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The role of ultrasonic vocalizations in the formation of rat groups in a semi-natural environment

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I contacted Eelke with another project that was about the basolateral amygdala's role in reward, but since the animal lab was closing due to construction of MH2, she suggested another topic. The role of ultrasonic vocalizations in a semi-natural environment, they had already investigated the role of USVs in the same semi-natural environment, but found no evidence for its role in sexual behavior. The question then became why do they emit USVs, which sounded like an interesting topic to pursue.

Eelke then showed me how to use the computer program Noldus to do the observations, and how the different behaviors looked. She also gave me several useful tips on writing the thesis. I did all of the statistical analysis, coding of behaviors, and writing. I also did almost all of the literature searches. Close to the end of the project the external hard drive containing the final raw data died, which was very disappointing. The main findings of the analysis should though still provide some interesting indications on our research question.

Supervisor

Student

Abstrakt

Denne studien undersøkte om ultralyd vokaliseringer (USV) hadde en effekt på dannelsen av grupper av rotter i en semi-naturell setting som tillot rotter p leve nærmere deres naturlige mønstre som de lever i villmarken. Dette ble gjort ved at fire grupper av rotter ble sluppet løs in i et semi-naturelt miljø. Før de ble sluppet løs i miljøet, gjennomgikk alle en operasjon. Hvor noen av rottene fikk den laryngale nerven kuttet, som gjorde dem ute av stand til å vokalisere USVs, noe som gjorde de devokalisert. Dette hadde en effekt som påvirket flere oppførsler sammenlignet med de som kunne vokalisere. Mest fremtredende forandret var mengden av mat som ble transportert in i undergrundsbasen, hvor rottene som kunne vokalisere ikke transporterte mye mat. Det var også en stor effekt for de devokaliserte rottene og løping. De rottene som kunne vokalisere flyktet derimot mere total, men de flyktet derimot ikke fra de devokaliserte rottene. Dette og effekter fra hver av kombinering av observasjoner vil bli diskutert

Nøkkelord: ultralyd vokaliseringer; semi-naturelt miljø; deokalisering; rotte grupper; gruppe oppførsel

Abstract

This study investigated if ultrasonic vocalizations (USV) had an effect on the formation of groups of rats in a semi-natural setting that allowed the rats to live closer to their natural state in the wild. This was done by letting four groups of rats into a semi-natural environment. Where prior to release into the environment, all the rats received surgery. Where some of the rats had the nerve to the laryngeal nerve cut, making them unable to vocalize the USVs, making them devocalized. With the effect that several behaviors differed between the rats that could vocalize, most prominently changed was the amount of food that was transported into the burrow, where the rats that could vocalize did not transport much food. There was also an effect that the rats that was devocalized ran more than the rats that could vocalize. The rats that could vocalize did though flee more, but not from the devocalized rats. This and effects in each of the combined observations will be discussed.

Keywords: Ultrasonic vocalization; semi-natural environment; Devocalization; Rat group; Group Behavior;

In the wild rats live in social groups varying in size consisting of both sexes, with varying ratios of males and females. Although, it is more common for these groups of rats to have more females than males, partly because fewer males survive to an older age than females do (Davis, 1953). Groups of rats also quite often end up living quite near each other, with some groups living with less than meter between them. As measured by their home base, which usually refers to either a nest or a burrow. This closeness of the rat groups makes it difficult to study rats group behavior, because it makes identification of which group each individual rat belongs to, in addition rat groups in the wild are more fleeting than ones created in a laboratory (Davis, 1953). Another difficulty with studying rat behavior in the wild, is that rats seem to prefer to be in their home bases, above the ground in a nest or below the ground in a burrow. An effect seen in an experimental setting that gave the rats the choice between spending time in an area resembling that of the outside world (open-area), and one that resembles an underground burrow area. Meaning that most of their behavior likely happens inside the confines of a burrow (Chu & Ågmo, 2015).

Meanwhile, some behavior tends to exclusively happen out in the open-areas, like paracopulatory behavior, spatially coordinated movements, and sexual behavior. With female rats spending much more time in the open-areas when they are willing to copulate, and they are only willing to copulate when in estrous (Chu & Ågmo, 2015; Weiss, Segev, & Eilam, 2015, 2017). The spatial movement that rats display in open areas with other rats, is behavior that happens when the rats start to move in relative relation to each other. This was first found in a dyadic setting, where two rats were allowed move around freely in a semi-natural environment, consisting of an open-area, and their movement patterns were measured. These duos then tend to find one area or corner that becomes a home base that they both spend time in, and return to after exploration. With one rat often leading more in terms of what areas and objects are explored first, when outside of the home base, with the other rat following, and exploring these areas and objects after the other rat (Weiss et al., 2015, 2017). Furthering the evidence for these movement patterns in groups, a quartet of rats was released into an open-areas. With the results supporting the finding that rats find a designated home base within the open-area, that the rats use to explore their surroundings. However, only rats in a group condition used home-bases in their open-area, as rats tested in solo conditions did not show a preference for a specific area, even when they were familiar with the semi-natural environment. The rats that were familiar with the environment did show a locomotor increase, but not as large as rats that were in the environment the first time with a duo, or quartet. Showing patterns that indicate rats both organize themselves, and behave differently in groups versus when they are alone (Weiss et al., 2015, 2017).

The way rats move in these patterns is probably because they are group animals, that could be using ultrasonic vocalizations (USV) to keep track of each other. USVs are sounds that are above the range of a human's ability to detect pitch, which is measured to be around a frequency of 20 000hz (20 kHz). Whereas a rat's ability for auditory perception of pitch has been measured up to as much as 80 kHz, and is therefore far beyond that of any human (Sales, 2010). That rats are not just able to hear USVs but also able to vocalize ultrasounds, something that has been known for a long time. Which when discovered rapidly caused the suggestion that these high pitched vocalizations are used to communicate with other rats (Sewell, 1970). With USVs short travel distance, due to higher frequencies of pitch dissipate more quickly, it would be an adaptive way to communicate. Especially, for rats since they are a species of animal that is more prey than predator (Staples, Hunt, van Nieuwenhuijzen, & McGregor, 2008). The travel distance is shorter even shorter for USVs in the wild because there are many more things for the USVs to bounce off than a in the average laboratory (Sales, 2010). Meaning USVs would be even more adaptive as a means of communication in the wild, because predators would be even less likely to hear the USVs and find the rats emitting them.

There was a controversy about the USVs being used as a socially communicative tool by rodents, because USVs correlated with movements and physical exertion of rats (Blumberg, 1992). This was in part, because their small size makes it possible, and easy for the rats to use their larynx to emitting USVs (Johnson, Ciucci, Russell, Hammer, & Connor, 2010; Sales, 2010). This created the hypothesis that USVs are byproducts of the movements of the thorax, rather than deliberate sounds made by the rats. The evidence, however, pointed out that USVs are also emitted during activities in which the rats are being immobile. Modifying the mechanical byproduct hypothesis to that some USVs were just byproducts of physical exertions, while others USVs were socially communicative (Blumberg, 1992). The current state of evidence makes the hypothesis that USVs are mechanical in nature, appear to be unlikely, as USVs have been found to be emitted in many different situations. and in many different types of rodents and subspecies of rats (Lahvis, Alleva, & Scattoni, 2011; Walker, Naicker, Hinwood, Dunn, & Day, 2009). They also seem to be involved in many different behaviors and different emotional states in rats (Sales, 2010), among them are affect (Knutson, Burgdorf, & Panksepp, 2002; Wöhr & Schwarting, 2013), anxiety (Rao & Sadananda, 2015), juvenile isolation (Sewell, 1970), fear(Yee, Schwarting, Fuchs, & Wöhr, 2012), sex differences (Chu, Snoeren, & Ågmo, 2017), reward (Opiol, Pavlovski, Michalik, & Mistlberger, 2015), isolation (Brudzynski, 2005), fighting and play fighting(Burke, Kisko, Pellis, & Euston, 2017), and social interactions (Portavella, Depaulis, & Vergnes, 1993),. The involvement of USVs in many different contexts therefore strongly indicates that USV’s are used for social communication by rats.

The investigations into how rats use USVs has lead much of the literature to divide USVs into three different classes of USVs, the 40-kHz class, the 50-kHz class, and the 22-kHz class. That each correspond to different types of situations, and age of the animals(Burke et al., 2017; Portavella et al., 1993; Wöhr & Schwarting, 2013). Though, in general, USVs have short durations, with the most recorded having a duration of 25-200 milliseconds (ms), with a total duration span between 2ms-3000ms. With small individual differences in pitch, between 1-7hz, within a rat's vocalizations (Snoeren & Ågmo, 2013). At the same time, there are also great individual difference in frequency of emitting USVs and duration of vocalizations (Rainer Schwarting, Jegan, & Wöhr, 2007) There are also clear species differences specific USV frequencies used in combination with behavior, and USVs usage, where are rather large between different types of rodents (Gomes et al., 2013). As an example of sub species-specific differences, as Sprague-Dawley rats tend to release fewer positive, 50-kHz vocalizations than Wistar-rats to the same behavior and consequences of that behavior (Walker et al., 2009). There are also some sex differences, where female rats seem to use 50-kHz USVs, to all sexual behavior, male rats vocalize the USV linked to aversiveness, the 22-kHz, in response to ejaculation (Snoeren & Ågmo, 2013). With rats also changing their responses as they mature, and learn to respond differently to USV signals over time with new group member also modulating their USVs when forming groups (Brudzynski, 2013; Weiss et al., 2015).

The three classes of pitch actually have a wider pitch than their names indicate as 40-kHz class of vocalizations are actually USVs with a pitch frequency of between 30-65-kHz, with a duration of about 80-150ms (R. Schwarting & Wöhr, 2012). Meanwhile, the measurements for the 50-kHz class of vocalizations are usually short with a duration of 20-100ms, and have a pitch that goes from 35-kHz to 70-kHz (Burn, 2008; R. Schwarting & Wöhr, 2012) Whereas the 22-

kHZ class is measured in the range of 18-32-kHz, with a duration around 300-3000ms (Wöhr & Schwarting, 2013). These classifications of USVs are then very different from each other i terms of what is measured for each of them, and they have all correspond with different types of situations, and behaviors.

The class of USVs called 40-kHz are vocalizations primarily emitted by pups that have become isolated from their littermates, meaning that it appears to signal distress and alarm. The 40-kHz USVs when heard by a mother rat, tends to make the mother rat come to the rat pups, and help the pups. Either because they become separated from the rest of the litter of pups, or because the pups need tending to. These USVs also seem to be going somewhat all over the place in terms of sonographic structures (Brudzynski, 2005; R. Schwarting & Wöhr, 2012). A playback experiment using the pitch of 40-kHz with a flat sinus tone investigated if these sonographic structure modulations of the 40-kHz convey any meaning. The findings showed that the sinus tone on its own was not enough to cause a behavioral reaction in the mother rat (Noirot, 1972) Strongly indicating that the sonographic structure being all over the place, conveys some form of meaning to the rat mother. Which would imply that timbre, the characteristic quality of a sound independent of pitch and loudness, conveys meaning for rats as well as humans (Burn, 2008; R. Schwarting & Wöhr, 2012). So, when rat pups release 40-kHz USVs, in response to an intruder coming into the nesting area, it is the combinations of timbre, pitch, and loudness that causes the meaningful content conveyed when a rat responds to the 40-kHz USVs. In a twist 40-kHz USVs are not necessary for the mother rat to hear for her to defend her pups from an intruder. Showing an instinct that goes beyond that of USVs alone (Kolunie, Stern, & Barfield, 1994). Testing if there are connections between the instinct to release 40-kHz calls is connected to similar adult behavior in rats have been attempted; where 40-kHz USVs have been investigated for connections with adult anxiety, alarm, and stress. The results of the studies into these connections have produced slightly mixed results, with some patterns found in rats selectively breed for anxiety, alarm and stress, or high USV vocalizations (Gomes et al., 2013; R. Schwarting & Wöhr, 2012), but no such pattern has been found for rats not breed for a specific phenotype. Meaning that they these 40-kHz USVs are probably not a good predictor for adult rat anxiety and stress for normal rats (R. Schwarting & Wöhr, 2012). Perhaps because the 40-kHz USVs, unlike the other two classifications of USVs, seems to disappear gradually as the rat matures (R. Schwarting & Wöhr, 2012).

Whereas the 50-kHz vocalizations start to appear as the rat matures, the 50-kHz USVs are primarily tied to positive events, situations, rewards, and anticipation of positive events. With a stronger connection to positive events when the 50-kHz USVs are below .3 seconds (Burn, 2008; R. Schwarting & Wöhr, 2012). A pattern that is probably in part learned and comes as maturation as juvenile rats do not necessarily vocalize them in anticipation of positive events; and the frequency of calls is modulated by the life of the adult (Brenes et al., 2016; Brudzynski, 2013). At the same time, juvenile rats appear to react more strongly to playbacks of 50-kHz sounds than an adult rat do (Wöhr & Schwarting, 2013). Rats also respond to being approached with emitting 50-kHz USVs (Willadsen, Seffer, Schwarting, & Wöhr, 2014), and social enrichment (SE) also increases 50-kHz vocalizations; with SE usually referring to rats that are housed with a group of rats, rather than as a duo or in isolation. There is also an increase in social behaviors, social exploration, and responses to 50-kHz vocalization for rats in an SE, showing how groups can change rat behaviors (Brenes et al., 2016). Surprisingly, rats also show a pattern of vocalizing as a response to isolation, with 50-kHz vocalizations being emitted when put in a cage in a room devoid of other rats. A decrease is however seen in rats that are socially deprived, but a difference that can be increased again with increased socialization (Brenes et al., 2016) An interesting finding is that there is also an increase in 50-kHz USVs when a rat is reintroduced to other rats (Willadsen et al., 2014). Showing that 50-kHz USVs potentially act as a signal for conveying a “desire for positive social engagement” to any rats nearby that they cannot sense yet (Rainer Schwarting et al., 2007). It seems then that 50-kHz USVs are important for socialization and that they are altered by amount of socialization (Brenes et al., 2016; Smith, Lacy, & Strickland, 2014)

The next class of vocalizations are the 22-kHz class, these USVs are the linked to aversive situations and are emitted in response to situations like fights or being attacked, foot shocks, or detection of predators. When the 22-kHz class of vocalizations is associated with aversive stimuli, it does make male rats emitting them in response to ejaculation quite strange. Especially when considering that adult rats that hear 22-kHz vocalizations they can show fear responses, like freezing, or escape behavior (Blanchard & Blanchard, 1989). Reactions that are increased the longer the duration of the 22-kHz USVs are, as duration appears to signal the intensity of a threat, with the closer or more immediate the threat the longer the alarm call appears to be (Brudzynski, 2005). The main modifier of which behavior is chosen, escape or freeze, in response to a 22-kHz alarm call appears to be if they can escape into a burrow, with freezing happening when a burrow is not available in response to a 22-kHz USVs, and escape when one is available (Kitaoka, 1994; Wöhr & Schwarting, 2013) However, juvenile rats do not necessarily react to 22-kHz vocalizations in the beginning of their youth, but do seem to go through an instinctive associative learning process; where they start recognizing 22-kHz USVs as a predictor of an aversive stimuli, as they mature. Where the juveniles rats later on then also vocalize 22-kHz as a response to aversive stimulation (Wöhr & Schwarting, 2013). Showing that these vocalizations too are not just predetermined species-specific reactions to stimuli, but contingent on if the rat has learned or matured enough to associate these calls with an appropriate valence and which behaviors are appropriate to express in response to them (Smith et al., 2014).

The field of rat USVs are constructed and used by rodents is even more complex than just the three different beforementioned classes, with the 40-kHz flat sinus experiment as an indication of this difference. In an analysis of these differences in rat vocalizations by Takahashi, Kashino, and Hironaka (2010) investigated differences in patterns of USV calls, by looking at USVs that have pauses and modulations. When in a sequence, these calls are called a step, which corresponds to an instantaneous change of pitch in the USV. With rapid oscillations of them being called trills, these two moderations of the USVs they called frequency modulated (FM). They used these to identify three different clusters of vocalizations, cluster 1 was around 20-30-kHz, and corresponded to the 22-kHz vocalizations. The cluster 2 vocalizations were between 35-50-kHz, did not correspond to a previously described classification. It did however, correspond to the flat and lower components of the step, and was related to feeding. The cluster 3 which were USVs above 50-kHz vocalizations corresponded to the thrill, upward ramp, and higher components of the step, and was found to correspond with movement. There are also situational differences for when vocalizations; when food is readily available USVs are not seen reliably in response to food. However, when rats are food deprived they start emitting high FM type 50-kHz USVs in response to the anticipation of food arriving (Opiol et al., 2015). There is also evidence to contradict the idea that 50-kHz vocalizations purely as vocalizations for positive stimuli as rats can respond with 50-kHz calls to a saline injection, or drug withdrawal (Burn, 2008; Rainer Schwarting et al., 2007).

Further complicating the study of USVs is that rats might not even use their ears to hear some USVs; with the finding that rats might be able to pick up USVs with their whiskers (vibrissae; Wolfe, Mende, & Brecht, 2011). Meaning a deafening of a rat might not remove the ability to hear some of the USVs of other rats. However, the whiskers primary function appears to be sensing things in close by, so they should not be good enough to compensate fully. They do though have effects on rat socialization, shown by the experiment of Wolfe, Mende, and Brecht (2011). They found that whiskers influence social interactions between rats, by trimming the whiskers of rats, and letting the rats interact with each other before a video analysis. The findings showed that that trimming the whiskers of rats caused an increase in ferocious aggressive encounters, and decreased the amount of social interactions. (Burn, 2008; Wolfe et al., 2011). The similarity to the results from a study on rats that are devocalized, rats that have had their ability to vocalize removed, is striking. With Burke et al. (2017) using devocalized rats, and finding that devocalization appeared to cause rats to display increased ferocity in fighting, with serious biting happening in fights that may have started as play fighting; but instead it appears that due to lack of vocalization, ended up escalating to ferocious fighting between rats. Strongly indicating that the ability to vocalize, and the ability to hear them are important. In line with these finding is that 50-kHz flat vocalizations usually being emitted during fights (Burke et al., 2017), juvenile rats do however seem to not need USVs to keep fights friendly. With vocalizations also being emitted by the attacked animal (Sales, 2010). Some other effects of devocalizations are that female rats do not find males that are devocalized as attractive as males that can, with no effect of this seen in amounts of copulation (Chu et al., 2017; Wöhr, Seffer, & Schwarting, 2016). With playbacks of 50-kHz calls near a male negating the lowered attractivity of devocalized males (Willadsen et al., 2014) Meanwhile, female rats that are devocalized on the other hand seem to alter their own behavior by increasing their paracopulatory behavior (Snoeren & Ågmo, 2013). Rats also seem to prefer rats that can vocalize over rats that are devocalized (Kisko, Himmler, Himmler, Euston, & Pellis, 2015). Effects that can have dire consequences for adult rats, since rats appear to be living in relationships of reciprocity (tit-for-tat). They are likely to give back similar amounts of grooming, food, and other positive experiences as they get from another rat. Indicating that rats that are devocalized must increase their attractivity as a partner rat. Perhaps by showing more dominance, because rats groom a more dominant rat more a than a submissive rat, showing that increased dominance makes other rats find it worth it to groom them. It is also important to note that rats seem to be able to remember these relationships for 6 days, an effect that is 5 days longer than most experiments using sniffing as the memory of another rat (Burn, 2008; Schweinfurth, Stieger, & Taborsky, 2017; Schweinfurth & Taborsky, 2018; Stieger, Schweinfurth, & Taborsky, 2017). Taken together with the results from devocalization increasing ferociousness in fighting situations, lack of USVs might lead to a negative spiral in the relationship between the rats.

With all these effects of USVs there are strong indications that USVs are socially communicative, and USVs seem to follow many criteria for being useful for survival:

> Rat USVs may relate to several biological functions (a) a locating function announcing presence of the emitter and enabling its localization, (b) an emotive function carrying information about the emitter's internal emotional valence, (c) a conative function mobilizing the recipient for action in a non-specific way, or activating its attention, (d) an alarming function informing about external danger (promoting for instance, freezing and other defensive responses), (e) agonistic function promoting escape, withdrawal or dispersion, (f) an affiliative function signaling approach and promoting conspecific

contacts, and (g) a phatic function maintaining connections between individuals and maintaining cohesiveness of social groups in gregarious animals. - (Brudzynski, 2005).

The evidence is mostly lacking for (g), and (f) where we do not have enough evidence to support USVs functionality in group behavior. Most of the evidence on USVs has been gathered in dyads and some of the few results from group contexts are that increased contact with more individuals causes more 50-kHz flat USVs, and that they appear to have little consequence in sexual behavior in a semi-natural setting (Brenes et al., 2016; Chu et al., 2017). Most of these studies mentioned are also over relatively short periods of time, meaning that USVs might have immediate effects, but are not important over longer periods of time. However, with rat's apparent reciprocity relationships, the negative effects of devocalization in fights, and how USVs are emitted in many dyadic social situations it does appear unlikely that there is not an effect of USVs over longer periods of time.

Most of the studies that are done on rats also do not investigate them in a setting that allows them to behave in groups, which is something they seem to seek out when in the wild. There might then be no effect of USVs in the setting that that allows rats to behave more naturally. There is also a gap in many of these studies on USVs, because they lack an environment that resembles how they chose to live in the wild, with both an open-area and an area that resembles a nest and a burrow, and barely any studies have investigated USVs in conjunction with a burrow-area. With an even greater gap in literature of the affiliative non-sexual behaviors and the role of USVs in group housed in a semi-natural environment.

The purpose of this study will then be to try and find indications to the role of USVs in the formation of rat groups, by investigating if the ability to vocalize has an effect on the formation of groups, over time, in a semi-natural environment.

Method

Subjects

12 males and 16 female Wistar rats (300 and 250g, respectively) were purchased from Charles river. Following arrival, they were housed in same-sex pairs with a rat that was not in their experimental group. The cages were Macrolon IV cages with commercial rat pellets and water ad lib. The room they were in used 12:12 reversed light cycles, and was kept at 21 +/- 1 degree Celsius and a relative 55 +/- humidity

Surgery

All the rats received surgery or a sham surgery, where some got the nerve to the vocal cords, the laryngeal nerve, cut and the sham surgery operated rats had the same surgery without the laryngeal nerve to the vocal cords cut. All of the females had an additional surgery where their ovaries were removed (ovariectomy), to control when they were receptive. They could then be made interested in sexual behavior by injecting hormones (progesterone and estrogen).
The rats were tested after the devocalization surgery for the ability to vocalize (See Chu et al., 2017 for specifics and results)

Apparatus

The semi-natural environment was based on descriptions of wild rat burrows and open area (Calhoun, 1962 cited in Chu & Ågmo, 2014) and on Blanchard and Blanchard (1989)s open-field and burrow design. The overall size of the environment is 2.1 x 2.4m, divided into two rectangular shapes where one half was designed as a burrow area and one was designed to simulate an open-field area. The room in which the environment was located was divided by a light blocking separation made of extruded polyethylene foam, firmly fitted to the open-area wall facing the burrow-area and the walls and celling of the room. This made it possible to vary the light intensity in the open area while maintaining complete darkness in the burrow

Burrow. The tunnel walls and nest boxes in the burrow were made of sheet steel with the inside cowered by a thin, black plastic coating. Plexiglas covered the burrow area so that the tunnels and nest boxes in the burrow were visible for the camera but closed to the rats.

Open. Similar sheet steel as used in the burrow area surrounded the open area to a height of 44cm. To make sure that the subjects could not escape that area, 31cm high plexiglass sheets were fixed to the wall making a total height of 75cm. No cover was used in the open area. In the open area, a lamp 2.5 m above the center provided a light of 180 lux from 11:00-until 23:00 h (a diagram and overhead photo of both the open-area and the burrow area of the semi-natural environment can be seen in Chu & Ågmo, 2015).

General about the apparatus. The floor in both areas were made of dark polyvinylchloride mounted on a steel frame. With the wall separating the two areas of the environment being opened for the rats by creating four small openings (8 x 8 cm). With the wall opposite the burrow having small holes drilled into it for the tip of drinking bottles to be inserted into, to allow the rats to drink.

Camera. Two different types of camera and lamps were used to capture the open area and the burrow area. Two infrared lamps (850nm; model sal-60, New sutway Digital Technology [Shenzhen] Guangdong, P.R. China) assured that a high resolution, digital B/W camera (JVJ-3331H) produced a clear image over the entire burrow.

A video camera (Sanyo VCC-6592P) equipped with a zoom lens (Computar T6Z5710-CS 5.7-34.2 mm) and automatic iris was installed in the ceiling, at the same height as the lamp in the open area and close to it to assure an undistributed view of the entire area.

The cameras were connected to hard disk drive DVD recorders (Sony RDR-HX780) with a capacity large enough to record 64 h of video of good quality. Every 48h the contents on the hard disk were transferred to DVDs for storage, before being transferred to external hard drives for usage in the study.

Design

Before each group was introduced to the semi-natural environment apparatus, the floor in the open area, tunnels and the nest boxes were covered with approximately 2cm of aspen wood chips (Tapvei, Harjumaa, Estonia). Approximately 2kg of food pellets were put on the floor close to one corner of the open area. Twelve aspen wood sticks 2x2 cm, 10cm long (Tapvei) were randomly distributed in the open area, and three polycarbonate huts (15x 16,5 cm, height 8.5cm; Datesend, Manchester, UK) were relatively irregularly placed close to the middle, and to the sides of the open area. In addition, six pieces of a small square mat of nonwoven hemp fibers (5 x 5cm, 0,5cm thick; Happi mat; Datesend) were put in the nest boxes of the burrow area as bedding material.

Procedure

The rats were divided into four groups, each with four females and three males that were unfamiliar with each other, and had one copulatory experience before entering the semi-natural environment. With the females being unreceptive to sexual approaches in the days used for this study (day 0 and 1). To help with identification between the rats, the males 1-3 were shaved and had their tails colored with a black marker with 1, 2, 3 or no lines. Rat 1-3 received lines where the males received thicker lines than the females. Female 1 and male 1 received shaving behind the neck, female 2 and male 2 received shaving on the back and female 3 and male 3 received shaving on their lower back towards the tail. Female 4 did not receive shaving or any marking and was identified from not having any markings or shaving. The rats were then let into a semi-natural environment and left alone for 8 days. Where the first and second day was used in this experiment and later days was used in (Chu et al., 2017)

Analysis

The videos were analyzed using the Noldus 12.5 software. Where 29 different behaviors were coded (see table 1 for descriptions). Each group had a total of 48 videos of 10minutes, where the burrow-area and open-area videos combined and synchronized to as close to no visual difference as possible. Creating 24 video combinations containing both the open-area and the burrow area for each group. With the total for all for all four groups being 96 videos. The start of the observations was from the 10first minutes of the rats in each group 1 to 4. Then the next sample of 10minutes was taken an hour later, and the next an hour after that again until this was done six times total for the nightlight session for day 0. The next set of sample observations was taken from when the light in the open-area was switched on around 2305, then a new sample was taken of 10minutes with the next 10minutes being taken an hour later, and so on until done six times total for the daylight session for day 0. Then the next set of videos were taken approximately the same time they were let into the environment 24hours ago, and the same procedure of 10minute samples taken six times was done again for the nightlight for day 1, and for the daylight session (See table 2 for a more visual explanation).

The results were analyzed in SPSS version 25, where the Shapiro-Wilks test showed that the data was not normally distributed in the dataset (data not shown). Therefore, the non-parametric test Mann-Whitney U was chosen to test for two-tailed differences between the sham-operated rats and the devocalized in both in frequency and duration. The video material of each session was combined into segments, creating four segments, and one combination for day 0 and one combination for day 1. A combination was also done with the two segments of nightlight data, and the two segments of daylight data (see table 2). This was done both for duration of the behaviors, frequency of behaviors, and who they did behaviors their behavior towards. (for the appropriate behaviors). With the total of everything not needing a combination variable. The rats were then given their corresponding code 1 for sham-operated and 2 for devocalized, creating the two groups used in the analysis.

Some analysis was not done due to time, and the space it would take to report them properly: differences between the sexes, open-area/burrow and frequency in behavior of being done to rat groups. An analysis into whether the sham-operated behave differently internally with each other and if the devocalized rats behave differently towards other devocalized rats.

Table 1
Descriptions of behaviors coded

Behavior	Descriptions of the behaviors
Boxing/wrestling	Boxing or wrestling with another rat; usually happens standing
Resting/immobile	lack of movement and any other behavior; can be sleeping
Resting with others	Resting or sleeping on another rat or in very close that is also resting or sleeping
Walk over/under others	Moving underneath or ontop of another rat
Running	Movement speed faster than walking
Non-social exploration	Examination of the area that the rat is in; this variable was also measured as location: open-area->wall/reflection, open-area ->open field, and burrow
Sniffing Anogenitally	Sniffing of the genitals; may also include licking of the genitals; usually identified by turning of the head and moving the head to the other rat's genitals
Self-grooming	Fixing or otherwise fiddling with one's own fur
Grooming others	Fixing or otherwise fiddling with another rat's hair; not including aggressive allogrooming
Drinking	Ingestion of water or other liquids
Pursuing/chasing	Movement to get closer to one or several rats with specific intent; may be to assist another rat; to follow another rat; to move to attack another rat; or to make sure that rat moves away from a given area or oneself
Approach	Movement towards another rat face first; usually friendly
Freezing	Stops to move or do anything as a response to a stimulus
Freezing with other	Like freezing just in proximity of another rat
Nose-off	Conflict with another rat where the snouts are used to decide the conflict; can be hostile, but can also not hostile be
Fighting with other	Ferociously battling another rat, everything a rat can do to hurt another rat, like biting, may be employed
Kicking	Punching or hitting the other rat with the paws
Sniffing other	Using their nose to smell the other rat(s) in close vicinity
Flee	Using a high amount of energy, usually only movement, to get away from another rat; can also include running over/under another rat at this speed; can also include energetic and forceful expulsion of energy to get to an area blocked by a rat
Mount	Being on top of another rat in a position that is usually sexual in nature
Food transport	Taking food pellets from one area to another; usually moved to a nest box in the burrow
Digging/moving bedding/nesting material/wood	Pushing/digging/moving or otherwise reposition what is on the floor
Carrying nesting materials/wood	Transporting nesting material/wood sticks
Chewing wood	Investigating or biting the wood sticks
Eating	Ingesting food or investing a food pellet in a way that is hard to distinguish from eating; only difference seen if pellet is dropped without having been eaten of
Hiding	Being inside the boxes provided in the open area without other behavior being visible
Aggressive stance	Aggressive posturing that does not include touching another rat
In opening	Being inside or clearly looking through the opening between the open area and burrow without doing anything else
Nose greeting	Nose to nose short sniffing; a nose to nose hello
Standing non-social exploration	Standing in the open area examining the surroundings this variable was also measured as location: open-area->wall/reflection, open-area ->open field, and burrow

Note: Some behaviors might not happen; the seemingly nonsensical order of the behaviors comes from the computer program Noldus 12.5

Table 2

Table for video observations

Day	Lighting	Segment	Group 1	Group 2	Group 3	Group 4
0.	NO	1	Day 0 1312-1322	Day 0 1305-1315	Day 0 1347-1357	Day 0 1318-1328
	L		Day 0 1412-1422	Day 0 1405-1415	Day 0 1444-1454	Day 0 1418-1428
	I		Day 0 1512-1522	Day 0 1505-1515	Day 0 1544-1554	Day 0 1527-1537
	G		Day 0 1612-1622	Day 0 1605-1615	Day 0 1644-1654	Day 0 1627-1637
	H		Day 0 1712-1722	Day 0 1705-1715	Day 0 1750-1800	Day 0 1727-1737
	T		Day 0 1812-1822	Day 0 1805-1815	Day 0 1850-1900	Day 0 1827-1837
0.	L	2	Day 0 2312-2322	Day 0 2305-2315	Day 0 2305-2315	Day 0 2305-2315
	I		Day 0 0012-0022	Day 0 0005-0015	Day 0 0005-0015	Day 0 0005-0015
	G		Day 0 0112-0122	Day 0 0105-0115	Day 0 0105-0115	Day 0 0105-0115
	H		Day 0 0212-0222	Day 0 0205-0215	Day 0 0205-0215	Day 0 0205-0215
	T		Day 0 0312-0322	Day 0 0305-0315	Day 0 0305-0315	Day 0 0305-0315
	S		Day 0 0412-0422	Day 0 0405-0415	Day 0 0405-0415	Day 0 0405-0415
1.	NO	3	Day 1 1312-1322	Day 1 1305-1315	Day 1 1344-1354	Day 1 1330-1340
	L		Day 1 1412-1422	Day 1 1405-1415	Day 1 1444-1454	Day 1 1430-1440
	I		Day 1 1512-1522	Day 1 1505-1515	Day 1 1544-1554	Day 1 1530-1540
	G		Day 1 1612-1622	Day 1 1605-1615	Day 1 1644-1654	Day 1 1630-1640
	H		Day 1 1712-1722	Day 1 1705-1715	Day 1 1744-1754	Day 1 1730-1740
	T		Day 1 1812-1822	Day 1 1805-1815	Day 1 1844-1854	Day 1 1830-1840
1.	L	4	Day 1 2312-2322	Day 1 2305-2315	Day 1 2305-2315	Day 1 2305-2315
	I		Day 1 0012-0022	Day 1 0005-0015	Day 1 0005-0015	Day 1 0005-0015
	G		Day 1 0112-0122	Day 1 0105-0115	Day 1 0105-0115	Day 1 0105-0115
	H		Day 1 0212-0222	Day 1 0205-0215	Day 1 0205-0215	Day 1 0205-0215
	T		Day 1 0312-0322	Day 1 0305-0315	Day 1 0305-0315	Day 1 0305-0315
	S		Day 1 0412-0422	Day 1 0405-0415	Day 1 0405-0415	Day 1 0405-0415

Note: No light refers to lo level lighting seen during nighttime; Lights refer to lighting resembling daytime; each combination of six videos is a segment

Results

Location of non-social exploration and standing-non-social exploration was non-significant for all permutations (data not shown).

The analysis for both days

When both days are combined in one analysis, most behaviors turned out to be not significantly different between sham-operated and devocalized rats.

Sham-operated. Sham-operated rats flee more times and for longer duration, a small effect size (see table 3 and table 4).

Devocalized. The rats that were devocalized had more instances of food transport (see table 3) and had a longer duration of food transport (see table 4), with an almost medium effect size (see table 3 and 4). There was a significant difference in frequency (see table 3) and duration (see table 4) of running, with a small effect size (see table 3 and table 4).

Nighttime data versus daytime

When testing for differences in the behaviors in the data for the nighttime, and the data for the daytime, only the same differences as already described for the total data was found (data not shown).

The data for day 0

When the data for day 0 was analyzed most behaviors turned out to be not significantly different between sham-operated and devocalized rats.

Sham-operated. There was also a significant effect found for flee on day 0 on frequency of flee with sham-operated rats fleeing more times (table 5), they also had longer durations of flee (see table 7) the effect size was small (see table 5 and 7). The sham-operated rats spent longer time on non-social exploration, with a small effect size (see table 12)

Devocalized. For food transport there was also a significant effect, with devocalized rats transporting food more times (see table 5), and for longer durations (see table 7) the effect size was medium (see table 5). The devocalized rats also stayed for longer periods in the openings, the effect size was small (see table 7) The devocalized rats were groomed for longer durations, with a large effect size (see table 18).

The data for day 1

When the data for day 1 was analyzed most behaviors turned out to be not significantly different between sham-operated and devocalized rats.

Sham-operated. The sham-operated rats had more instances of running (see table 6), the effect size of this was small (see table 6 and table 8). There was also a significant effect for frequency sniffing anogenitally with a close to medium effect size (see table 6).

Devocalized. The devocalized rats ran for longer durations of (see table 8), the effect size was small. The rats that were devocalized had longer durations of being pursued/chased, with a small effect size (see table 19).

Data for segment 1 (No light)

When the data for segment 1 was analyzed most behaviors turned out to be not significantly different between sham-operated and devocalized rats.

Sham-operated. The sham-operated rats fled more times (see table 13) and for longer times than devocalized rats (see table 9), small effect size (see table 13 and table 9), The sham-operated rats had longer durations of non-social exploration, small effect size (see table 9)

Devocalized. There was a difference in duration in chewing wood, small effect size (see table 9). For food transport there was a significant difference in frequency (see table 13) and duration (table 9) with large effect sizes (see table 13 and table 9). The devocalized rats were also mounted for longer duration, with a medium effect size (see table 20).

Data for segment 2 (Light)

When the data for segment 2 was analyzed most behaviors turned out to be not significantly different between sham-operated and devocalized rats.

Sham-operated. The sham-operated rats hid more times, with a large effect size (see table 14) The sham-operated rats were pursued/chased more, with a medium effect (see table 21).

Devocalized. There was a significant difference for duration of running where devocalized rats ran for longer, small effect size (see table 10) –and walking there was a difference for more walks for devocalized rats, small effect size (see table 14).

Data for segment 3 (No light)

When the data for segment 3 was analyzed most behaviors turned out to be not significantly different between sham-operated and devocalized rats.

Sham-operated. There was a difference in frequency of anogenital sniffing with sham-operated rats sniffing more times, medium effect size (see table 15)

Devocalized. There was a significant difference in duration of running where devocalized rats ran more. The devocalized rats were also chased more, with a small effect (see table 22)

Data for segment 4 (Light)

When the data for segment 4 was analyzed most behaviors turned out to be not significantly different between sham-operated and devocalized rats.

Devocalized. There was however a difference in duration of hiding where devocalized rats hid for longer periods of time, with a medium effect size (see table 12). The devocalized rats were also sniffed more anogenitally, with a large effect size (see table 23).

Table 3

Frequency of behavior that they are doing for both days

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Aggressive stance	2.77	89.2	2	102.3	3275	-1.650	.099	
Approach	5	213	5	202	18863	-.8891	.374	
Boxing/wrestling	2	81.7	2	84.13	2827.5	-.3159	.752	
Carrying nesting materials/wood	1	2	3	4	0	-1.414	.157	
Chewing wood	1	39.5	2	46	688	-1.273	.202	
Digging/moving bedding/nesting material/wood	3	181.79	3	171.44	13772	-.927	.354	
Drinking	2	65	2	68.7	1977	-.564	.573	
Eating	1	32	1	31.98	429.5	-83	.993	
Fighting with other	1	7.8	1	8.4	23	-.414	.679	
Flee	1	68.2	1	54.28	1442.5	-2.25	.025	.04
Food transport	2	27.91	3.5	40.66	347	-2.726	.006	.11
Freezing	1	3	1	3	3	0	1	
Grooming others	1	34.4	1	38.71	514.5	-1.048	.29	
Hiding	1	92.3	1	81.6	3125	-1.405	.160	
Hiding with others	1	15.3	1	12.65	73.5	-1.108	.268	
In opening	6	160	7	18.45	11133	-1.906	.057	
Kicking	2	122	2	123	6917	-.087	.931	
Mount	2	27.9	4	31.05	329	-.705	.481	
non-social exploration	21	264	23	273.2	31522	-.634	.526	
Nose greeting	1	17	1	15.89	117.5	-.375	.707	
Nose-off	3	145	3	145.3	9284	-.427	.966	
Pursuing/chasing	3	98.7	2	98.05	3809.5	-.0702	.944	
Resting with others	1	18.9	2	23.85	132	-1.491	.136	
Resting/immobile	3	288	3	285.8	37630	-.1351	.893	
Running	3	142	6	169.4	875.5	-2.662	.008	.02
Self grooming	3	21.6	3	22.57	19493	-.2519	.423	
Standing non-social exploration	3	96.7	4	94.66	4091	-.2519	.801	
Walk over/under others	2	75.7	2	66.53	2051.5	-1.318	.188	
Walking	1.01	23.7	3	246	23405	-1.158	.247	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 4

Total duration of behavior being done for both days

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta-squared (η2)
Aggressive Stance	5.56	92.69	7.6	95.16	3711.5	-.293	.77	
Approach	9.68	213.49	8.99	200.83	18697.5	-.0262	.305	
Boxing/wrestling	7.18	81.47	6.72	84.72	2796.5	.4082	.683	
Carried nesting materials/wood between	.88	2	15.68	4	0	1.342	.18	
Chewing wood	3.96	39.11	6.3	46.61	688.5	-1.38	.168	
Digging/moving bedding/nesting material/wood	24.2	184.4	16.84	166.92	13185	-1.546	.122	
Drinking	9.36	64.31	10.12	69.76	1920.5	-.8031	.422	
Eating	33.28	34.91	14.46	25.75	1.8457	1.846	.065	
Fighting with other rats	.96	7.5	1.62	9	20	-.612	.54	
Flee	2.76	68.03	2.21	54.58	1457.5	2.052	.04	.03
Freezing	1.86	3.5	1.52	2.67	2	-.477	.057	
Running	7.93	141.88	10.91	169.25	8763.5	2.625	.009	.02
Grooming others	4.6	35.83	5.54	36.29	577.5	-.0895	.929	
Hiding	5.98	13.75	6.6	14.27	87.5	-.1699	.865	
In opening	18.27	160.40	22.36	178.87	11321	1.677	.094	
Kicking	2.68	121.78	2.8	123.71	6851	.2063	.837	
Food transport	7.4	27.66	16.26	40.94	338	2.787	.005	.12
Mounts	4.88	28.54	9.26	29.85	353	-.2843	.776	
non-social exploration	131.68	273.54	125.28	256.48	30519.5	-.1.222	.222	
nose-offs	9.91	145.03	10.08	144.95	9307	-.75	.994	
Pursued/chased	7.72	98.34	6.7	98.93	3811	-.065	.948	
Self grooming	31.58	216.23	26.88	209.66	19816	-.521	.602	
Standing non-social exploration	8.2	97.21-	8.875	93.8	4032.5	-.409	.683	
Walk over/under others	4.74	74.63	4.06	68.5	2150	-.839	.401	
Walking	53.8	231.03	58.4	245.47	23494	-1.094	.274	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 5

Table for frequency of behaviors day 0

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean Rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta-squared (η2)
Aggressive Stance	2	54.08	2	60	1206	-.956	.339	
Approach	7	122.61	6	114.81	5945	-.825	.409	
Boxing/wrestling	2	53.51	2	58.28	1163.5	-.753	.451	
Carrying nesting materials/wood	1	1	3	2	0	-1	.317	
Chewing wood	1.5	26.69	2	31.3	312.5	-1.096	.273	
Digging/moving bedding/nesting material/wood	3	88.09	3	83.45	3153.5	-.591	.555	
Drinking	2	38.05	2	39.37	627.5	-.261	.794	
Eating	1	23.32	1	22.37	215.5	-.251	.802	
Fighting with other	1	4.71	1.5	6	5	-.801	.423	
Flee	2.5	48.18	1.5	37	666	-2.127	.033	.05
Food transport	1	13.41	4	21.45	5.5	-2.385	.017	.18
Freezing	1	3	1	3	3	0	1	
Grooming others	1	22.38	1.5	26.07	188	-12	.316	
Hiding	2	48.57	2	41.49	808	-1.277	.202	
Hiding with others	2	6	1	4.2	6	-1.095	.273	
In opening	6	85.09	7	10.25	2914.5	-1.837	.066	
Kicking	3	81.65	3	79.87	2942.5	-.238	.812	
Mount	3	23.58	4	27.68	226.5	-.969	.333	
non-social exploration	24	144.86	24	142.32	9052	-.245	.806	
Nose greeting	1	16.97	1	15.89	117.5	-.375	.707	
Nose-off	2	72.19	2	74.99	2079.5	-.3744	.708	
Pursuing/chasing	4	63.71	4	66.6	1554	-.396	.692	
Resting with others	1	6.1	2	10	6	-1.75	.08	
Resting/immobile	3	15.31	2	149.46	10296	-.083	.933	
Running	3	81.5	6	95	2736	-1.693	.09	
Self grooming	3	112.71	3	115.19	5422.5	-.2693	.787	
Sniffing anogenitally	2	44.59	2	41.13	732.5	-.613	.54	
Sniffing others	12	129.15	11	124.34	6989.5	-.495	.62	
Standing non-social exploration	4	62.02	5	6.44	1597.5	-.233	.815	
Walk over/under others	2	45.51	2	4.46	701	-.893	.371	
Walking	2	125.72	26.5	14.88	6447	-1.511	.131	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 6

Table day 1 frequency of behavior

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Aggressive Stance	1	35.83	3	42.34	516.5	-1.304	.192	
Approach	4	88.97	4	90.35	3682	-.175	.861	
Boxing/wrestling	1	28.89	1	26.17	300	.691	.861	
Carrying nesting materials/wood	3	1.5	1.5	0	0	-1.414	.157	
Chewing wood	1	13.24	2.5	15.3	72	-.696	.486	
Digging/moving bedding/nesting material/wood	4	94.62	3	87.87	3682.5	-.849	.396	
Drinking	2	27.95	2	29.09	375.5	-.270	.787	
Eating	1	9.27	1	10.1	29.5	-.354	.723	
Fighting with other	1	3.5	1	3.5	4.5	0	1	
Flee	1	21.07	1	17.1	136.5	-1.208	.227	
Food transport	2	16.18	3.5	19.98	121.5	-1.074	.283	
Grooming others	1	12.04	1	14.04	65.5	-1.066	.286	
Hiding	2	44.33	1	40.57	752	-.716	.474	
Hiding with others	1	9.85	1	9.06	36.5	-.479	.632	
In opening	6	75.16	7	81.07	2619	-.806	.420	
Kicking	1	40.01	2	45.17	714.5	-1.056	.291	
Mount	1	4.80	1	4	6	-.775	.439	
non-social exploration	18	118.67	23	132.97	6304.5	-1.521	.128	
Nose-off	3	73.94	3	70.16	2319	.539	.589	
Pursuing/chasing	2	35.1	2	32.95	436	.423	.672	
Resting with others	1	13.41	1	15	75	.606	.545	
Running	3	74.75	5	60.6	1663.5	-2.112	.035	.03
Resting/immobile	2.5	137.81	2	136.94	8569	-.09	.929	
Self grooming	3	98.17	3	105.96	4310	-.929	.353	
Sniffing anogenitally	2	21.72	1	12.14	57	-2.128	.033	.11
Sniffing others	8	102.52	6	97.2	4461	-.633	.526	
Standing non-social exploration	2	33.93	2.5	36.57	530	-.554	.579	
Walk over/under others	2	30.52	1	26.92	346	-.858	.391	
Walking	13	103.86	15.5	109.5	4960	-.651	.515	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 7

Day 0 Duration

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Aggressive Stance	6.04	56.78	5.14	54.38	1291.5	-.369	.712	
Approach	13.36	120.80	12.24	118.41	6233.0	-.252	.801	
Boxing/wrestling	7.88	53.87	8.52	57.5	1190.0	-.556	.578	
Carrying nesting materials/wood	.88	1	15.68	2	0.0	-1.000	.317	
Chewing wood	3.84	26.16	6.16	32.11	294.5	-1.334	.182	
Digging/moving bedding/nesting material/wood	23.04	91.72	13.56	76.5	2743.5	-1.903	.057	
Drinking	5.22	38.38	5.16	38.73	644.0	-.066	.948	
Eating	31.5	25.40	14.56	18.20	153.0	-1.734	.083	
Fighting with other	0.88	5	7.28	5	7.0	0.000	1.000	
Flee	3.40	48.40	2.18	36.69	655.0	-2.145	.032	.05
Hiding	9.52	45.23	10.48	47.35	912.5	-.367	.713	
Food transport	3.64	13.5	14.92	21.25	52.5	-2.219	.027	.16
Freezing	1.86	3.5	1.52	2.67	2.0	-.577	.564	
Grooming others	4.64	23.25	7.30	24.07	216.0	-.191	.849	
Hiding with others	5.98	6.75	1.88	3.60	3.0	-1.722	.085	
In opening	18.24	84.57	24.42	101.34	2851.5	-2.026	.043	.02
Kicking	3.58	80.24	4.04	82.32	2931.0	-.274	.784	
Mount	5.32	23.94	9.56	27	238.0	-.714	.475	
Non-social exploration	145.55	149.51	122.92	133.20	8167.5	-1.575	.115	
Nose-off	7.64	72.34	9.92	74.61	2095.5	-.294	.769	
Pursuing/chasing	9.92	65.36	8.80	62.21	1547.5	-.428	.669	
Resting with others	131.40	6.5	173.04	8.67	10.0	-.845	.398	
Resting/immobile	125.24	146.49	165.92	156.12	9688.0	-.927	.354	
Running	7.89	82.10	10.93	93.81	2804.0	-1.458	.145	
Self grooming	33.32	115.81	25.46	108.56	5188.5	-.776	.438	
Sniffing anogenitally	5.96	44.03	4	42.35	765.5	-.288	.773	
Sniffing others	39.28	128.40	37.96	125.78	7115.0	-.269	.788	
Standing non-social exploration	10.07	61.49	12.12	61.51	1639.5	-.003	.998	
Walk over/under others	5.12	45.92	3.88	39.5	676	-1.085	.278	
Walking	65.84	125.08	80.25	142.27	6332.5	-1.714	.087	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 8

Day 1 Duration

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Aggressive Stance	4.48	36.75	9.96	40.5	562.5	-.702	.482	
Approach	8.34	91.95	7.40	85.54	3471	-.805	.421	
Boxing/wrestling	4.52	27.89	4.62	28.22	329	-.072	329	
Chewing wood	4.84	13.47	12.20	14.90	76	-.452	.651	
Digging/moving bedding/nesting material/wood	25.94	93.24	22.24	90.05	3837.5	-.397	.692	
Drinking	15.80	27.72	13.36	29.33	369	-.369	.712	
Eating	41.32	9.69	11.52	9	30	-.246	.805	
Fighting with other	1.04	3	1.62	4	3	-.655	.513	
Flee	2.24	20.39	2.12	18.13	152	-.612	.540	
Food transport	8.55	16.82	16.34	19.57	130.5	-.763	.446	
Grooming others	3.92	13.04	4.16	12.96	77.5	-.027	.978	
Hiding	63.94	45.39	61.64	38.62	693.5	1.209	.227	
Hiding with others	10	8	25	11.38	25	1.333	.183	
In opening	18.68	76.85	20.44	78.48	2776.5	-.222	.825	
Kicking	1.56	41.38	1.58	42.98	784.5	-.295	.768	
Mount	2.04	5.20	1.52	3.33	4	-1.043	.297	
non-social exploration	126.60	123.69	127.54	124.52	7082.5	-.087	.930	
Nose-off	14.12	74.76	10.56	68.85	2246.5	-.826	.930	
Pursuing/chasing	5.12	33.13	5.84	38.03	398.5	-.916	.360	
Resting with others	148.36	13.82	198.94	14.30	82	-.151	.880	
Resting/immobile	294.04	140.38	266.82	132.33	8117	-.806	.420	
Running	8.16	60.06	10.88	75.52	1621.5	-2.289	.022	.04
Self grooming	30	101.11	29.63	100.81	4658	-.035	.972	
Sniffing anogenitally	6.16	21.64	2.24	12.5	59.5	-1.922	.055	
Sniffing others	24.28	102.61	22.56	97.05	4450	-.659	.510	
Standing non-social exploration	6.12	34.96	5.10	35.05	572.5	-.018	.985	
Walk over/under others	4.45	29.02	4.30	28.98	395.5	-.008	.994	
Walking	40.68	104.62	44.59	108.26	5059.5	-.419	.675	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 9
Segment 1 duration

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Aggressive Stance	5.28	38.27	4.32	37.46	611.5	-.152	.879	
Approach	15.72	80.97	15.24	76.75	2681.5	-.551	.582	
Boxing/wrestling	8.08	46.03	7.80	49.14	866	-.514	.607	
Chewing wood	2.62	20.36	6.18	28.39	164	-1.981	.048	.09
Digging/moving bedding/nesting material/wood	11.68	46.55	7.68	42.36	837	-.739	.460	
Drinking	4.64	28.39	4.14	30.13	347.5	-.376	.707	
Eating	34.80	21.26	18.06	15.29	109	-1.628	.103	
Fighting with other	0.88	5	7.28	5	7	.000	1.000	
Flee	5.78	43.85	2	32.53	512.5	-2.198	.028	.06
Food transport	3.46	8.43	12.35	15.33	13	-2.392	.017	.29
Freezing	1	2	1.52	2.67	1	-.447	.655	
Grooming others	4.58	17.31	7.52	19.5	115.5	-.586	.558	
Hiding	8.58	37.61	7.98	37.27	594.5	-.064	.949	
Hiding with others	5.98	6.75	1.88	3.60	3	-1.722	.085	
In opening	18.23	58.24	25.78	71.45	1322	-1.914	.056	
Kicking	4.18	61.30	4.68	60.49	1687	-.123	.902	
Mount	5.32	21.81	9.56	22.31	211	-.126	.900	
non-social exploration	175.24	89.05	143.51	72.13	2485.5	-2.181	.029	.03
Nose-off	6.44	44.86	9.76	47	810	-.357	.721	
Pursuing/chasing	11.48	53.33	12.06	52.17	1100	-.177	.859	
Resting with others	32.90	1.5	107.04	3	0	-1.225	.221	
Resting/immobile	21.58	67.07	28.88	77.15	2027	-1.429	.153	
Running	9.24	61.32	9.96	63.22	1684.5	-.282	.778	
Self grooming	29.28	68.99	20.92	65.95	1912	-.422	.673	
Sniffing anogenitally	5.24	35.13	3.98	34.73	511	-.077	.938	
Sniffing others	45.56	85.91	40.14	76.27	2712.5	-1.243	.214	
Standing non-social exploration	10.32	51.01	14.36	52.52	1105	-.240	.811	
Walk over/under others	4.68	35.76	3.31	28.67	371	-1.398	.162	
Walking	85.31	79.77	95.65	83.30	2811	-.458	.647	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 10

Segment 2 duration table

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Aggressive Stance	13.16	18.68	11.72	18.09	133	-.155	.877	
Approach	7.5	40.99	6.12	41.02	699.5	-.005	.996	
Boxing/wrestling	7.56	8.18	9.60	9.20	24	-.397	.692	
Chewing wood	18	6	4.40	4.75	9	-.640	.522	
Digging/moving bedding/nesting material/wood	35.08	44.91	18.36	35.62	575	-1.630	.103	
Drinking	9.44	10.38	8.32	9.17	34	-.439	.661	
Eating	25.08	5	3.20	1	0	-1.528	.127	
Flee	2.84	4.67	4.12	5.67	7	-.516	.606	
Food transport	7.96	5.36	40.14	7.13	9.5	-.852	.394	
Grooming others	6.54	6.5	5.36	4.67	8	-.816	.414	
Hiding	28.74	8	599.56	9.89	28	-.770	.441	
In opening	18.24	26.34	20.30	31.42	271.5	-1.103	.270	
Kicking	1.96	19.48	3.60	22.39	155.5	-.752	.452	
Mount	2.40	3	18.32	6	0	-1.464	.143	
non-social exploration	105.28	63.63	86.60	56.79	1417	-.990	.322	
Nose greeting	0.88	1	1.56	2	0	-1.000	.317	
Nose-off	10.74	27.69	10.76	28.83	287.5	-.236	.813	
Pursuing/chasing	7.10	12.78	3.20	9.20	31	-1.043	.297	
Resting with others	203.60	5.13	386.44	7	5	-.783	.433	
Resting/immobile	385.40	77.27	442.13	83.79	2576.5	-.849	.396	
Running	6.36	22	11.12	31.23	140	-2.030	.042	.09
Self grooming	37.52	47.32	27.58	43.04	799	-.714	.475	
Sniffing anogenitally	13.96	9.42	13.48	8	25	-.527	.598	
Sniffing others	18.12	43.95	30.72	48.76	790	-.816	.415	
Standing non-social exploration	7.44	10.69	8	10.14	43	-.198	.843	
Walk over/under others	5.26	10.84	6.80	11.5	37.5	-.206	.836	
Walking	31.88	46.90	53.97	58.69	723	-1.797	.072	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 11
Segment 3 duration

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Aggressive Stance	4.76	27.32	10.86	33.31	259	-1.225	.220	
Approach	8.44	62.56	7.92	55.79	1475.5	-1.038	.299	
Boxing/wrestling	3.64	20.02	3.56	18.23	134.5	-.451	.652	
Chewing wood	5.36	9.64	12.20	10.5	40	-.330	.741	
Digging/moving bedding/nesting material/wood	18.64	53.96	22.24	58.31	1275	-.684	.494	
Drinking	12.72	19.29	13.32	19.76	174	-.132	.895	
Eating	41.32	6.89	5.5	2	1	-1.886	.059	
Fighting with other	0.84	1.5	2.88	3	0	-1.225	.221	
Flee	2.27	15.77	1.98	11.79	63.5	-1.293	.196	
Food transport	8.55	11.25	21.14	15.43	57	-1.389	.165	
Grooming others	3.92	8.79	3.68	8.28	29.5	-.212	.832	
Hiding	63.69	25.09	136.76	27.82	258	-.619	.536	
Hiding with others	15.60	4.60	25.64	6.40	8	-.940	.347	
In opening	23.60	52.15	24.29	53.08	1245	-.151	.880	
Kicking	1.24	27.30	1.74	29.23	325.5	-.429	.668	
Mount	2.78	3.5	1.52	2.67	2	-.577	.564	
non-social exploration	149.62	70.84	154.52	74.10	2239	-.451	.652	
Nose-off	14.74	47.81	12.57	45.60	955.5	-.379	.705	
Pursuing/chasing	4.92	24.49	5.66	28.11	215.5	-.789	.430	
Resting with others	12.76	5.71	35.62	6.5	12	-.378	.705	
Resting/immobile	164.52	67.45	139	64.66	1874.5	-.398	.690	
Running	9.5	39.52	17.32	50.66	677	-2.017	.044	.02
Self grooming	29.40	59.54	33.48	62.35	1543.5	-.421	.674	
Sniffing anogenitally	9.76	14.55	1.10	4.88	9.5	-2.410	.016	.23
Sniffing others	26.86	68.39	21.76	57.80	1588.5	-1.557	.119	
Standing non-social exploration	6.40	24.90	8.08	26.33	287	-.344	.731	
Walk over/under others	4.20	18.32	3.40	16.47	127	-.538	.591	
Walking	41.84	64.48	50.87	67.31	1865.5	-.412	.680	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 12

Segment 4 duration behavior

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Aggressive Stance	2.88	9.83	2.92	9.17	37.5	-.265	.791	
Approach	5.68	29.60	6.88	3.63	399.5	-.225	.822	
Boxing/wrestling	7.32	8.40	7.61	9.86	29	-.586	.558	
Chewing wood	3.38	4.17	9.64	5.5	4	-.667	.505	
Digging/moving bedding/nesting material/wood	32.04	39.66	23.42	33.59	547	-1.212	.226	
Drinking	16.32	9	21.64	9.90	36	-.355	.722	
Eating	55.16	3.5	143.16	4.67	4	-.707	.480	
Fighting with other	4.32	3	1.45	1.5	0	-1.225	.221	
Flee	1.52	5.75	3.44	6.67	10	-.408	.683	
Food transport	13.34	7	8.08	5.13	5	-.783	.433	
Grooming others	4.32	4.83	4.28	5.33	8	-.258	.796	
Hiding	169.84	2.64	6.88	12.42	7.5	-2.341	.019	.16
Hiding with others	4.40	4	24.36	5.33	5	-.745	.456	
In opening	11.70	24.61	16.68	26.64	283	-.489	.625	
Kicking	1.92	15.03	1.36	13.79	87.5	-.395	.693	
non-social exploration	87.37	52.89	78.24	51.90	1267	-.163	.871	
Nose-off	13.22	27.48	9.04	23.88	27.5	-.852	.394	
Pursuing/chasing	6.16	9.38	6.08	9.80	31	-.148	.882	
Resting with others	521.05	8.60	531.91	8.33	29	-.109	.914	
Resting/immobile	542.71	74.44	40	66.56	2096.5	-1.105	.269	
Running	5.40	2.82	8.53	25.73	195.5	-1.245	.213	
Self grooming	35.48	42.16	29	39.22	727	-.551	.582	
Sniffing anogenitally	3.88	7.23	4.92	8.5	13.5	-.468	.640	
Sniffing others	17.88	34.42	3.45	39.59	534	-1.028	.304	
Standing non-social exploration	3.22	1.42	3.20	9.29	37	-.423	.672	
Walk over/under others	5.74	11.29	7.16	13.11	53	-.630	.529	
Walking	31.78	39.98	27.98	42.48	743	-.471	.638	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 13
Segment 1 frequency

	Median sham-operated	Mean Rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Aggressive Stance	2	37.10	2	39.80	580	-.540	.589	
Approach	10	81.94	9	74.93	2581	-.919	.358	
Boxing/wrestling	2	46.04	2	49.12	866.5	-.526	.599	
Carrying nesting materials/wood	1	1	3	2	0	-1.000	.317	
Chewing wood	1	21.68	2	26.33	201	-1.240	.215	
Digging/moving bedding/nesting material/wood	2	43.74	3	47.14	853.5	-.615	.539	
Drinking	2	28.24	2	30.40	342	-.495	.621	
Eating	1	19.26	1.5	18.57	155	-.204	.838	
Fighting with other	1	4.71	1.5	6	5	-.802	.423	
Flee	3	43.66	2	32.79	521	-2.188	.029	.06
Food transport	1	8.21	4	15.83	10	-2.790	.005	.39
Freezing	1	2.5	1	2.5	1.5	.000	1.000	
Grooming others	1	16.73	2	20.77	101.5	-1.261	.207	
Hiding	2	37.53	2	37.44	598.5	-.018	.986	
Hiding with others	2	6	1	4.20	6	-1.095	.273	
In opening	7.5	58.91	8	70.04	1378.5	-1.615	.106	
Kicking	4	62.65	3	58.21	1584.5	-.683	.495	
Mount	3	21.57	4	22.72	204.5	-.293	.770	
non-social exploration	29	85.12	30	79.19	2902.5	-.764	.445	
Nose greeting	1	15.94	1	14.92	103	-.359	.720	
Nose-off	2	45.06	2	46.54	822.5	-.254	.799	
Pursuing/chasing	4	52.27	5	54.82	1070.5	-.389	.697	
Resting with others	2	2	2	2	1	.000	1.000	
Resting/immobile	3	70.94	2	71.10	2359.5	-.024	.981	
Running	4	60.57	6	64.57	1625	-.599	.549	
Self grooming	3	67.87	2.5	68.27	1990	-.057	.954	
Sniffing anogenitally	2	35.63	2	33.66	487.5	-.392	.695	
Sniffing others	18	85.17	15	77.62	2791	-.974	.330	
Standing non-social exploration	4	51.11	6	52.32	1111.5	-.194	.846	
Walk over/under others	2	35.70	2	28.79	373.5	-1.414	.157	
Walking	26	80.72	29.5	81.53	2910.5	-.105	.917	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 14

Segment 2 frequency

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta-squared (η2)
Aggressive Stance	2	17.62	3	20.5	115.5	-.787	.431	
Approach	3	41.71	2	39.4	660	-.414	.679	
Boxing/wrestling	2	7.59	2	10.5	17.5	-1.218	.223	
Chewing wood	3	5.83	2.5	5	10	-.439	.661	
Digging/moving bedding/nesting material/wood	4	44.18	3	37.21	616.5	-1.237	.216	
Drinking	1	10.15	1.5	9.67	37	-.19	.849	
Eating	1	4.71	1	3	2	-.756	.45	
Flee	2	5.17	1	4.67	8	-.272	.785	
Food transport	3	5.64	11.5	6.63	11.5	-.479	.632	
Grooming others	1	6.13	1	5.67	11	-.239	.811	
Hiding	2	11.75	1	6.56	14	-2.389	.017	.34
In opening	5	26.65	6.5	30.78	283	-.903	.366	
Kicking	2	19.69	2.5	22	161	-.627	.531	
Mount	2	3	15	6	0	-1.508	.132	
non-social exploration	11.5	62.29	12.5	59.76	1530	-.366	.715	
Nose greeting	1	1.5	1	1.5	0.5	0	1	
Nose-off	3	27.65	2	28.93	286	-.271	.787	
Pursuing/chasing	3	12.33	2	10.8	39	-.458	.647	
Resting with others	1	4.75	2	8.5	2	-1.936	.053	
Resting/immobile	2	79.95	2	78.64	2761.5	-.175	.861	
Running	3	22.2	6	30.69	147	-1.894	.058	
Self grooming	2	45.75	3	46.55	866.5	-.136	.892	
Sniffing anogenitally	3	9.38	2	8.1	25.5	-.488	.625	
Sniffing others	6	45.3	5	45.93	872	-.109	.914	
Standing non-social exploration	4	11.31	2	9	35	-.847	.397	
Walk over/under others	1	10.63	2	12.2	34	-.55	.582	
Walking	10	46.4	18	60.1	686.5	-2.09	.037	.04

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 15
Segment 3 frequency

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Aggressive Stance	1	26.67	3	34.97	232.5	-1.799	.072	
Approach	4.5	59.89	4	60.19	1656.5	-.047	.963	
Boxing/wrestling	1	20.5	1	17.05	121.5	-1.057	.29	
Chewing wood	1	8.68	2.5	11.81	29.5	-1.305	.192	
Digging/moving bedding/nesting material/wood	4	54.27	4	57.73	1297.5	-.548	.584	
Drinking	2	19.12	2	19.97	170.5	-.244	.808	
Eating	1	6.22	1	5	7	-.703	.482	
Fighting with other	1	2	1	2	1	0	1	
Flee	2	16	1	11.5	60	-1.654	.098	
Food transport	2.5	10.79	8	15.82	51.5	-1.683	.092	
Grooming others	1	7.79	1	9.06	26.5	-.697	.486	
Hiding	2	27.12	1	23.76	251	-.797	.426	
Hiding with others	1	6.1	1	4.9	9.5	-.775	.439	
In opening	7	50.73	7	55.45	1152.5	-.775	.438	
Kicking	1	26.49	2	30.65	297	-1.029	.303	
Mount	1.5	3.75	1	2.5	1.5	-1.225	.221	
non-social exploration	21	67.1	27	80.83	1895.5	-1.899	.058	
Nose-off	3	46.63	3	47.65	981	-.178	.859	
Pursuing/chasing	2	26.1	2	23.96	230.5	-.489	.625	
Resting with others	1	5.21	1.5	7.38	8.5	-1.327	.185	
Resting/immobile	3	67.7	2	64.18	1853	-.512	.609	
Running	3	39.8	7	50.24	691.5	-1.902	.057	
Self grooming	3	57.87	3	65.57	1411.5	-1.168	.243	
Sniffing anogenitally	2	14.29	1	6.25	15	-2.121	.034	.18
Sniffing others	9	67.71	6	58.97	1643.5	-1.288	.198	
Standing non-social exploration	2	24.52	3	26.86	276	-.577	.564	
Walk over/under others	2	19.13	1	15.43	111.5	-1.189	.235	
Walking	14	63.99	19	68.16	1825.5	-.606	.544	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 16
Segment 4 frequency behavior

	Median sham-operated	Mean rank Sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Aggressive Stance	1	9.72	1	9.28	38.5	-.195	.846	
Approach	3	29.24	3	31.20	386.5	-.433	.665	
Boxing/wrestling	1.5	8.90	1	9.14	34	-.107	.915	
Chewing wood	2	4.67	2	4	5	-.344	.731	
Digging/moving bedding/nesting material/wood	4	40.71	3	32.25	504	-1.715	.086	
Drinking	3	9.31	2.5	9.65	38.5	-.136	.892	
Eating	1.5	3.5	2	4.67	4	-.764	.445	
Fighting with other	1	2	1	2	1	.000	1.000	
Flee	1	5.69	2	6.83	9.5	-.572	.567	
Food transport	2	5.5	2	5.5	8	.000	1.000	
Grooming others	1	5	1	5	9	.000	1.000	
Hiding	1	17.57	1	17.38	135	-.059	.953	
Hiding with others	1	4.5	1	4.5	7.5	.000	1.000	
In opening	5	24.68	6.5	26.55	285	-.451	.652	
Kicking	1	14.03	1.5	15.13	88.5	-.388	.698	
non-social exploration	11	50.94	10	54.89	1193.5	-.653	.514	
Nose-off	2	27.73	1	23.52	263	-1.047	.295	
Pursuing/chasing	2	9.5	2	9.5	32.5	.000	1.000	
Resting with others	1	8.80	1	8	27	-.381	.703	
Resting/immobile	2	70.21	2	73.67	2243.5	-.500	.617	
Running	2	21.16	3.5	25.30	204	-1.066	.286	
Self grooming	2	40.82	3	41.28	775	-.089	.929	
Sniffing anogenitally	1	7.82	1	6.33	13	-.608	.543	
Sniffing others	4	34.79	6	39.03	550	-.849	.396	
Standing non-social exploration	2	9.79	2	10.36	39.5	-.219	.826	
Walk over/under others	2	11.82	2	12.28	60.5	-.162	.871	
Walking	10	39.83	10	42.70	736	-.539	.590	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 17

Being done to Duration both days

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Approach	1.40	495.41	1.44	506.25	107661	-.56	.58	
Boxing/wrestling	2.44	55.59	2.46	53.69	1230.5	-.29	.77	
Fighting with other	1.84	6.67	1.62	6	12	-.28	.78	
Flee	1.58	57.42	1.28	48.90	1020	-1.27	.20	
Grooming others	3.92	13.16	5.5	16	60	-.85	.40	
Hiding with others	5.52	8.33	17.80	9.75	30	-.577	.564	
Kicking	1.16	145.02	0.98	133.73	8322.5	-1.12	.26	
Mount	1.04	30.19	1.24	33.44	414	-.70	.49	
Nose-off	3.24	221.83	3	211.09	20629	-.85	.39	
Pursuing/chasing	2.16	117.54	2.30	128.16	3887	-.92	.36	
Sniffing anogenitally	2.20	48.10	1.92	47.70	8210	-.061	.951	
Sniffing others	1.92	1008.94	2	1033.16	461481.5	-.889	.374	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 18

Being done to behavior duration day 0

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Approach	1.36	280.08	1.44	302.32	30966.5	-1.458	-.145	
Boxing/wrestling	2.28	35.17	1.96	30.81	383.5	-.813	-.416	
Flee	1.62	40.88	1.34	33.35	500.5	-1.380	-.167	
Grooming others	4.24	6.09	16.70	12	1	-1.974	-.048	.29
Hiding with others	5.52	3	1.88	1	0	-1.342	-.180	
Kicking	1.16	102.42	1	95.44	4170.5	-.814	-.416	
Mount	1	27.42	1.24	31.02	349.5	-.813	-.416	
Nose-off	2.92	91.44	2	77.74	2867	-1.732	-.083	
Pursuing/chasing	2.28	81.02	3.20	78.48	2029	-.280	-.779	
Sniffing anogenitally	2	30.26	1.78	29.50	380	-.160	-.873	
Sniffing others	1.90	590.32	2.16	621.99	152687.5	-1.496	-.135	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 19

Done to behavior duration day 1

	Median sham-operated	Mean rank sham-operated	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Approach	1.48	217.41	1.44	206.06	20304.5	-.928	-.353	
Boxing/wrestling	2.44	20.9	3.34	22.47	192.5	-.402	-.688	
Fighting with other	2.14	3	1.62	4	3	-.655	-.513	
Flee	1.42	17.19	1.24	16.29	86	-.22	-.826	
Grooming others	3.62	7.63	4.48	7.33	23	-.129	-.897	
Hiding with others	14.8	6.33	24.36	7.57	17	-.571	-.568	
Kicking	1.24	43.21	.96	38.69	703.5	-.832	-.405	
Nose-off	3.42	130.76	4.20	134.26	7695	-.356	-.722	
Pursuing/chasing	2.28	37.08	3.20	54.23	206.5	-2.326	-.02	.07
Sniffing anogenitally	2.76	18.03	4.92	23.67	34	-.887	-.375	
Sniffing others	1.96	419	1.88	413.70	80447	-.308	-.758	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 20

Duration of being done to segment 1

	Median sham-operated	Mean Rank sham-treatment	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Approach	1.24	203.45	1.40	227.93	15980.5	-1.872	.061	
Boxing/wrestling	2.28	27.45	1.80	21.29	177.5	-1.255	.21	
Fighting with other	2.14	3.5	1.58	0	370.5	-1.474	.14	
Flee	1.26	36.78	2.02	29.03	0	-1.225	.221	
Grooming others	3.99	5.6	16.70	11.00	1	-1.934	.053	
Hiding with others	5.52	3	1.88	1.00	0	-1.342	.18	
Kicking	1.16	75.15	.78	63.36	1798	-1.580	.114	
Mount	.94	19.87	1.08	22.97	1	-2.861	.004	.20
Nose-off	2	4.63	1.60	35.98	593.5	-.870	.384	
Pursuing/chasing	2.16	64.18	2.36	7.52	1255	-.778	.437	
Sniffing anogenitally	1.96	17.52	2.88	21.44	95	-.969	.333	
Sniffing others	1.88	429.73	1.76	441.10	80905.5	-.629	.529	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 21

Duration of being done to segment 2

	Median sham-treatment	Mean rank sham-treatment	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Approach	1.64	76.69	1.56	76.02	2355	-.085	.932	
Boxing/wrestling	2.32	8	3.76	9.33	25	-.542	.588	
Flee	2.08	4.75	1.9	4.25	7	-.289	.773	
Kicking	1.16	28.03	1.36	30.24	369	-.499	.618	
Mount	1.82	9.67	1.42	7.8	23	-.759	.448	
Nose-off	4.74	51.91	2.6	41.3	815.5	-1.809	.07	
Pursuing/chasing	2.76	17.23	1.44	8.58	30.5	-2.153	.031	.15
Sniffing anogenitally	2.34	13.54	1.56	10.32	47.5	-1.139	.255	
Sniffing others	2.28	162.03	2.92	178.97	11508.5	-1.529	.126	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 22

Duration segment being done to segment 3

	Median sham-treatment	Mean rank sham-treatment	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Approach	1.64	142.73	1.48	13.46	8157.5	-1.246	.213	
Boxing/wrestling	2.44	12.18	2.48	13.7	41.5	-.427	.670	
Fighting with other	.84	1.5	2.88	3	0	-1.225	.221	
Flee	1.48	13.42	1.2	11.67	49	-.509	.610	
Grooming others	3.62	5.25	3.68	4.8	9	-.245	.806	
Hiding with others	15.9	3.5	25.64	4.2	4	-.387	.699	
Kicking	1.22	29.15	1	26.14	318	-.676	.499	
Nose-off	3.36	87.7	4.48	9.02	3415	-.288	.774	
Pursuing/chasing	2.4	28.88	6.12	42.79	99.5	-1.980	.048	.06
Sniffing anogenitally	2.48	11.74	.96	6.5	5.5	-.788	.431	
Sniffing others	1.84	267.89	1.72	259.3	30224	-.611	.541	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Table 23

Duration being done to segment 4

	Median sham-treatment	Mean rank sham-treatment	Median Devocalized	Mean rank Devocalized	Mann-Whitney U	Z-score	*p*-value	eta squared (η2)
Approach	1.24	75.24	1.36	75.9	2661	-.090	.928	
Boxing/wrestling	3.76	9.57	3.36	9.45	38	-.045	.964	
Fighting with other	4.32	3	1.45	1.5	0	-1.225	.221	
Flee	1.36	4.14	1.96	7	1	-1.091	.275	
Grooming others	8.32	3	5.72	3	2	.000	1	
Hiding with others	14.8	3.5	13.72	3.5	4	.000	1	
Kicking	1.6	14.35	0.84	13.40	79	-.302	.763	
Nose-off	3.76	43.57	3.76	44.73	856.5	-.207	.836	
Pursuing/chasing	1.82	8.29	3.12	13.75	11	-1.808	.071	
Sniffing anogenitally	2.78	6.58	6.86	13	1	-2.008	.045	.29
Sniffing others	2.22	152.43	2	152.58	11413	-.014	.988	

Note: eta squared (η2) denotes significance; no variable has .001 significance

Discussion

This study investigated the role of USVs in formation of groups in a semi-natural environment consisting of both an open-area and a burrow-area. The results from our study does seems to indicate that there is an effect on the behavior in the groups, with differences between the devocalized and the sham-rats, with some overall effects, and several other effects that were dependent on when they were performed during the two days used in this study. There were though three overall differences between the sham-operated and the devocalized rats, food transport, running, and fleeing.

Where the largest differences in the data for both days are the transportation of food where the rats that were devocalized transported larger amounts of food to the burrow than the sham-operated rats. The devocalized rats also ran for longer and more often than the sham-operated rats, in part because they were running back to the food pile in the open area. While the sham-operated rats fled more than the devocalized rats. This could then indicate that the sham-operated rats were more likely to expend more explosive energy on movement, where the devocalized rats ran more for longer periods of time instead. It can also indicate different coping styles adopted, with the devocalized rats eventually adopting an active coping style that includes movement, while the rats sham-rats adopt a passive style where they avoid other rats more Walker et al. (2009) Another possible explanation is that being that the devocalized rats were less anxious than the sham-operated animals, as greater mobility has been indicated as a trait of less anxiety, and fleeing being a an acute form of anxiety that does not increase mobility for long (Walker et al., 2009). Another option is that the sham-operated rats might recognize the burrow and huts placed in the open area as safe places to escape into more readily than the devocalized rats (Blanchard & Blanchard, 1989; Kitaoka, 1994). At the same time, the sham-operated rats fled more, they did not flee more from the devocalized rats, indicating that devocalized rats do not cause fear for other rats. There was however a clear disruption of in the group inside the semi-natural environment.

The disruption of the group as a whole becomes more clear when comparing food with results from Chu and Ågmo (2014) that used the same semi-natural environment without devocalized rats all the food had been transported into the burrow area by the end of 24hours into the experiment. something did not happen in any group in this study, while the amount left was not very large at the end of the study, it was still there, 48hours later. Suggesting, that it is not necessarily that devocalized rats gather more food, but that it somehow is disruptive for the rats that are able to vocalize, and that the sham-operated rats therefore did not do as much of the gathering of the food. It can suggest that they do not feel as much a part of the group or that the burrow is not a safe home base. It might also be a reverse effect where they are confident that they do not have to gather food as much, because the other rats are doing it. A third option is that since the devocalized rats seek out something that rewards them (Brenes & Schwarting, 2015).

Discussion for day 0

The devocalized rats spent more time overall in the first day being in the openings between the burrow and the open area. Could indicate that they are looking for predators or other rats that may be hostile before going to gather food. In particular that they are looking out the openings to look for them since they cannot use USVs to move in conjunction with other rats (Weiss et al., 2017). That spending time in the opening primarily happens on the first day can be an indication of an increased underlying insecurity from that lack of ability to communicate with the other rats and therefore know where other rats are, and use them to stay safer. As they do seem to stay in openings to investigate the environment, or look at the rats being in the open-area (Blanchard & Blanchard, 1989).

The first hours (Segment 1). There was a difference in duration of non-social exploration in the first day where the sham-operated rats explored the semi-natural environment more. A lack of interest that could be an indication of depression as Wistar-Kyoto rats that have several biological, and behavioral indication of being depressed, Also display a lower activity level as a response to being introduced novel environments (Rao & Sadananda, 2015). Another behavioral difference only seen in the first hours is that devocalized rats are mounted more than sham-operated rats. A difference that might be because the devocalized rats are less able to indicate that they do not want to be mounted. Its lack of significance later though might be because it happens fewer times as the rats start to spend more of their time in the burrow, where mounting another rat is rarer.

Since the rats that are devocalized are groomed more in the first videos, it might be that the other rats are trying to comfort them in the beginning. With lower amounts of USVs being found in a rat strain suspected to mimic depression (Rao & Sadananda, 2015) Another indication that devocalization causes different behavior in a group context is that they ended up chewing wood sticks more than the sham-operated rats did, which could be a self-reassuring behavior like some grooming is(Kalueff & Tuohimaa, 2005).

Daylight (Segment 2). The sham-operated rats went more time into the huts placed in the open area, possibly as a response to being chased more than the devocalized rats in the first night. Another indication that might be linked with depression is that the devocalized rats had more instances, but not of duration of walking, indicating a more disrupted sleep pattern during the first night (Baiden, Stewart, & Fallon, 2017).

Discussion for day 1

Devocalized rats were pursued/chased for longer durations. This does then in combination with the overall running indicate that the devocalized rats ran from the rats that were after them, but did not escalate their effort of going away to more than a run. Rather strangely the sham-operated rats ran more times, meaning their runs would be many more short runs. Perhaps indicating that they were a place and heard the devocalized rat approaching and did a short run to protect their tunnel area as it would be adaptive to protect oneself by using the tunnel (Blanchard & Blanchard, 1989; Kitaoka, 1994)

The sham-operated rats sniffed anogenitally more times in the last day, but not for long durations, indicating that they did not manage to sniff the other rat for a very long time. Which could mean that they moved away from the anogenitally sniffing rat.

Second darkness (Segment 3). The devocalized rats were chased more, which is the opposite pattern to first night. When combined with rats being more active during the dark periods it is probably an effect that is a stronger indicator of their behavior as a response to devocalized rats over time.

Second Daylight (Segment 4). That the devocalized rats are sniffed more anogenitally the last night could indicate that they are not truly recognized as a part of the group, and that they need to be investigated as a potential new female that may be in estrous. It might also be that it signals that the rat being sniffed anogenitally is not able to vocalize that they do not want to be sniffed anogenitally, something the sham-operated rat would be able to.

The devocalized rats also started spending more time in the huts in the open-area in the last night. Indicating that they did not want to spend as much time in the burrow, but still wanted to be in a spot where they could feel safe.

General discussion

Since rats live in reciprocal relationships (Schweinfurth et al., 2017; Schweinfurth & Taborsky, 2018; Stieger et al., 2017), it might be that they are trying to trade gathering food for socialization. However, against this possibility is that direct gifts of food were though uncommon in the samples of video that was used. And only in one instant in the open area, did a devocalized rat clearly seem to gift a piece food to another. With the apparent effect that it appeared to increase their amount of positive engagements afterwards between the recipient and the giver rat. This was so rare though that it is not a likely cause for this discrepancy either. It is then more likely that it is because the devocalization does not just affect the rat that is devocalized, but how they behave as a group unit. While clearly also some rats are more affected than others from being devocalized. Where some individual rats end up changing their own behavior as a response to being devocalized. A similar effect was found in Snoeren and Ågmo (2013), where the female rat that was devocalized changed her behavior more than the males changed their responses or preference towards a specific female. As a clear example of individual change seen in this study was seen in one female rat that spent almost all of her time hiding in the open-area huts (data not shown). Where a similar behavior that would indicate depression in humans (Baiden et al., 2017; Kurtz, Kurtz, Given, & Given, 1995). This might though be antromorpizing so there might be another reason for these differences, like increased mental capacity due to loss of sensory input and output (González-Garrido et al., 2017), causing an increased ability focus to the bedding spot, making it uncomfortable.

That the ability to vocalize 50-kHz USVs got little support in our study. The indications for us if anything is that devocalized rats are approached more (see table 20). This goes against the idea that 50-kHz USVs signal for “a desire for positive engagement” (Rainer Schwarting et al., 2007). This does not negate a potential for the 50-kHz USVs being signs of wanting positive engagements with other rats, as the devocalized rats or the sham-operated rats have no indication of a pattern in the rest of the observations. Showing that they are equally much approached. Potentially, it is because they are not giving of the 50-kHz calls that investigated/approached.in the first set of observations.

That the effect of non-social exploration seen in the first day, in the first segment suggest that the devocalized rats investigate their environment less than the sham-operated rats do in the beginning but then relatively increases for them. Although, this might also be because it is more important to learn ones surrounding early on, and the devocalized rats are again displaying symptoms of depression. As spatial behavior increases activity in the hippocampal area, depressed people have less activity there. In concordance with this the devocalized rats chew more on the wood sticks which if one is to extrapolate from a human perspective again, a sign of frustration manifesting itself early on. It might also be that some of the difference between the fleeing and running between the sham-operated, and devocalized rats comes from a lesser energy spent when moving away from the rat chasing them.

That there was no difference between the rats in regard to boxing/wrestling, might be due to no distinction between that and rough-and-tumble play. However, there was at the same time very few actual fights in the observations so even though the pattern outside of a context such as the one used in this study is that devocalized rats have more ferocious fights (Burke et al., 2017), it was not the case for our data, where barely any ferocious fights happened throughout the observations. Meaning that the situation of being forced into a cage contributes to the fight escalation. It can though be because their analysis actually used slow-motion videos to detect biting differences. A more careful analysis of these fights could though lead to a pattern not detected in our study. It might also represent a pattern in fighting more similar to juvenile rats, where the fights do not escalate in the absence of USVs (Kisko, Euston, & Pellis, 2015).

Limitations and future directions

Since one of the main objective of this study was to find indications if USVs through devocalization, would affect behavior in a rat groups, there are several avenues to continue investigating. There are however limitations to how generalizable these results are because for most behaviors no difference was found at all, and while some behaviors are relatively close to being significant. The study did not measure the USVs of the rats in the semi-natural environment during the study to see what behaviors were correlated with the release of USVs, or what happened with amount of USVs directed toward she devocalized rats, did they increase or decrease. Was the same pattern found for an increase in USVs that was found in Brenes et al. (2016) where there was an increase in 50-kHz in response to being housed with more rats, a potential new study could investigate if devocalized rats interrupt this effect, this would continue the elucidation of what effects the rates of USVs.

However, the most obvious limitation of this study is that there was no definition of what would constitute a cohesive group that functions well, versus a group that has no group cohesiveness. However, this might then be something a future study does, not only to cover this but as a continuation as the data does suggest that the lack of ability to vocalize disrupts the behavior of the rats in the groups. In addition, several variables were not measured in discrete enough fashion so that some effects that may have affected the results and shown a clearer picture was not done. One of them dominance was however not measured at all, which can have large effects on rat's social lives, more so for males than females. There also was no measurable difference between meaningful digging/moving material, and meaningless, ie seemingly moving miniscule amounts of flooring material, which could be a self-comforting behavior. There also was no distinction made between the different types of self-grooming, which can have several different functions and meaning (Kalueff & Tuohimaa, 2005). Some of this does come from the resolution of the videos being too poor to make some accurate distinctions between friendly and non-friendly encounters, and some distinctions between behaviors.. It would also be difficult to distinguish if they noticeably move their whiskers differently, with the resolution provided by the videos. With improved cameras though it would be interesting to see if there was a noticeable difference between whisker trimming and devocalizing in a group setting. Since the results were relatively similar between Burke et al. (2017) and (Wolfe et al., 2011), which does obscure the potential cause of USVs a little, since it might be that the lack of the ability to control the larynx or the surrounding area, changes how the rat moves its whiskers.

We also did not run the prosocial behaviors together as a variable, non-social behaviors (these could've also been made into anxiety/stress and non- stressful), and socially negative behaviors for differences. There also was no difference is strength given to each of the different variables as some non-friendly boxing is not as detrimental as a ferocious fight, meaning that a fight should've been given a greater value due to its increased importance. So, a study going into more detail in its measurements could find a more detailed pattern of the rats behavior, and if there was differences between other variables, like grooming, and larger classifications of behavior.

Another future direction that could be done is that since there was no group with only devocalized or sham-operated rats, there was comparison between those two rather extreme cases this could then continue to work on the exact same research question and almost the same method. This could then cause a large difference or none at all because the rats are in the same situation. Which could then strengthen the evidence for rat empathy if that ended up being the pattern.

That the rats in this study only has one burrow means that the rats cannot achieve true avoidance of another rat or rats due to the small size of the burrow environment (Chu & Ågmo, 2014) and that there is no possibility to enter or create another group in another burrow that the other rat(s) do not enter without being intruder rats. Meaning they have to socialize with other the other rats in the environment whether they want to or not. a study where the burrow was split in half, or had two different burrows would allow the rats to avoid each other. While living close to each other like they do in the wild (Davis, 1953).

Another solution to this would be to attach a new burrow-area to the semi-natural environment. Perhaps also a slightly larger open-area to allow for more rats to interact with each other while keeping the potentially 2groups large enough. The larger open are is though of less importance since they tend to live in small areas in the wild (Davis, 1953). This would then test if devocalized rats would be more ostracized and primarily stay in another burrow.

It could then be interesting to see if rat's sub-species that prefer to stay in a nest would also be affected in the same way if not given the choice, to stay in a nest, and if there was created a nest that the rats could go to in addition or instead, if this would be affected by the USVs. In part because the nest would have to have different characteristics that would alter sound patterns. The experiment could also be done with another species to investigate if they would respond differently to being devocalized in a semi-natural setting. It would could also be done with the Sprague-Dawley rats, that has a different reaction pattern (Walker et al., 2009), to see if the pattern would be different for this sub-species.

That the rats receive all the food at once, which might make them stay more inside the burrow area instead of going outside to look for food pellets. Might be a contributing factor to the pattern of devocalized rats doing more food transport for the group. Identifying if the rats would still follow this pattern if the food was delivered periodically, could identify if the rats use USVs indicate that the food has arrived. To deliver the food in a similar semi-natural environment. The potential solution would be to install a pellet releaser that drops the food at random times.

The rats also cannot change any part of the burrow systems if so desired, with some non-measured effects like rats building up a pile of bedding material to keep other rats away, being very easy to remove for the other rats. A potential study could then fill up the burrow-area with more bedding material to the roof in some areas, and see if this alters any patterns. It would then be interesting if the rats would end up dividing themselves in the burrow. It would be even more interesting if one could monitor the USVs in the burrow both because there is a relatively small litterature on USVs in burrow, and with more bedding the USVs might bounce differently creating different sonographic patterns, and if this would change any aspect of the USVs being emitted.

A possible elongation of this study would be to see if the rats that are devocalized over time start displaying more signs of isolation/autism (Brenes et al., 2016; Brudzynski, 2005; Casarrubea et al., 2017) if they would continue to be chased in a timeframe that continues after this experiment. Which could then indicate that devocalization over time truly is detrimental for a social animal like rats are, rather than just important in the beginning of a group being formed.

Another direction would be to see if rats do seem to use USVs when moving together in patterns, as they do release USVs when moving, seeing if they do use USVs to coordinate these movements between each other would elucidate some of the findings that they use USVs. If they however do not use USVs at all to coordinate between themselves, how do they do it?

A possible continuation of this study would be to see if rats that cannot smell, or vocalize are still are able to learn to recognize other rats, do they behave as if unfamiliar when meeting or would there be a cumulative negative effect as many studies show that rats use olfaction as the strongest social memory inducer, but the results from (Stieger et al., 2017)suggests that they can to a degree remember other rats for longer, with other senses. This could then help elucidate how they remember who is in their group and who is not, and if memory of other rats is intact without sniffing. But that the sniffing more likely creates a cohesive feeling,

Interestingly, if the rats cannot meet another rat when hearing playbacks of 50-kHz they lose interest after about a minute, showing that they understand rather quickly that the playback is not going to lead to a social situation for them. Perhaps indicating that think it is for someone else (Willadsen et al., 2014). Which shows that it might be possible to habituate rats to different kinds of USVs. Something that could be used to elucidate what the USVs and how rats would change their behavioral patterns as a response. If the USVs would have to be played rather constant, it should be something that should be possible in the semi-natural environment used in this study, would this then change behavior in any noticeable way in the group setting or not.

Making rats deaf instead of mute could also help with the understanding of USVs role in rat groups. Permanently or by using earplugs this could then be used as a method for elucidating if hearing the USVs would cause the same interaction, or if they are more self-reinforcing. With an earplug it would also be possible to see if removing it would alter how the rats interact with each other. Perhaps it would also be possible to design some earplugs that filter out specific vocalizations allowing for studies that investigate specific parts of the USV spectrum.

Conclusion

This study of the role of USVs in group formation in a semi-natural environment suggests that USVs or the lack of this ability in some of the group members changes the dynamics in rat groups. While it also suggests that rats USVs affect groups in the wild too. More so for some rats than for others, this study is then a clear indication that some patterns of USVs being disruptive for socialization found in duos are found in groups too, however, there are also other patterns that are not found like the transportation of food into the burrow being disrupted. There is though still much that need to be elucidated about rats and their use of USVs in group settings, there is however stronger indications of Blumberg (1992) (f) and (g) being correct in regards of USVs being socially communicative in rats. With our semi-natural environment design increasing the probability that this pattern is found in the wild.

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