) as between-subject factor and reward zone (social and nonsocial; we pooled USV data across all three sucrose zones) as a within-subject factor. In addition to investigate possible group differences in the relationship between the frequency of calls and the time spent in the social reward zone, or the sucrose reward zone, respectively, we ran mixed linear model analyses for each zone. The frequency of calls was quantified as a number of calls in the respective zone/[calls in the social zone + calls in the sucrose reward zone] × 100.

Finally, as an exploratory analysis (see Supporting Information section, Figure **S1**, and Table **S1**), we ran a three-way ANOVA to find out whether the number of 50-kHz USVs of groups differed over 8 min (each trial duration). In this analysis, we took group as a between-group factor, reward zone (social and nonsocial), and time (in full minutes) as within-subject factors and the mean number of 50-kHz USV emitted per minute in all conditions and repetitions as the dependent variable. The significance level at $p < .05$ was set for all statistical analyses, and all the post hoc tests were Bonferroni-corrected for multiple comparisons.

5 RESULTS

tgDISC1 rats show reduced interest in social contact

Details of the rats' choice behavior are described elsewhere (Seidisarouei et al., **2022**). Briefly, we found that tgDISC1 rats differed from WT rats in their social, but not in their nonsocial reward preferences: Compared to WT rats, DISC1 rats spent less time in the social reward zone that offered the opportunity to interact with the juvenile conspecific, and more time in the nonsocial reward zones that offered the opportunity to consume sucrose solution. The reduced time spent interacting with the conspecific was unlikely due to a hypersensitivity for sugar solution in the sucrose zones because we found no difference in sucrose preference and sucrose reward-seeking behavior between tgDISC1 and WT rats in the SDT. We conclude that the reduction in time spent interacting with conspecifics reflects genuinely reduced interest in social contact, that is, social anhedonia.

50-kHz USVs

5.2.1 Characterization of all 50-kHz USVs

In total, $n = 30{,}092$ 50-kHz USVs were identified.

There was large individual variability in vocalization activity between animals in both groups and all reward zones (Figure **2A**).

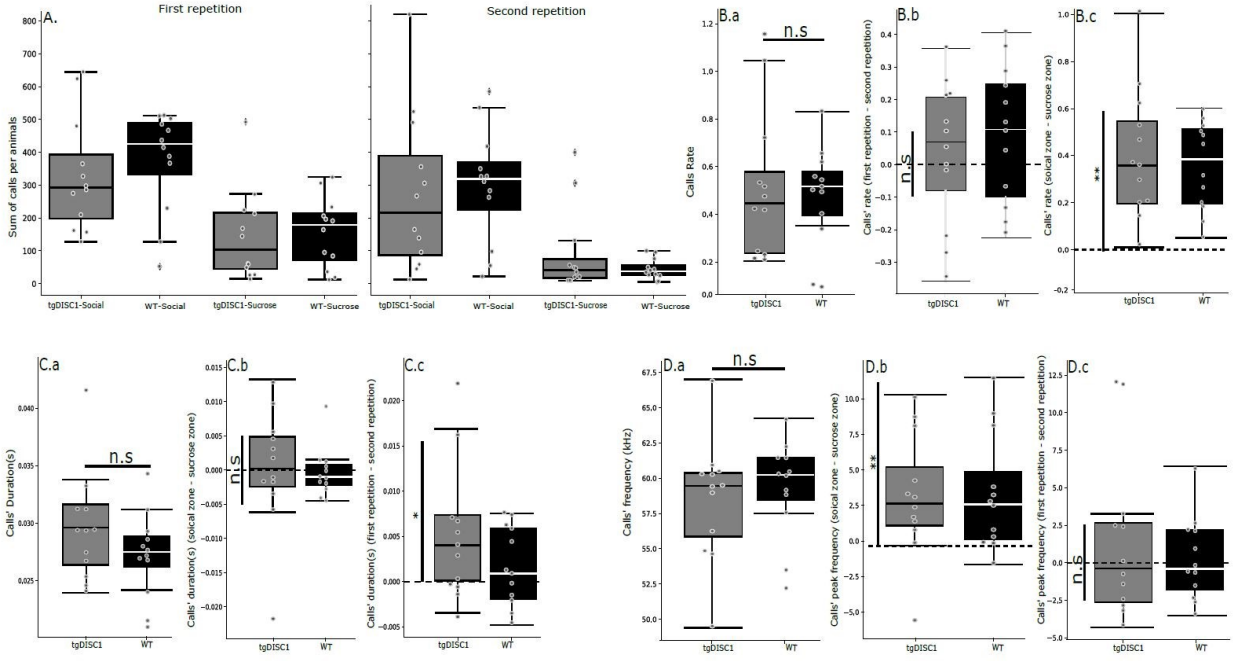

FIGURE 2

*Illustration of the number of ultrasonic vocalizations (USVs) (A), call rate (B), duration (C), and peak frequency (D) of disrupted-in-schizophrenia 1 (DISC1) and wild-type (WT) rats in all reward zones and across both repetitions. *p < .01 **p < .001.*

5.2.2 Call rate

The three-way ANOVA did not reveal a between-group difference in the rate of calls vocalized per group in both reward zones over two repetitions (F [1,22] = 0.86, p = .77; for more details, see Figure **2B**, Table **2A**). Likewise, we found no significant results for the within-subject factor *repetition* (F [1,22] = 1.7, p = .194, Figure **2B**, Table **2B**). However, the analysis showed a significant main effect of the reward zone (F [1,22] = 65.3, $p \leq .001$), showing an expected higher rate of calls in the social reward zone than in the sucrose zones (Figure **2B**. a and Table **2C**). Furthermore, there was an interaction effect of reward zone × repetition (F [1,22] = 8.3, $p \leq .009$, Table **2D**), demonstrating that animals produced fewer calls in the second repetition than the first repetition in the sucrose zone. No other interaction effect reached significance.

Call duration

The results of the three-way ANOVA analysis revealed no significant difference between tgDISC1 and WT rats in the duration of calls (F [1,22] = 1.7, p = .196, Figure **2C** and Table **2E**). Moreover, we found no significant difference in the duration of calls between the reward zones (F [1,22] = 0.1, p = .920, Figure **2C**, Table **2F**). However, the analysis showed that rats' calls did have a longer duration in the first repetition than the second repetition (F [1,22] = 7.6, p = .011, Figure **2C**. a, Table **2G**). Again, no significant interaction effect was found.

Call peak frequency

Analyzing the peak frequency of calls emitted by rats through a three-way ANOVA did not yield a significant difference (F [1,22] = 0.28, p = .597, Figure **2D** and Table **2H**). There was a significant effect of the within-subject factor *reward zone* on call peak frequency (F [1,22] = 10.4, $p \leq .004$, Figure **2D**. a, Table **2I**), showing that animals in the social reward zone vocalized with a higher frequency compared to the sucrose reward zones. The other within-subject factor, repetition, did not yield a significant effect (F [1,22] = 0.301, p = .589, Figure **2D**, Table **2J**). No significant interaction effect was found in this analysis.

Mixed linear model analysis

This analysis showed no significant difference between groups ($\beta_i = -.62$, SE = 0.46, $z = -1.34$, CI [−1.52, 0.28], p = .177; for more information see Table **3**) in the percentage of 50-kHz calls vocalized in the social reward zones as a function of percent time spent in the social reward zone.

6 DISCUSSION

Our findings did not demonstrate significant between-group differences in 50-kHz USV vocalization patterns between tgDISC1-rats and WT controls. This null effect is inconsistent with our prediction that differences in USVs between tgDISC1 and WT rats would reflect or even mediate, and the difference in social motivation reported earlier (Seidisarouei et al., **2022**; Wang et al., **2022**). In the following, we will offer a tentative explanation for these null-results.

Regional specificity of tgDISC1-induced DA transmission effect

Studies have shown that the dorsal striatum (caudate and putamen) plays a critical role in assigning value to a social object and encoding it as a reward (Acevedo et al., **2012**; Clements et al., **2022**; Klein & Platt, **2013**). In fact, a deficit in dorsal striatum function is associated with low-value attribution for social interaction in autism (Clements et al., **2022**), demonstrating the crucial role of the dorsal striatum in valuing and encoding a social object. On the other hand, findings show a significant role of the ventral striatum (Burgdorf & Knutson, **2001**; Mulvihill & Brudzynski, **2019**) and not the dorsal striatum (Burgdorf & Knutson, **2001**; Costa et al., **2019**) in the emission of 50-kHz USVs. More specifically, although microinjections of DA agonists into the nucleus accumbens shell increased the emission of 50-kHz USVs (Mulvihill & Brudzynski, **2019**), microinjection of AMPH into the dorsal striatum or DA denervation in the dorsal striatum did not result in changes in the number of 50-kHz USVs (Costa et al., **2019**). In this context, findings suggest that 50-kHz USV can release phasic DA (Willuhn et al., **2014**) and that DA release is not always followed by USV production (Simola et al., **2012**). On the other hand, DA release in the nucleus accumbens accompanies the perception of 50-kHz USVs which induce social approach in rats (Willuhn et al., **2014**). These findings may suggest that USV production is less DA-dependent than previously thought. For example, a study by Wright et al. (**2013**) demonstrated that the frequency of 50-kHz USVs and the distribution of call subtypes in response to AMPH treatment are linked to the action of DA on D1- and D2-like receptors. However, blocking the reuptake of DA is not enough to trigger the emission of calls. Moreover, at this point, it should be noted that several studies have shown the importance of non-dopaminergic transmissions such as serotonin (Wöhr et al., **2015**), glutamate (Costa et al., **2015**; Panksepp & Burgdorf, **2000**), norepinephrine (Branchi et al., **2001**; Grant et al., **2018**), adenosine (Simola et al., **2016**), and glucocorticoids (Popik et al., **2014**) in the emission of 50-kHz USVs, indicating that USVs are a compound behavior that does not depend on DA alone. This may explain why tgDISC1 rats do not differ from WT rats in 50-kHz USV behavior despite their DA deficiency.

Moreover, the reduced DA levels in the amygdala and HPC of tgDISC1 rats may disrupt social interactions (Allsop et al., **2014**; Davis et al., **2009**; Hernandez-Lallement et al., **2016**), but not the production of 50-kHz USVs. As shown, the amygdala is involved in the perception of 50-kHz USVs and social approach behavior (Schönfeld et al., **2020**), but to the best of our knowledge, there is limited research on the role of the amygdala in the production of 50-kHz USVs. Similarly, the HPC's role in 50-kHz USV production remains unknown.

In addition, previous research has demonstrated that prior experience can reduce the duration of rat USVs (Wöhr et al., **2008**) and this might be the reason why we detected a longer call duration in the first compared to the second task repetition in the SSPT phase, where actors were confronted with a juvenile conspecific in the maze for the first time. As our analysis showed, the duration of the calls decreased during the second repetition when the animals were already familiar with the context of the SSPT phase.

Last but not least, in the acoustic features' analysis of 50-kHz calls, we found that both groups' peak frequency of calls in the social reward zone was significantly higher than the call frequency in the sucrose zone (Figure **S2**). To our knowledge, no study has yet compared the 50-kHz call frequency in concurrent social and nonsocial reward contexts; therefore, this finding may open a new avenue for future relevant research.

Limitations

We only used male rats. A recent study (Uzuneser et al., **2019**) reported the significant importance of sex on dopaminergic, serotoninergic, and noradrenergic changes in the dorsal striatum of tgDISC1 rats, and this study showed no change in DA levels in the dorsal striatum in male tgDISC1, which is in contrast to previous findings. This study highlights the role of considering sex in studying the DISC1 phenotype and translational research in general. Therefore, future studies using tgDISC1 rats should consider male and female rats.

In addition, in the behavioral data analysis, we only considered the time animals spent in reward zones (social and nonsocial). However, this time does not provide information about the time animals spent on specific behaviors (e.g., exploratory sniffing or rearing). In this regard, it has been shown that there is a positive correlation between highly active behaviors (jumping or playing) and specific 50-kHz USV subtypes and a negative correlation between less active behaviors (sniffing and rearing) and 50-kHz USV (Burke et al., **2017**); therefore, analyzing certain behaviors and their association with 50-kHz USVs could be a more efficient approach.

Because of the study design, 50-kHz USVs in the social reward zone could be emitted by both the actor and social partner rats (Table **1**). Hence, during social interaction, there were always two rats that emitted USVs, while during sucrose consumption, we measured the USVs of only one rat. Although USV source allocation was applied, it is impossible to rule out with certainty that the difference in USV call rates between the social and nonsocial reward zones also partly reflected the difference in numbers of animals emitting USVs. Therefore, the results of this study, although replicating previous results (Mulvihill & Brudzynski, **2018**; Seidisarouei et al., **2021**), should be interpreted with caution.

TABLE 1. Between-group differences in the number of 50-kHz calls in the different zones of the setup.

Group	Zone				
	Social reward	**Sucrose reward**	**Neutral**	**Out**[1]	**Total**
tgDISC1	7238	2845	2327	2580	14990
WT	8060	2413	2417	3022	15912

[1] *Calls in the Out column were vocalized outside of any of the reward or neutral zones.*

TABLE 2. The result of post hoc tests on all 50-kHz ultrasonic vocalizations (USVs)

A. **Dependent Variable**	**Group**		**Mean**	**Standard Err.**	**P-value**
Call rate	tgDISC1		.513	.092	.771
	WT		.481	.060	
B. **Dependent Variable**	**Repetition**		**Mean**	**Standard Err.**	**P-value**
Call rate	First		.527	.053	.194
	Second		.467	.065	
C. **Dependent Variable**	**Reward Zone**		**Mean**	**Standard Err.**	**P-value**
Call rate	Social		.694	.071	.001
	Sucrose		.300	.203	
D. **Dependent Variable**	**Reward zone*Repetition**		**Mean**	**Standard Err.**	**P- value**
Call rate	Social	First	.671	.062	.506
		Second	.716	.091	
	Sucrose	First	.382	.056	.002
		Second	.217	.048	
E. **Dependent Variable**	**Group**		**Mean(s)**	**Standard Err.**	**P-value**
Call duration	tgDISC1		.030	.014	.196
	WT		.027	.010	
F. **Dependent Variable**	**Reward Zone**		**Mean(s)**	**Standard Err.**	**P-value**
Call duration	Social		.028	.008	.920
	Sucrose		.029	.001	
G. **Dependent Variable**	**Repetition**		**Mean(s)**	**Standard Err.**	**P-value**
Call duration	First		.030	1.2	.011
	Second		.027	1.0	
H. **Dependent Variable**	**Group**		**Mean(kHz)**	**Standard Err.**	**P-value**
Call frequency	tgDISC1		58.5	.085	.597
	WT		59.3	.067	
I. **Dependent Variable**	**Reward Zone**		**Mean(kHz)**	**Standard Err.**	**P-value**
Call frequency	Social		60.3	.008	.004
	Sucrose		57.5	.001	
J. **Dependent Variable**	**Repetition**		**Mean(kHz)**	**Standard Err.**	**P-value**
Call frequency	First		59.2	.909	.589
	Second		58.7	.934	

TABLE 3. The mixed linear model regression results; % of calls and time spent in the social reward zone

Model:	**MixedLM**	**Dependent Variable:**	**% of calls in social**
No. Observations:	24	Method:	REML
No. Groups:	2	Scale:	116.6
Min. group size:	12	Log-Likelihood:	-85.4
Max. group size:	12	Converged:	Yes
Mean group size:	12.0		

	Coef.	**Std.Err.**	**z**	**P>|z|**	**[0.025**	**0.975]**
Intercept	61.026	20.169	3.026	0.002	21.49	100.5
group [T. tgDISC1]	33.684	28.915	1.165	0.244	22.98	90.3
% of time in social	0.271	0.305	0.374	0.374	-0.326	0.86
% of time in social: group [T. tgDISC1]	-0.623	0.461	-1.349	0.177	-1.527	0.28
Group Var	116.658					

7 CONCLUSIONS

We recently reported social anhedonia in tgDISC1 rats. However, here, we found no group-dependent association between social interaction and 50-kHz USV emission. We, therefore, have no evidence to assume that 50-kHz USVs are related to, or mediate, the DISC1 deficit in social motivation.

ACKNOWLEDGMENTS

Open Access funding enabled and organized by Projekt DEAL.

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4. General Discussion

4.1 Summary of results

4.1.1 Study 1

In our first study, to broaden the current view of rat USVs, we focused on rats' 50-kHz USVs to determine whether the type and magnitude of reward were associated with a change in the proportional emission of 14 identified subtypes of USVs[53]. We found that rats vocalize, in general, more 50-kHz calls in the presence of other conspecific, which is in the same direction as findings that attributed the social function to 50-kHz calls. Indeed, rats vocalize 50-kHz calls extensively in a social context, such as mating and sexual behavior[66] or when they want to elicit a social approach[67]. In this regard, digging down into 50-kHz subtypes, our results showed that rats vocalize specific subtypes like Trill and Complex more significantly in the proximity of another rat. These two subtypes have been categorized in previous studies as frequency modulated (FM) calls [68] that serve a communicative function within social play[69], vocalizing in expectation of social interaction[70] and after administration of psychostimulant drugs[53]. In general, especially in a social context, rat USVs are like a chain of subtypes with different frequency (number of occurrences) of use of each subtype. In this regard, our result showed that the 14 identified subtypes of 50-kHz USVs do not have the same level of functionality and importance, and some of them, such as Trill, Flat, Composite, and Complex, are the main rings of the chain. Presumably, rat signals different codes to the outside world by changing the number, acoustic features, and position of these main rings under different emotional and motivational states. Furthermore, we found that increasing sucrose concentration (2, 5, 10%) could enhance the chattiness level of rats in the social condition. Put another way, the number of 50-kHz USVs, vocalized in the social zone was significantly different as a function of sucrose concentration. This effect could be mediated by DA increase which can be released in the nucleus accumbens by drinking sucrose[71].

4.1.2 Study 2

As mentioned in the introduction, DA plays a critical role in the pathogenesis of major neuropsychological disorders such as SCZ, ASD, and MDD. Currently, patients with these mental diseases suffer from controversial diagnostic manuals[72] (the fifth revision of the Diagnostic and Statistical Manual of Mental Disorders [DSM-5][54]), which cannot set a valid boundary between disorders and lack of consistently in the expected efficiency of pharmacotherapy interventions[73,74]. In this context, a possible approach could involve establishing animal models with specific neuronal features that produce phenotypes similar to those of neuropsychological disorders to achieve a biologically based classification of mental disorders. Social anhedonia caused by social dysfunctions is a typical phenotype in neuropsychological disorders that, not surprisingly, severely affects patients' psychological well-being[54]. Discovering this phenotype's neural underpinning could remove some current barriers to efficient diagnosis and treatment. To contribute to this end, in our second study, we used the tgDISC1 rat (see "Introduction") as an established animal model to determine how dopaminergic dysfunction in specific brain regions (DS, AMY, and HPC) potentially alters the animal's evaluation of two different reward types (social and non-social). Unlike for non-social reward, we found a reduced motivation in tgDISC1 rats for social interaction, which resembles the social withdrawal seen in MMD patients as a consequence of social cognition deficit, mainly caused by altered DA turnover in the striatum[75], AMY[76], and HPC[77]. This finding becomes more interesting when we compare it with previous studies, which revealed a selective dysfunctional process only for social rewards in SCZ[78], ASD[79], and MDD[80].

4.1.3 Study 3

Considering the results of our first study (particular vocalization of 50-kHz USVs and their subtypes depending on the type of reward) and the second study (decreased social motivation and deficit in dopaminergic transmission in tgDISC1 rats), in our third study, we wanted to know whether the neural and behavioral phenotype of tgDISC1 rats can be manifested in their 50-kHz USVs. This curiosity was triggered mainly by current knowledge about the language abnormalities (alogia[81], monotone speech[82] and discursive[83]), in addition to the sensory deficit, especially the auditory deficit, in SCZ[32] and also difficulties by ASD patients in using language in social situations[84]. For example, SCZ is characterized by deficits in generating mismatch negativity (MMN)[85]. MMN is initiated only in response to stimuli that vary from a predictable pattern and can be based on auditory variables such as pitch and duration. Because alterations in the pitch and duration of vocalizations may be associated with changes in emotions that may precede social interactions, such a deficit may contribute to the abnormal, aberrant patterns of social behavior and social judgment that account for the disorder.

However, in our third study, despite tgDISC1 rats' dopaminergic dysfunction (reduced DA level in DS, AMY, and HPC) and decreased social motivation, we did not detect any change in the quantitative (call rate) and qualitative (frequency and duration) USV of tgDISC1 rats. To find out why, we compared the functions of each region in two domains (social behavior and USVs) and concluded that the importance and functions of each region might differ depending on each domain. For example, AMY plays a very important role in social cognition and functionality[76,86], whereas its role in the 50-kHz USV is insignificant or undiscovered until now[87]. This domain-dependent functionality of the regions was also evident when we cast about in literature to know how a decreased DA level in DS can alter social motivation while not affect the 50-kHz USV. In short, neural and behavioral alterations could not be detected through 50-kHz USV of tgDISC1 rats.

5.The current view on the neural basis of social reward processing

As I mentioned in the introduction, there is currently no consensus on how or whether the brain processes social rewards differently from non-social rewards. However, following the curiosity of scientists who consider a unique brain mechanism for processing social rewards, findings have been made that I summarize in the following pages.

As a leading theory, social motivation theory has provided a promising angle of view for perceiving social reward processing and the deficits caused by disruption of this reward processing. This theory suggests that the evolution of humans as social beings has placed a particular emphasis on social motivation, which can be described as a collection of psychological dispositions and biological mechanisms[30] that lead humans to orient to the social world preferentially, to enjoy social interactions, foster and maintain the social bonds. All these social actions ultimately could enhance the chance of better fitness in the social environment. At the biological level, social motivation results from multi-directional communications among specific brain regions such as AMY, VS, OFC, and vmPFC. Notably, each region is involved in a particular function, but no region acts individually[21]. In this context, the signaled socially relevant information (verbally or non-verbal) is received by guided attention through activating AMY[88].The subcortical structures measure the rewarding utility[89] of the received cue, and to fulfill this process, the cortical activations are required to signal the conscious hedonic representations of the cue[90]. In this process, the involvement of AMY plays a crucial role in the computation and updating of the social information value. In addition, AMY also plays a vital role in computing the salient value of social stimuli in conjunction with VS and OFC[91].

To assign an incentive salience to a reward, which causes the motivational magnet (wanting) to attract attention[91], VS is involved in both social and non-social stimuli[92]. On the other side, OFC exchanges the social information value to a common currency (DA) which informs the executive system and guides goal-directed action[89]. Interestingly, there is a positive correlation between the

activation of the OfC-Str-AMY network and higher social response to the social stimuli[93]. In contrast, the weaker activation of this network is associated with more anti-social traits[94].

Another alternative to the social motivation theory is a recently established view that opens up room for considering a new transdiagnostic domain that assumes the same biological root for different psychological disorders[32].

In an influential review, Stefano Porcelli and his colleagues[32] recently summarized brain regions, networks, and specific neurotransmitters with their different functions in the so-called social brain.

The social brain is a specialized brain that, under the force of evolution, has created social functionality for different brain systems in addition to their original functions. Based on primarily animal experiments and also considering human experiments, five major social networks have been delineated. Three of them (social perception [detection and processing of social stimuli], affiliation [pro-social behavior], and aversion [aversive behaviors]) are influenced by the anchored role of AMY (Figure 3).

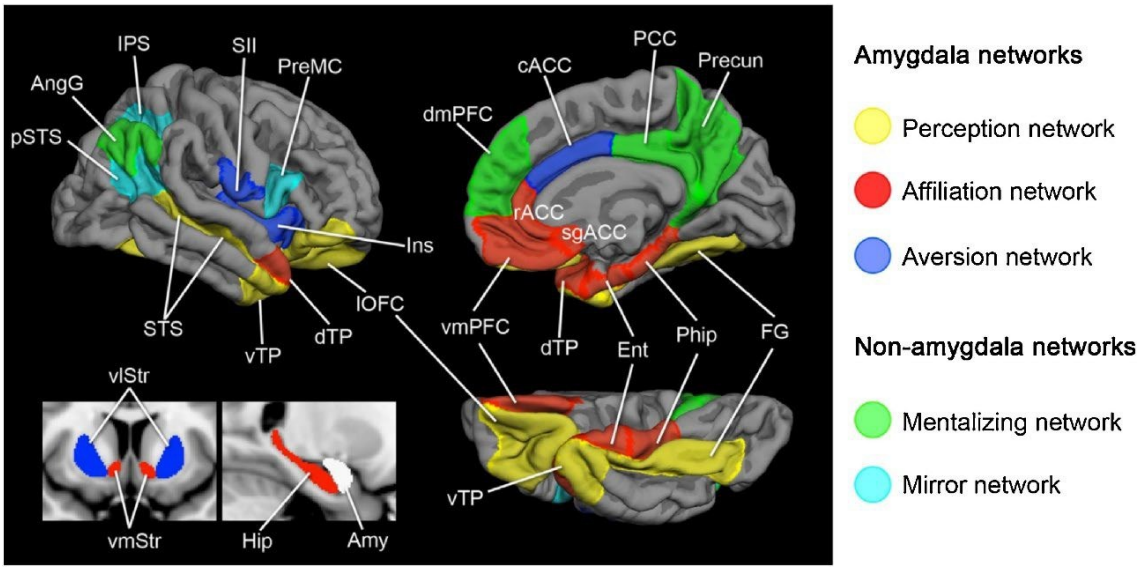

Figure 3. The five large-scale brain networks sustain processes important for social behavior. Perception network: *lOFC*=lateral orbito frontal cortex; *vTP*=ventro lateral temporal pole; *FG*=fusiform gyrus; *STS*=superior temporal sulcus. Affiliation network: *dTP*=dorso medial temporal pole; *rACC*=rostral anterior cingulate cortex; *sgACC*=subgenual anterior cingulate cortex; *vmPFC*=ventromedial prefrontal cortex; *Ent*=entorhinal cortex; *PHip*=para hippocampal cortex;

vmSt=ventro medial striatum. Aversion network: *cACC*=caudal anterior cingulate cortex; *Ins*=insula; *SII*=somatosensory operculum; *vlSt*=ventro lateral striatum. Mentalizing network: *dmPFC*=dorso medial prefrontal cortex; *PCC*=posterior. cingulate cortex; *Precun*=precuneus; *AngG*=angular gyrus (temporoparietal junction). Mirror network: *pSTS*=posterior superior temporal sulcus; *IPS*=intraparietal sulcus; *PreMC*=premotor cortex[32].

In social perception, after detecting the stimuli, memory systems contribute to the valuation of the stimuli's salience by communicating to AMY and other regions of the salience network, such as the dACC and dorsolateral OFC[95]. Concerning social perception, the most relevant social information can be derived from facial expressions. Consistent anatomical studies have revealed the link between AMY and face perception regions such as the Fusi-form area and superior temporal sulcus[96,97]. Unsurprisingly, the lesion in AMY can impair facial-emotional recognition, which clearly can determine the misinterpretation of social signals in social interactions[98].
After assigning salience to a social stimulus, the next step is the involvement of the social affiliation or aversive network to detect the social stimuli's valence[32]. With respect to the emotional output (positive or negative) of both networks, the significant contribution of AMY is necessary[99]. Social affiliation is vital in building and maintaining social relationships that require constant emotional regulation. For this purpose, there is two-way signal transmission between AMY, vmPFC, and ACC. In this context, several studies have shown that disruption in a region such as the vmPFC can produce a variety of socially inappropriate behaviors such as lack of empathy, guilt, remorse, apathy, indifference, and unfavorable (social) decision-making[100–102].
In addition to brain networks, recent evidence suggests that most neurotransmitter systems (DA, opioids, GABA) may be influenced by systems that are mainly specialized for social stimuli such as oxytocin (OXT) or vasopressin and, to some extent, serotonin (5-HT)[32]. For example, OXT interacts with DA (in the central AMY) in response to a social stimulus to determine the salience, regardless of its valence[103]. In the same vein, in releasing OXT, it is shown that 5-HT plays an important role. Considering this interplay of different neurotransmitter systems, we can speculate that when we see a person, 5-HT releases OXT, which modulates DA that determines the salience

of that person for us, and any dysfunction in this process potentially causes social dysfunction such as reduced attention to a socially significant person like a friend.

As mentioned earlier, after social perception, the valence of the social stimuli must be evaluated as positive (rewarding) or negative (aversive) to fulfill the function of social affirmation or aversive network. Here it is may necessary to briefly explain why we need to perceive social stimuli as rewarding. Indeed, perceiving social stimuli as rewarding is thought to be crucial for developing a social brain[104]. Furthermore, solid social ties (as a phenomenon facilitated by the social brain) bring us various benefits, from the protection and support we receive from our parents in childhood to the affective input from our loved ones in adulthood. Not surprisingly, our survival and well-being can be severely threatened without these social ties.

In processing the stimuli's salience, DA plays a primary role, particularly DA from the ventral tegmental area (VTA), which projects to Vstr. Concerning this process, it is notable that there is a positive correlation between downstream DA release and duration of social interaction[104,105]. Interestingly, OXT was found to enhance the VTA activation in response to social[106] but not social stimuli[107]. In addition, 5-HT1b[108] and μ-opioid receptor[109] receptors also modulate the DA released from VTA. These modulations dictate the magnitude and duration of DA release, which encodes the subjective feeling of pleasure due to the social stimulus (e.g., a friend's happy face) and reinforce the desire for continued interaction with the reward source (e.g., our friend). Again, this was an example of how socially specialized neural systems modulate the process of stimuli salience, and it is clear that due to a disruption of this process (social stimuli salience through the social affirmation network), some maladaptive social responses may occur, similar to those observed in MMD patients.

In this step, it is time to return to the question of whether social and non-social stimuli are processed in a distinct neural system.

According to current evidence and what was mentioned earlier, it seems that the brain may process these two different types of stimuli mainly in overlapping neural processing systems,

with social stimuli having a certain uniqueness. This difference could be evolved due to the different sensitivity of social stimuli to contextual factors such as social values and principles, which affect the processing of social stimuli differently than non-social stimuli. Furthermore, as previous studies showed, human social functioning severely relies on brain processing speed, attention, working memory, and executive functioning, which can affect our social competence[32], but whether this processing pattern performs on non-social stimuli in the same way as on social stimuli remains to be elucidated.

As mentioned several times in this thesis, the social deficit is a common phenotype in various neuropsychological disorders, especially MDD, SCZ and ASD. In this context, in recent years, many studies using current imaging and electrophysiological methods have suggested several brain regions and different neurotransmitters with different contributions as major players in social deficits. Interestingly, there is a large overlap between brain regions (those discovered so far for social deficits) in different neuropsychological disorders (see Figures 1 and 2 in the review by Porcelli et al.[32]), in addition to similarity in neurotransmitters associated with social deficits across these disorders. These overlaps and similarities may suggest that social dysfunction is a continuum caused by defects in multiple neural networks that maintain social functioning[99,105] rather than defects in single regions, supporting the hypothesis that social dysfunction may be due, at least in part, to the dysfunction of specific transdiagnostic neural circuits.

In considering a unique brain mechanism for social stimuli, our results may also confirm this, as we found that rats use a different type of 50-kHz USVs for social rewards and the effects of DISC1 may differ depending on the type of reward. Indeed, these specific outputs for social rewards compared with nonsocial rewards should be executed by a particular brain process that functions only for social stimuli or context.In summary, multiple factors such as psychological and biological mechanisms supporting social motivation theory, the overlap between different brain regions discovered for the social deficit in different neuropsychological disorders, and also the (partial) similarity of the neurotransmitter system of different neuropsychological disorders

with the social deficit are crucial indicators that should be seriously considered in future research. In other words, these promising shreds of evidence should be considered to practically change the current prevailing view of social deficits in various neuropsychological disorders' diagnosis and treatment.

6. Methodological limitations

As with any research, a new perspective on what could have been done better emerges after the study has been conducted. The same is true for our studies. We could achieve deeper insights if we considered some additional aspects when designing our studies, which are briefly mentioned below.

What I observed while examining the rats' 50 kHz USVs gave me the idea that there are very individual vocalization patterns among the rats. In other words, some rats vocalized more than others in all conditions, while others were somehow introverted and vocalized less. Therefore, we could collect more behavioral (e.g., locomotion, social behavior, food intake, and home cage hierarchy) and neural information (OXT or DA content in brain regions associated with vocalization, such as the striatum) about each rat and use new analysis methods such as machine learning to discover a possible pattern between their behavioral and neural characteristics and their vocalization pattern. In addition, as mentioned in this study, we considered the subtypes of the 50-kHz USVs individually and associated them with reward zones. However, this method does not account for the possibility that rats use 50-kHz subtypes in different order or sequences for different conditions. For this, we could use special machine learning algorithms to find out the probable association of the arrangement of 50-kHz USV subtypes with a particular condition based on the characteristics of individual rats.

Another serious limitation I should point out here is that lab rats grow up in a very restricted environment compared to wild rats. As known, the environment impacts vocal communications, and it is impossible to claim that lab and wild rats have or use their USVs in the same way, which

is a major obstacle to generalizing the results. For future research, it is very important to create similar living conditions (as much as possible) for laboratory rats as for wild rats.

In the studies we performed with the tgDISC1 rats, we did not collect neural data such as OXT or DA in some influential brain regions such as striatum or AMY in each subject, which could help us consider different explanations and reach better conclusions.

7. Future directions

In this dissertation, I reported the results of studies covering two important dimensions of translational studies: USVs in rats and behavioural changes in the established transgenic animal model (tgDISC1). In general, any psychological disorder can be caused by complex interactions between genes and environmental factors that lead to neural and behavioural phenotypes that we refer to as symptoms in various mental disorders. To discover these symptoms' genetic pathways and neural bases, we need to rely mainly on natural structures such as genes or tools such as USVs. Since the rat is the most commonly used species in our translational studies, discovering its communication and developing animal models that can represent specific mental disorders with all their features is the optimal way to overcome most of the difficult obstacles we currently face in the diagnosis and treatment of mental disorders.

The remarkable advances in neurotechnology and genetic engineering are promising. They will provide scientists with deeper and more precise insights into the development of various mental disorders in the future, and this technological progress should be used to take a more global perspective (brain networks/complex interaction between different neurotransmitters) than a local one (specific neurotransmitters or brain regions), in our case in exploring the meaning and function of USVs as well as neural changes associated to social deficits in tgDISC1 rats. It should be noted, however, that the shift from a local to a global perspective should not be interpreted as the insignificance of individual brain regions or neurotransmitters.

8. Conclusion

8.1 USVs

Our findings on 50-kHz USVs, such as more USVs in social contexts, effects of non-social reward on social calls of rats, and different use of subtypes based on conditions and reward types, show the hidden potential in this non-invaisive tool that should be further explored. Identifying the patterns and associations of each USV with a particular situation signals a very important message that these calls are codes that can decode rat behavior in different experimental designs with different hypotheses. The use of specific subtypes for social interactions may facilitate the exploration of neural, emotional and motivational aspects of social deficits in translational studies and provide a reliable tool to demonstrate the validity and efficacy of preclinical pharmacological interventions.

8.2 DISC1

The detailed behavioral phenotypes of the DISC1 gene, which was discovered decades ago, have not yet been elucidated. Therefore, our new finding that the processing of social rewards is impaired as a result of DISC1, while the evaluation of non-social rewards remains intact, is a significant finding. This finding strengthens previous findings that the brain reward system processes social rewards differently. It underscores the discovery of genetic and neural bases that may function differently for social reward, ultimately paving the way for better prevention, diagnosis, and treatment of neuropsychological disorders characterized primarily by social deficits.

8.3 USV of tgDISC1 rats

We did not detect any change in the qualitative and quantitative aspects of USVs of tgDISC1 rats characterized by DA dysfunction and social anhedonia. This null finding signals the critical fact that social behavior and social USVs are independent mechanisms that are differentially controlled by specific brain regions such as DS, AMY, and HPC. In other words, the functions of these brain regions are different depending on whether they are responsible for social behavior or social calls. This consideration suggests the complexity of the brain regions' performance concerning their tasks.

9 References

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