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RATS LACK A PREFERENCE FOR SOCIAL FEEDING: A STUDY ON THE BEHAVIOR OF RATS WITH *AD LIBITUM* ACCESS TO FOOD

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Resumo

Os ratos são animais altamente sociais. Os primeiros estudos realizados com grupos de ratos a viverem juntos revelaram um complexo sistema social. Em ambientes naturalistas (ou semi-naturalistas) os ratos cavam túneis no substrato, que normalmente abrigam várias fêmeas e poucos machos. Quando vivem em colônias eles compartilham os mesmos locais de nidificação e alimentação, sendo capazes de adaptarem o seu comportamento para diferentes contextos sociais, observando poucos casos de comportamento agressivo. É comummente aceite que os ratos gostam de comer juntos, desde que foram observadas algumas circunstâncias onde isso acontecia. No entanto, esses relatórios não testam diretamente se os ratos mostram preferência para alimentação social. Além disso, o comportamento alimentar dos ratos permanece muito pouco estudado devido ao facto deste comportamento ser muito difícil de quantificar com precisão e de forma objectiva. Portanto, nós projetamos um experimento para estudar o comportamento alimentar de pares de ratos com *ad libitum* acesso à comida. Confirmando estudos anteriores, em nossas condições ratos mostraram um padrão diário de alimentação com maior atividade no período noturno do que no diurno, e que a sua alimentação consiste em comer poucas quantidades de cada vez, várias vezes ao dia. Os ratos mostraram uma preferência por um local de alimentação onde a alimentação social era possível, mas nossos dados sugerem que esta preferência não era devido a informação social. Os ratos parecem não ter preferências para alimentação social, visto que o tempo passado a alimentar sozinho corresponde à aproximadamente 90% de toda a atividade alimentar observada.

Palavras-chave: Ratos; Comportamento alimentar; Alimentação social.

Abstract

Rats are highly social animals. Early studies done with groups of rats living together revealed a complex social system. In naturalistic (or semi-naturalistic) environments rats cave burrows in the dirt that usually house many females and few males. When living in colonies they share the same nesting and feeding sites, being capable of adapting their behavior for different social contexts, with few instances of aggressive behavior. It is commonly accepted that rats enjoy feeding together since they were observed eating together in some circumstances. However, these reports do not test directly whether rats show preference for social feeding. In addition, the feeding behavior of rats remains vastly understudied, as this behavior is very difficult to quantify accurately and objectively. Therefore, we designed an experiment to study feeding behavior of pairs of rats with *ad libitum* access to food. Confirming previous studies, in our conditions rats showed a daily pattern of feeding with higher activity in the dark than in the light period, and their feeding is organized in grazes rather than meals. Rats did show a preference for a feeding site where social feeding was possible., but our data suggests that this preference was not due social information. However, rats appear to lack preferences for social feeding as time spent feeding alone corresponds to roughly 90% of all feeding activity observed.

Keywords: Rats; Feeding behavior; Social feeding.

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Introduction

The social environment and its influences on behavior

Rowland (2012) stated that "(...) the animal and its behavior are a complex interaction among many environmental variables", the social environment offers many variables of use to others which are known as social information, i.e. information provided by the activities of other individuals (Lindeyer, Meaney, & Reader, 2013).

Social behavior is a prominent ethological feature of animals living in groups, which involves any activity that directly influences the behavior of other individuals (Barnett & Spencer, 1951; Young, Liu, & Wang, 2008). Animals are capable of modulating their behavior as a function of the current social environment and previous social interactions. This behavioral trait, known as social plasticity, like any other trait, impacts the animal's Darwinian fitness. Darwin's (1859) theory of evolution on the struggle for life and the survival of the fittest by means of Natural Selection, implies that the most adaptable individuals should thrive, therefore this plasticity on behavior that optimizes animals' social interactions seems to be a excellent survival tactic of animals living in heterogeneous environments (Oliveira, 2012).

Animals in their daily routines provide information of use to others, that could be actively (e.g. vocalizations) or passively (e.g. pheromones) (Williams, 1966). This social information can influence the behavior of animals either subtly or prominently. The usage of such information can lead to synchrony in the performance of a task (i.e. contagious behavior), disinhibition (i.e. social facilitation) and may even offer the animals a learning opportunity (i.e. social learning) (Nicol, 1995). The nuances between the first two parameters are hard to define and are often combined and referred to as social facilitation.

Social interactions are essential for the well-being of social animals, individuals' impairments on social interactions are used as diagnostic tools for various psychological disorders - that affect humans - as autism (Pletnikov, 2002), schizophrenia (Jones, Watson, & Fone, 2011) and bipolar disorder (Malatynska & Knapp, 2005). These interactions are so important that social isolation is considered a risk factor for morbidity and mortality due its pronounced effects on the animals' physiology, neurochemistry and behavior (Cruces, Venero, Pereda-Péeez, & De la Fuente, 2014; Lopak & Eikelboom, 2004; van Leeuwen, Bonne, Avraham, & Berry, 1997).

Rats are social animals

Early studies done with groups of rats living together revealed an amazing social system between them (Barnett & Spencer, 1951; Calhoun, 1949; Chitty & Shorten, 1946; Pisano & Storer, 1948; Thompson, 1948). In naturalistic (or semi-naturalistic) environments rats cave burrows in the dirt and establish nests in them (Calhoun, 1963; Chu & Ågmo, 2015). These burrows usually house many females and few males (Fig. 1). The number of males tends to vary positively with variations in the population densities (Moore, 1999).

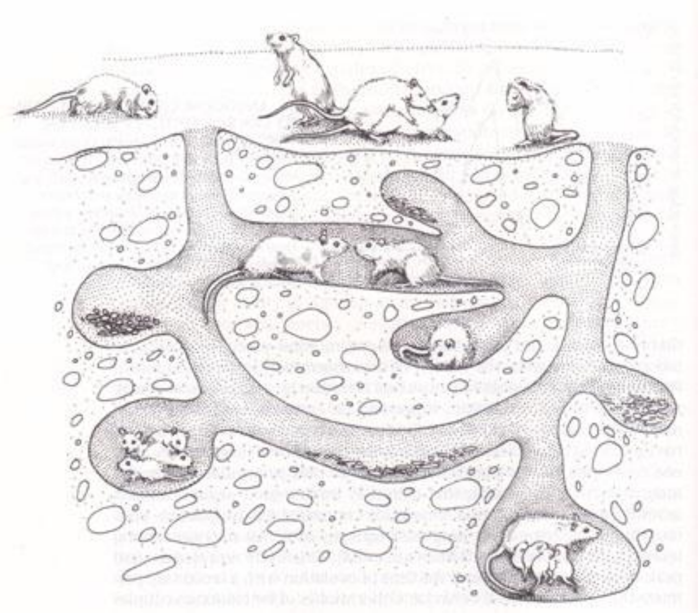


Figure 1 - Illustration of a typical burrow system of wild Norway rats. These burrow systems house a few females, a small number of males and their many subadult offspring (McClintock, 1987).

Social information, specially olfactory clues, are an important factor for food orientation in rats. Norway rats when traveling from their burrows to forage for food and water create trails that influences the movements of conspecifics that come after them (Calhoun, 1963; Telle, 1966). The breath of rats from recent eaten foods is used as cues by naive animals when selecting what to eat, this preferences were shown to last for weeks (Galef, 1989), and can even reverse learned aversions to food (Galef, 1986; Heyes & Durlach, 1990; Yoerg, 1991), but when reared in social isolation, rats do not exhibit signs of this kind of social learning (Galef, 1981).

Juvenile rats tend to approach feeding sites where an adult conspecific is present rather than feeding sites with others juvenile rats (Gerrish & Alberts, 1995) suggesting discrimination regarding the reliability of social information. Passive transmission of feeding preferences were shown between generations trough nursing, as the flavor of foods that mothers ingests while nurturing are detected by the pups in the milk and when weaned, they show preferences for foods with those flavors (Galef & Sherry, 1973). This effects are so robust that permit cultural traditions to be formed that can last for generations (Galef & Allen, 1995; Laland & Plotkin, 1993; Laland, 1999).

Rats have been shown to be sensitive to the conspecifics display of food-seeking behavior. Márquez, Rennie, Costa, & Moita (2015) when studying prosocial behavior in rats that had to decide between two-choices, they demonstrated that rats significantly prefer the option where both rats receive food rewards and that this preference depends upon displays of food-seeking behavior by the conspecific. Dopamine (DA) has been implicated in the mediation of a variety of natural rewards (Wise & Rompre, 1989; Bressan & Cripa, 2005). Kashtelyan, Lichtenberg, Chen, Cheer, & Roesch, (2014) recorded DA release in the ventral striatum, and saw that when rats are hungry, the observation of a food reward delivery to a conspecific initially increases the DA release but soon it decreases to levels lower than basal. Which suggests that rats are also sensitive to the consumption of food by a conspecific.

The usage of social information is not restricted to food foraging. A example of that is the social transmission of fear (Atasak et al., 2011; Bruchey, Jones, & Monfils, 2010; Kim, Kim, Covey, & Kim, 2010): a normal behavior of rats in fear situations is to freeze, the silence resulting from the cessation of movement by one individual (due freezing) is perceived by others as indication of danger, leading them to freeze, even if themselves haven't felt threatened by the situation (Pereira, Cruz, Lima, & Moita, 2012).

These reports show that rats are highly social animals. When living in colonies they share the same nesting and feeding sites, being capable of adapting their behavior for different social contexts, with few or, even in some cases no, aggressive behavior being observed between individuals living together.

The feeding behavior of rats

To this day the feeding behavior of rats remains vastly understudied, the main reason is that this behavior is very difficult to quantify accurately and objectively (B. L. Nielsen, 1999). Rats' feeding patterns seems to be highly affected by exogenous (e.g. resources availability, social environment) and endogenous stimuli (e.g. hunger, previous experience) (Clifton, Popplewell, & Burton, 1984; Collier, Hirsch, & Hamlin, 1972).

Despite scarce, there is information on the rats' feeding behavior dating back to the mid twenty century (see below). In these early studies, colonies of rats were kept in semi-naturalistic environments, the feeding behavior, unfortunately, constitutes only a small portion of these reports.

In all colonies the majority of activity registered was during the night period, showing that rats are nocturnal animals. Feeding orientated behavior arose shortly before sunset, being almost always the first orientated behavior after a resting period (Chitty & Shorten, 1946; Thompson, 1948). Individual differences in the feeding behavior were observed, some rats eat at the feeding site whilst others rely on scavenging fragments left by other rats on the floor around the feeding point (Barnett & Spencer, 1951). Small sized foods were usually eaten at the feeding point whilst large ones were carried to the nest where it could be hoarded.

With *ad libitum* access to food, rats' feeding behavior occurs in bouts, around ten meals per day (Le Magnen & Devos, 1980), but when food restrictions were imposed, animals modulated the meal size and frequency of feeding according the cost of feeding (Collier, Johnson, & Mitchell, 1999; Houston & McNamara, 1989; Kaufman & Collier, 1983). Even the colonies' social organization was modulated by food availability, which when restricted gave rise to individual behavioral differences when food is difficult to obtain: carrier rats that bring food to the nest, and non-carrier rats that steal food from other members of the group (Grasmuck & Desor, 2002; Thuller, Desor, Mos, & Krafft, 1992).

The problem with those reports is that the researchers organize feeding in meals by restring the availability of food (Collier et al., 1999; Houston & McNamara, 1989; Kaufman & Collier, 1983) or the way the organize the data (Le Magnen & Devos, 1980), stating that two to ten meals are normal behavior for rats, but rats are grazers rather than true meal eaters, therefore forcing the analysis of data into meals may not be a true reflection of the organizational units of the feeding behavior (Rowland, 2012). Goulding et al. (2008) home-cage observations of mice concluded that they are almost continuously active for several hours during the night and feeding occurs in short bouts throughout that prolonged activity period.

Due to increasing number of reports on the influences of the social environment in the behavior, including on feeding (Barnett & Spencer, 1951; Calhoun, 1963; Calhoun, 1963; Calhoun, 1966; Galef, 1981, 1986, 1989; Galef & Sherry, 1973; Gerrish & Alberts, 1995; Heyes & Durlach, 1990; Moore, 1999; Telle, 1966; Yoerg, 1991, and others), it is commonly accepted that rats enjoy feeding together since they were observed eating together in some circumstances. However these reports do not test directly whether rats show preference for social feeding, therefore further investigation on this matter is still to be done.

The present project aimed to address some of those questions above discussed: 1) how is the pattern of feeding of rats with *ad libitum* access to food without forcing meals? 2) Do rats show preferences for specific feeding sites? 3) Do rats have a preference for social feeding? To do so, we developed a new behavioral paradigm with three different feeder types, that allows us to constantly monitor the feeding behavior of a pair of rats for five consecutive days, at the same time contemplates three different feeding scenarios: feeding alone, simultaneous at different feeding sites or feeding together at the same feeding site.

Methods

Animals

Eight males and eight females Long Evans, weighting 250g to 275g were obtained from Charles Rivers Laboratories in Italy. Upon arrival the animals were kept pair-housed with *ad libitum* access to water and food, automatic controlled reversed 12h dark-light cycle (10 a.m. to 10 p.m. - dark (with red light)), temperature (20 - 22ºC) and humidity (40 - 70%), for two weeks before experiments, giving them time to habituate to our vivarium and the reserved light cycle.

Three days before experiments the animal's regular food chow was replaced for Bio-Serv Dustless Precision Pellets®, grain-based 1g with the same composition as the pellets used in the experiments. Since rats are neophobic (Barnett, 1958; Calhoun, 1949), this was important to habituate the rats to the food type before the experimental procedure.

Experimental setup

Experimental boxes

Four boxes measuring 400mm x 400mm x 600mm were made of light colored, opaque, 5mm thick acrylic containing four feeding and water sites each (Fig. 2 & 3).

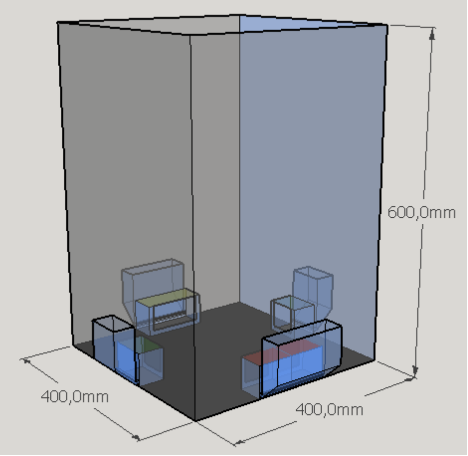


Figure 2 - 3D model of the experimental boxes with the four feeding sites.

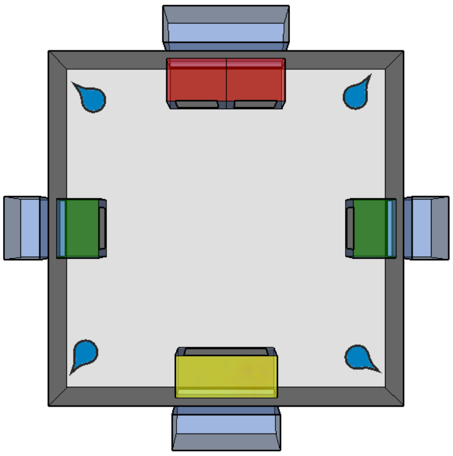


Figure 3 - Top view of the experimental boxes with the four feeding (colored boxes) and water (blue drops) sites.

Feeding sites

Three types of feeders (material: transparent, anti-reflex, 3mm acrylic) were made to provide three possible feeding scenarios:

1. **Non-social feeder:** the small entrance only allows for one rat to feed at a time, each box has two of this type of feeder in opposite sites so both rats can feed at the same time in different non-social feeders (dimensions: 70mm x 50mm x 60mm) (Fig. 4);
2. **Social no-competition feeder:** two small separated entrances with a middle partition, that isolates each side, allows both rats to feed at the same time without possible competition for the same pellet of food (dimensions: 140mm (70mm + 70mm) x 50mm x 60mm) (Fig. 5);
3. **Social competition feeder:** a large entrance that allows both rats to feed at the same time with possible competition for the same pellet of food (dimensions: 140mm x 50mm x 60mm) (Fig. 6).

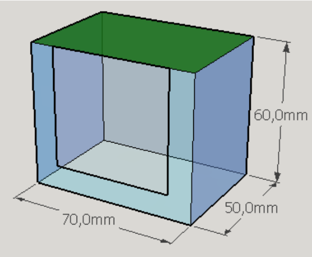


Figure 4 - 3D model of the non-social feeder.

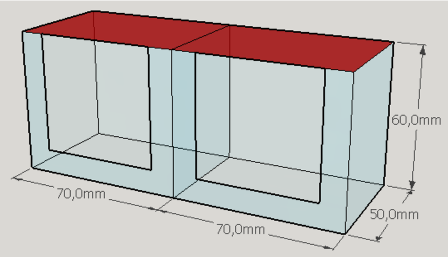


Figure 5 - 3D model of the social no-competition feeder.

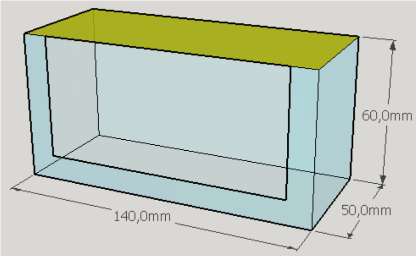


Figure 6 - 3D model of the social competition feeder.

Video recordings

Each cage was equipped with a camera (camera: Flea3 1.3 MP Mono USB3 Vision (Sony IMX035); lens: Fujinon YV2.8×2.8SA-2, 2.8mm-8mm, 1/3", CS mount Lens) recording the entire experiment at 3fps (frames per second) controlled by the Bonsai (an open source behavior analysis and control software developed by Gonçalo Lopes (Bonsai v. 2.1.4, Gonçalo Lopes, Champalimaud Center for the Unknwon(Lopes, et al. 2015)).

Experimental procedure

In each session four pairs of animals were placed at 12 p.m. in the four experimental boxes for five consecutive days with *ad libitum* access to water and excess food (Bio-Serv Dustless Precision Pellets®, grain-based 500mg). Each non-social feeder was filled daily with forty pellets, the social no-competition feeder with eighty pellets (forty in each side) and the social competition feeder with another eighty pellets. The filling was done through the outside access with minimum disturbance to the animals.

The boxes were intercalated with pairs of females and males for standardization. At day two of the first session, animals from one cage escaped and entered another cage, therefore both cages were excluded from the analysis. In the second session problems with the camera were observed in one cage, that cage was also excluded from the analysis. The remaining five cages were used in the analysis (Table 1).

Table 1 - The five cages from the two sessions used in the analysis with rats' gender.

1st Session		**2nd Session**		
Cage 1	Cage 2	Cage 3	Cage 4	Cage 5
♀♀	♂♂	♀♀	♂♂	♀♀

Video analysis

Automatic analysis

Our goal was to detect when one rat had its head inside a feeder giving us indication of feeding behavior, a similar method was used before to analyze the feeding behavior. Reid, Bacha, & Morán (1993) used a infrared beam to monitor "head-in" and "head-out" in the feeding sites. With that in mind we used Bonsai to define ten regions of interest (ROI): five regions corresponding to each feeder (social no-competition feeder had two defined ROIs, one for each side) (Fig. 7 - outlined in green) and five regions right above the previous ones (Fig. 7 - outlined in red), both green and red ROIs are important for a accurate analysis of the "head-in" the feeders behavior (see below, parameter 2). The Bonsai outputs the number of black pixels (Long Evans rats typically have their head covered with black fur) inside a ROI in each frame.

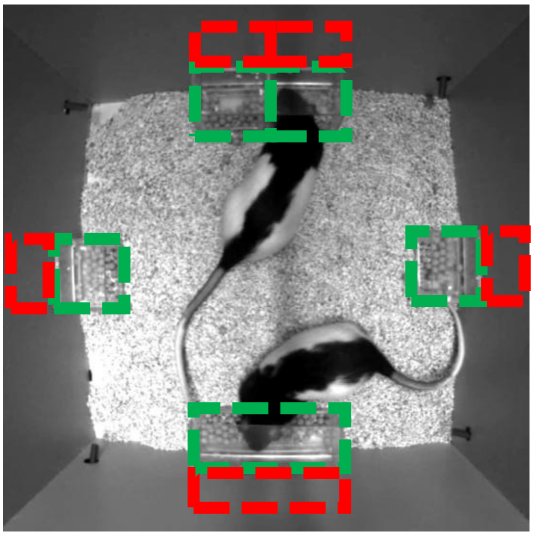


Figure 7 - Representation of the ROIs used for video analysis in the Bonsai.

The Bonsai's output is processed in Python, where information from ROIs is converted according the following parameters:

1. If the sum of black pixels inside each green ROI is within 300 000 to 1 200 000, for at least three seconds to a maximum of sixty seconds, each frame of that particular ROI is scored as one feeding event.
    a. **Size threshold:** the minimum of 300 000 pixels excludes any noise present in the analysis, the maximum of 1 200 000 pixels excludes presences bigger than the head (e.g. if the animals jumps to the top of the feeder that should be excluded as it would sum up to higher than 1 200 000 pixels);
    b. **Time threshold:** the minimum of three seconds was imposed after manual verification that animals walking fast on top of the feeder would sometimes activate the ROI even taking into account the size threshold. The maximum of sixty seconds was also imposed after observation of animals resting inside the feeder activating the ROI (the maximum time spent feeding per bout observed in manual analysis was forty-one seconds).
2. If black pixels were detected simultaneously in both green and red ROIs that bout of activity would be excluded as indication that the activity registered was outside the feeder.

The resulting data is divided in five columns corresponding to each green ROI, coded for each frame (rows) as '0's for no activity and '1's for activity. With this data base we can extract time spent feeding (i.e. the sum of frames with activity), number of visits (i.e. bouts of frames with activity) and average duration of visits per hour (i.e. the time spent feeding divided by the number of visits in one hour bins).

Manual Analysis

Manual analysis was done for three different days (cage 1 day 1, cage 2 day 2 and cage 1 day 3), for the first fifteen minutes of every hour. This analysis was used to validate the automatic analysis (above). With the criteria set above for automated scoring roughly 96% of the frames automatically scored were correspondent to the frames from manual scoring (correlation between automatic and manual scoring: cage 1 day 1 - R^2=0,9315; cage 2 day 2 - R^2=0,9865; cage 1 day 3 - R^2=0,9642).

Social feeding behavior

We defined and analyzed three different social feeding scenarios:

1. **Feeding alone:** only one green ROI is active at a time, which means that only one rat is at any of the feeding sites (Fig. 8);
2. **Simultaneous feeding:** two different green ROIs are active at the same time, which means that both rats are in different feeding sites (Fig. 9);
3. **Feeding together:** both rats are at the same feeding site at the same time, this can only occur at the social feeders:
    a. Social no-competition feeder: the two green ROIs corresponding to this feeder are active at the same time, which means that both rats are in same feeding site (Fig. 10);
    b. Social competition feeder: this situation was manually scored (i.e. manual identification of frames where both rats are at this feeding site), after filtering the frames where this feeding site was active (Fig. 11). The filtering was done with Bonsai, the program edits the videos by selecting the frames where the animal was with the head inside this feeder and outputs a video with only the frames where activity was registered.

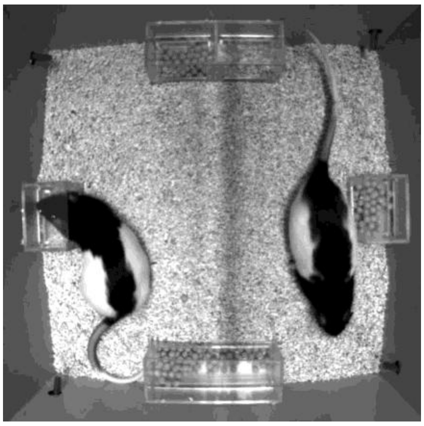


Figure 8 - Example of a rat feeding alone.

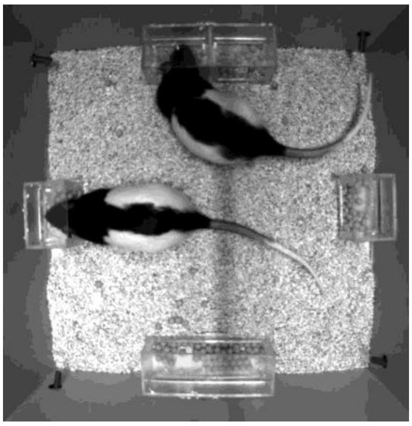


Figure 9 - Example of both rats feeding simultaneously in different feeding sites.

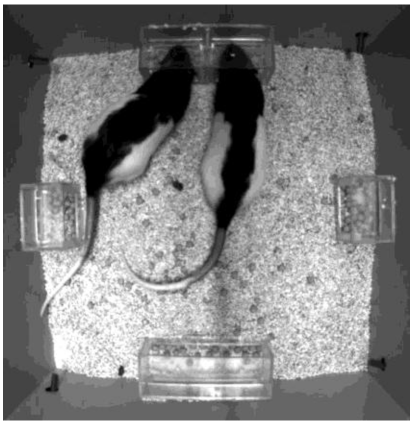


Figure 10 - Example of both rats feeding together at the social no-competition feeder.

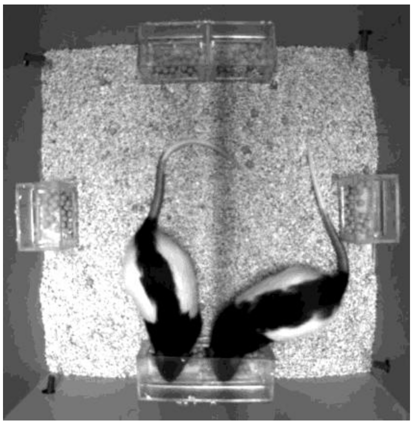


Figure 11 - Example of both rats feeding together at the social competition feeder.

Statistical analysis

All collected data were analyzed with the software IBM SPSS Statistics (v. 22, SPSS Inc., Chicago, IL). Wilcoxon-Mann-Whitney test was used to verify statistical differences between two groups. Kruskall-Wallis test was used to compare data from more than two groups, followed by pair-wise comparisons. Significant statistical effects were considered for results with p-value ≤0,05 (shown as "*"), p-value ≤ 0,1 was shown as indication of marginal significance (#); *** - p-value ≤ 0,001.

Ethics statement

All animal procedures were performed under the guidelines of the Animal Welfare Body of the Champalimaud Center of the Unknown, Portugal, which are in accordance with the EU guidelines.

Results

Feeding pattern

We first analyzed the feeding pattern of rat dyads in the five cages throughout the five experimental days. For this analysis we used one hundred and twenty data points per cage, from the five days (corresponding to one hour time bins). All the analyzed parameters (time spent feeding, number of visits, and average duration of visits per hour) showed a daily pattern throughout the days, with increasing activity in the beginning of the dark period (denoted by grey background) and a significant decreased during the light period (white background).

In the first two parameters (time spent feeding & number of visits), the pattern of the feeding activity is consistent in most of the days, but in the third and fifth dark periods a large variability between the cages was observed, probably due external perturbations (such as change of cages of rats housed in the same room) (Fig. 12 & 13). The last parameter (average duration of visits per hour) showed a high variability in the first day (dark and light periods), but it was consistent in the remaining ones (Fig. 14), which goes along with a study reporting that rats when facing a new environment adapt the feeding behavior within twenty-four hours (Raslear, Bauman, Hursh, Shurtleff, & Simmons, 1988).

Despite some variance between days (day three and day five), no significant statistical differences were found between days within the parameters: time spent feeding - $X^2_{KW}(4)=2{,}932$; p=0,569; N=25 (supplementary fig. 2); number of visits- $X^2_{KW}(4)=5{,}191$; p=0,268; N=25 (supplementary fig. 3); and average duration of visits per hour - $X^2_{KW}(4)=2{,}031$; p=0,730; N=25 (supplementary fig. 4). But significant statistical differences were found between the cages within the five days: time spent feeding - $X^2_{KW}(4)=10{,}398$; p=0,034; N=25 (post-hoc pair-wise comparisons reveled a significant difference between cage 3 and cage 4, p=0,035); number of visits - $X^2_{KW}(4)=9{,}526$; p=0,049; N=25 (post-hoc pair-wise comparisons reveled marginal differences between cage 1 & cage 4, p=0,063); and average duration of visits per hour - $X^2_{KW}(4)=10{,}442$; p=0,034; N=25 (post-hoc pair-wise comparisons reveled marginal differences between cage 4 & cage 5, p=0,099).

In addition, we also compared the feeding behavior between genders. Statistical significant differences were observed on the time spent feeding showing that females have higher feeding activity than males: time spent feeding - U=125, W=245; p=0,012; N=25; marginal differences on the number of visits - U=116, W=236; p=0,069; N=25; in the average duration of visits per hour no significant differences were found - U=107, W=227; p=0,24; N=25. P-values presented with Bonferroni correction (p-value multiplied by three) (Supplementary fig. 5).

Remarkable differences in the activity between dark and light periods were observed: time spent feeding - U=0, W=325; p<0,001; N=50; number of visits - U=0, W=325; p<0,001; N=50 and average duration of visits per hour - U=20, W=245; p<0,001; N=50. P-values presented with Bonferroni correction (p-value multiplied by three). This differences show that roughly 80% of the feeding activity happens during the dark period, which was expected, since rats are nocturnal animals (Lore & Flannelly, 1977) (Fig. 15).

To compare the proportion of feeding activity during the light and dark periods, , the data of the time spent feeding and number of visits from each cage were normalized for dark and light periods (sum of the values for each period divided by the total daily value), due to the differences found in the feeding activity between cages and periods of light. The values of the average duration of visits per hour were averaged for the duration of each period, i.e. twelve hours, giving us ten points per cage per day, five dark and five light periods. Differences between cages faded in the proportion of time spent feeding between dark and light periods ($X^2_{KW}(4)=1,191$; p=0,88; N=50), proportion of number of visits between dark and light periods ($X^2_{KW}(4)=0,824$; p=0,935; N=50), and average duration of visits per period ($K^2_{KW}(4)=8,418$; p=0,077; N=50), therefore normalized values will be used. Due differences between the periods, comparisons will done within each period (dark or light) in further analysis.

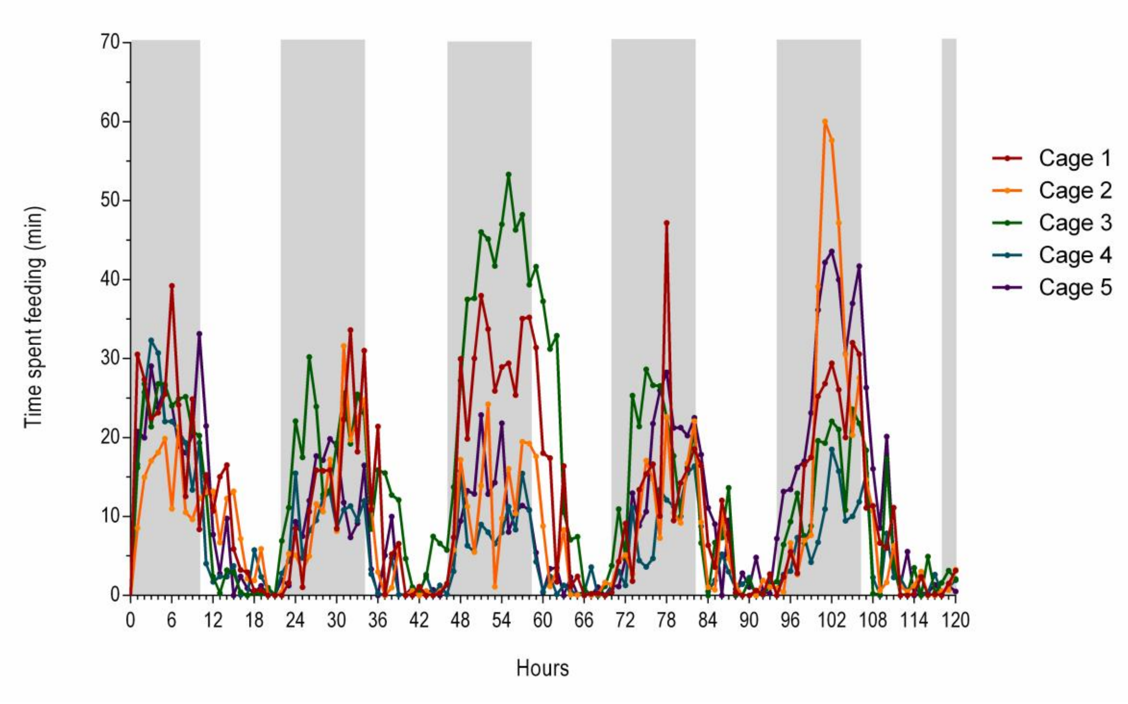


Figure 12 - Progression of the time spent feeding (in minutes) per cage, throughout the five days in one hour intervals. Each dot corresponds to the cumulative value of one hour. Dark periods denoted in grey background and light periods corresponds to the white background.

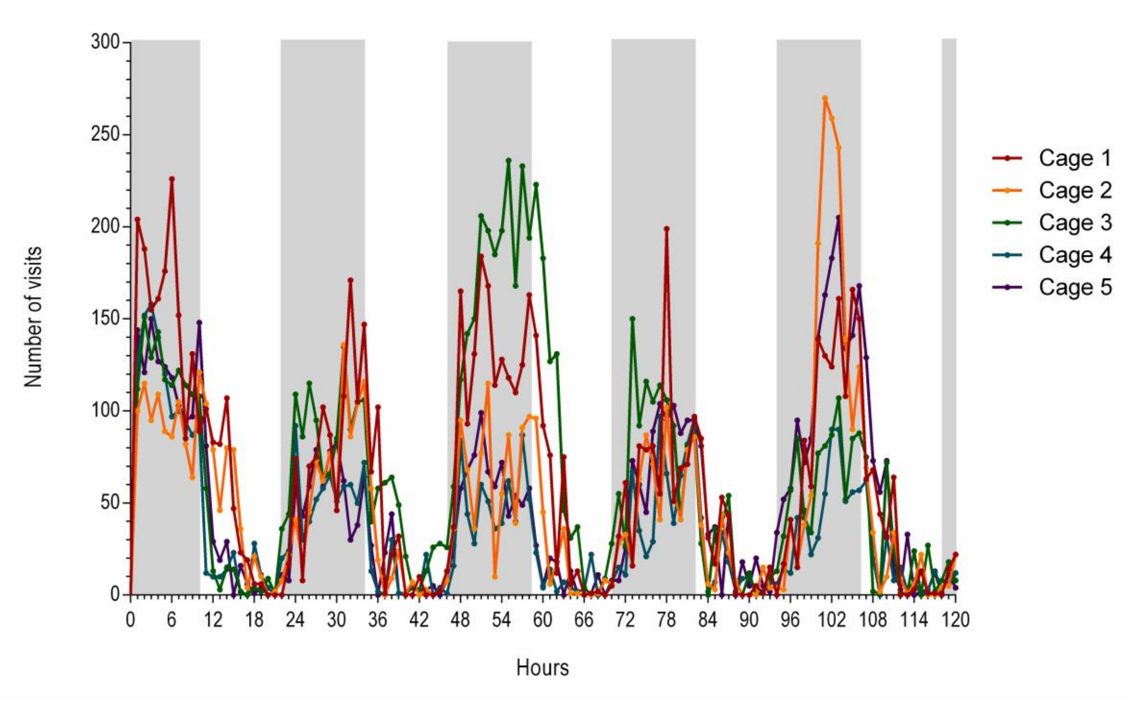


Figure 13 - Progression of the number of visits per cage, throughout the five days in one hour intervals. Each dot corresponds to the cumulative value of one hour. Dark periods denoted in grey background and light periods corresponds to the white background.

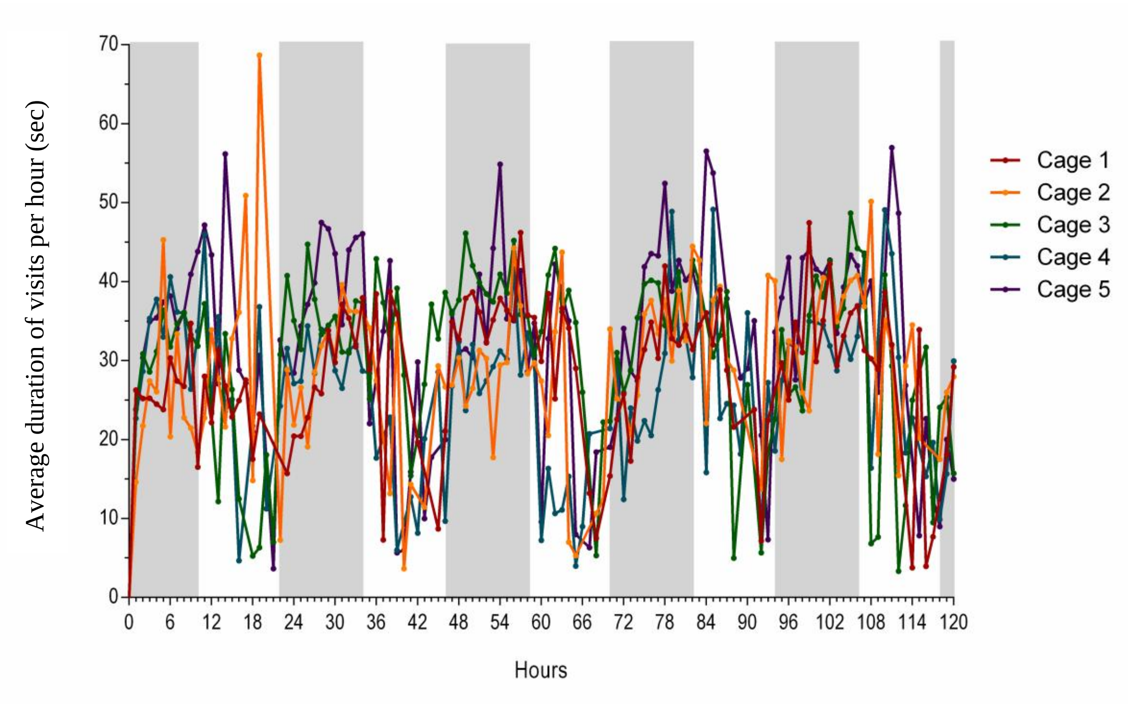


Figure 14 - Progression of average durations of visits per hour (in seconds) per cage, throughout the five days in one hour intervals. Each dot corresponds to the mean value of one hour. Dark periods denoted in grey background and light periods corresponds to the white background.

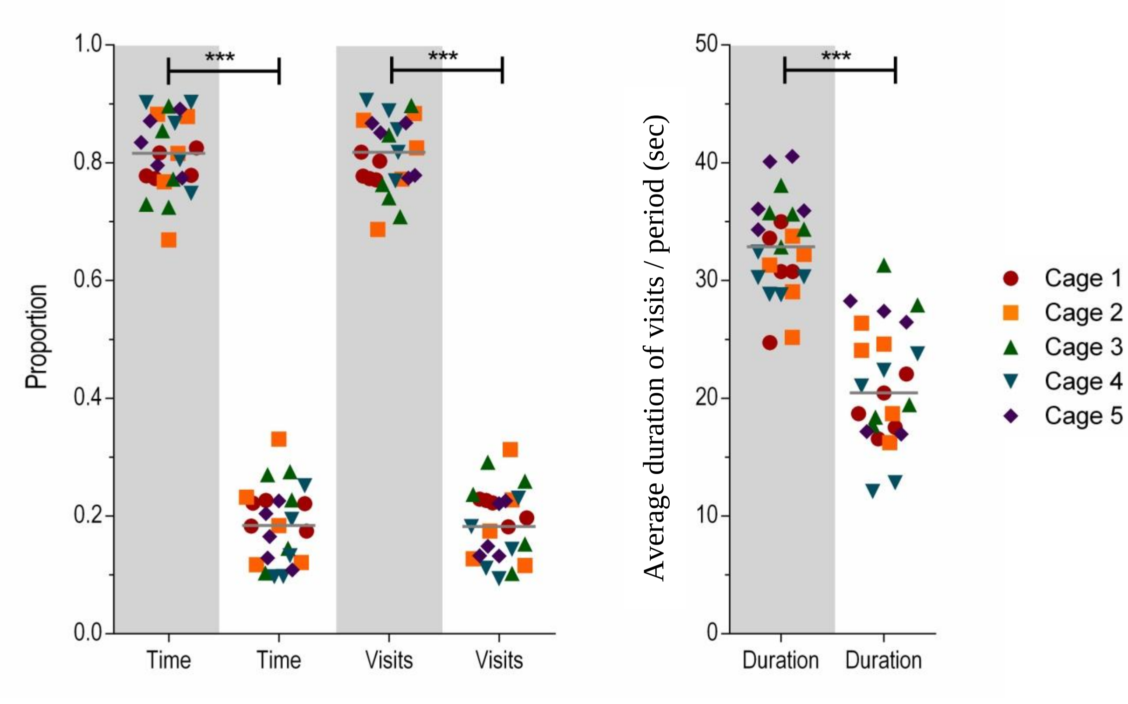


Figure 15 - Proportion of time spent feeding and number of visits, and average duration of visits per period (in seconds), in the dark and light periods. Each dot corresponds to the value of one day of each cage. Grey bars are median between all dots. Dark periods denoted in grey background and light periods corresponds to the white background.

Preferences for feeding sites

After characterizing the rats' feeding pattern, we started looking into the preferences for feeding sites. Since the feeder sites had different amounts of food, we chose to analyze the preferences for the type of feeder instead each feeder separately (i.e. the two non-social feeders, and the two social no-competition feeders were pooled together), to match the amount of daily food available in each one (eighty pellets) (for comparison between each feeder separately see supplementary data 2).

Significant statistical differences between feeder types were found in all three analyzed parameters in both dark and light periods: proportion of time spent feeding (dark period: $X^2_{KW}(2)=21,706$; $p<0,001$; N=75; light period: $X^2_{KW}(2)=10,454$, $p=0,005$; N=75) (Fig. 16), porportion of number of visits (dark period: $X^2_{KW}(2)=19,566$; $p<0,001$; N=75; light period: $X^2_{KW}(2)=8,964$; $p=0,011$; N=75) (Fig. 17), and average duration of visits per period (dark period: $X^2_{KW}(2)=18,697$; $p<0,001$; N=75; light period: $X^2_{KW}(2)=7,344$; $p=0,025$; N=75) (Fig. 18).

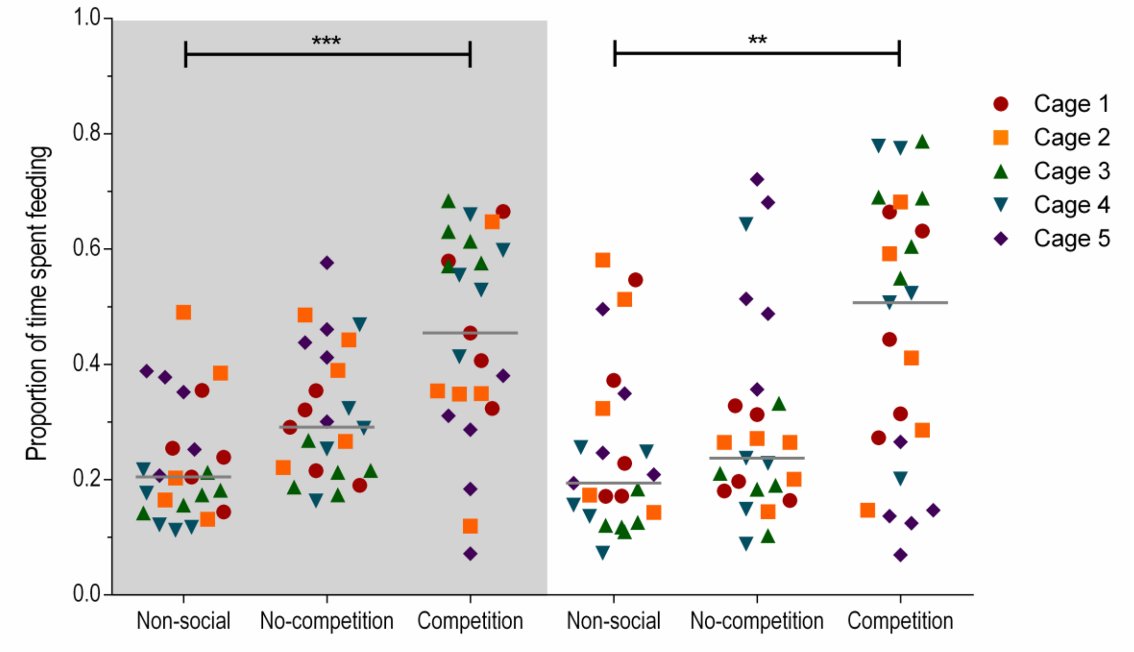


Figure 16 - Proportion of the time spent feeding in each feeder type in both dark (denoted in grey background) and light (white background) periods. Each dot corresponds to one dark or one light period of each cage for each day. Grey bars are median of all points.

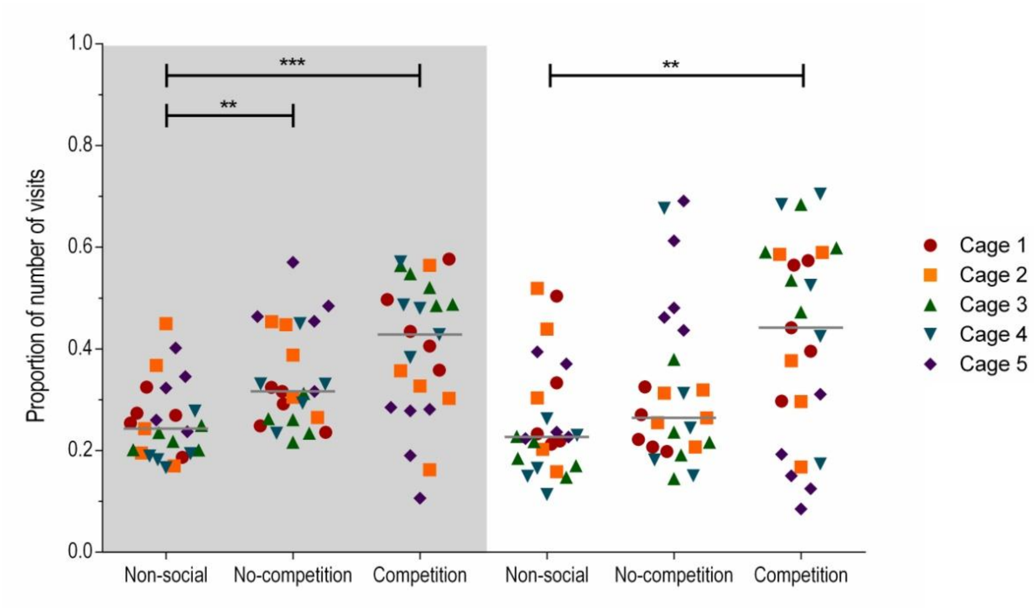


Figure 17 - Proportion of the number of visits in each feeder type in both dark (denoted in grey background) and light (white background) periods. Each dot corresponds to one dark or one light period of each cage for each day. Grey bars are median of all points.

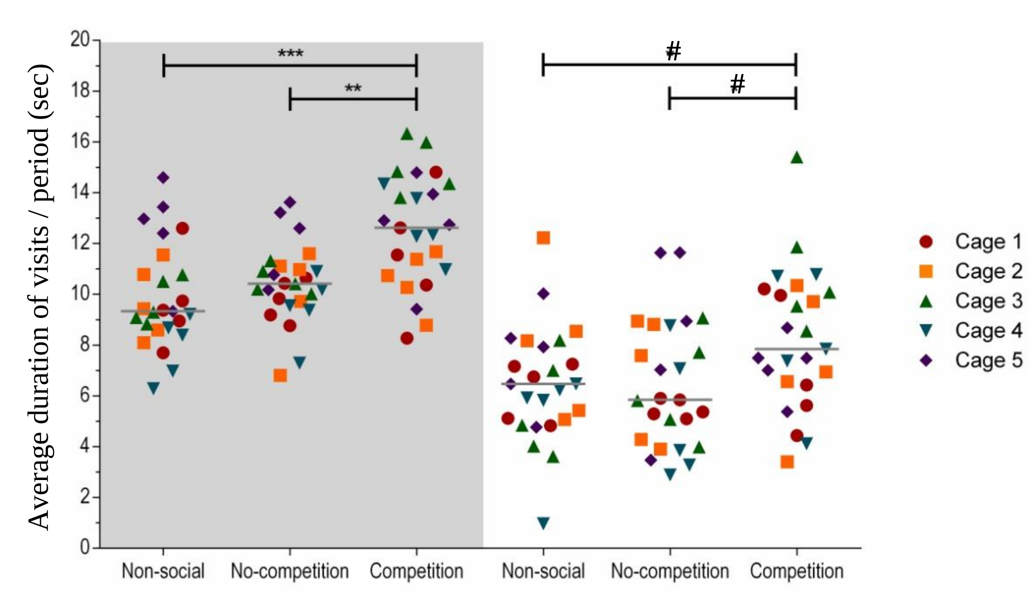


Figure 18 - Average duration of visits per period (in seconds) in each feeder type in both dark (denoted in grey background) and light (white background) periods. Each dot corresponds to one dark or one light period of each cage for each day. Grey bars are median of all points.

These results show that social feeders are preferred over the non-social feeders, with a clear preference for the social competition feeder. Almost 50% of all the activity registered was in the social competition feeder, roughly 30% in the social no-competition and 20% in the non-social feeders. But preferences for the social feeders doesn't necessarily mean preferences for social feeding, as animals can feed at these sites alone (which will be addressed below). Some non-social reasons can explain this differences (e.g. the size of the social competition feeder is bigger than the remaining feeders or availability of food that is higher in the social feeders than in non-social feeders).

Social feeding behavior

Finally, we looked at the rats' social feeding behavior, analyzing the proportion of time that both rats spent feeding alone, simultaneously, and together at one feeding site. Despite our finding that rats prefer to eat in the social competition feeder, they do not do it socially as significant statistical differences show a clear preference for feeding alone in both dark ($K^2_{KW}(2)=64,958$; $p<0,001$; N=75) and light ($K^2_{KW}(2)=60,367$; $p<0,001$; N=75) periods (Fig. 19).

Our results show that rats eat together at the same feeding site roughly 1% during their active period and less than that during the light period, at the same time in different feeding sites (simultaneous) around 9% of the time during the dark period and around 5% during the light period. In summary, our results shows that rats in our experimental conditions do not show a preference for social feeding as 90% of the activity registered at the feeders during dark periods and 95% during the light corresponds to solitary feeding.

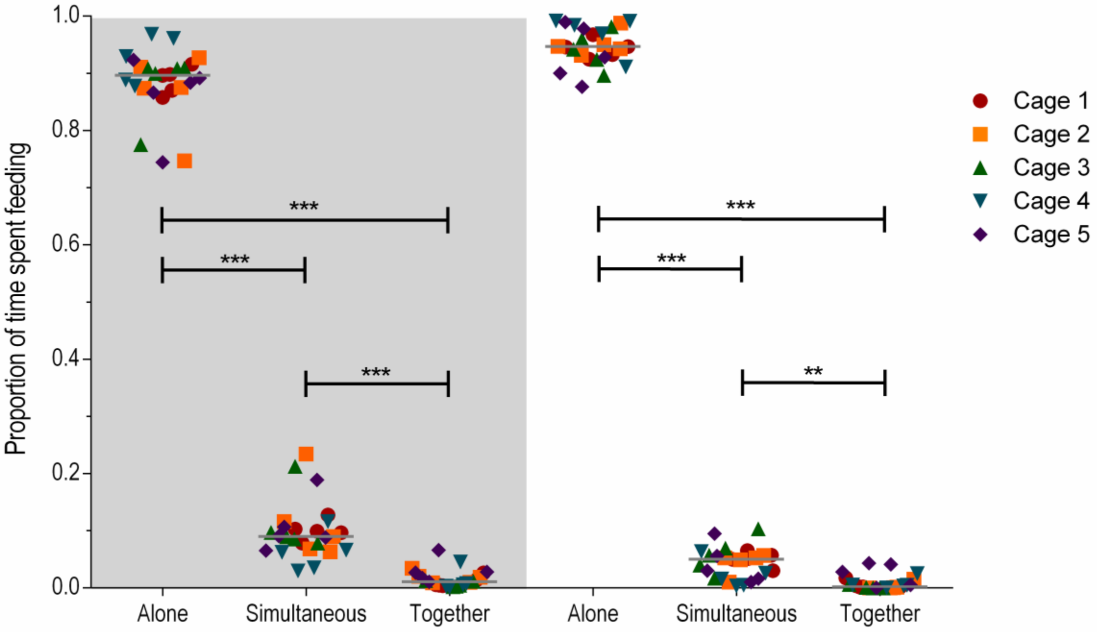


Figure 19 - Proportion of the time spent feeding alone, simultaneous in different feeding sites and together at the same feeding site, in both dark (denoted in grey background) and light (white background) periods. Each dot corresponds to one dark or one light period of each cage for each day. Grey bars are median of all points.

Discussion

With the automatic analysis we were able to track the animals' activity at the feeding sites throughout the five experimental days. We cannot state that all the activity registered at the feeding sites were for feeding purposes, animals when exploring their environment can explore the feeding sites without occurrence of actual feeding. Reid, Bacha, & Morán (1993) used a infrared beam to track the animals activity at the feeding point, to what they called food related behavior, like us they were not able to distinguish between actual feeding and exploratory behavior.

Many reports have observed rats dragging the food from the source to their nests, where they ingest it (Bindra, 1948a, 1948b; Gandolfi & Parisi, 1973; Grasmuck & Desor, 2002; Morgan, Stellar, & Johnson, 1943; Nieder, Cagnin, & Parisi, 1982), but when comparing the dragging behavior with the food size, Barnett & Spencer (1951) noticed that rats tend to drag big sized food to the nest whist small sized food are eaten on site. The food we used was sphere shaped with roughly 0,9 centimeter in diameter, during the experiments animals were observed eating at the feeding sites and taking the food out of the feeders, feeding outside them. Pisano & Storer (1948), on the food hoarding behavior, stated that when food is hoarded, and new food is available, rats do not rely on the hoarded one but rather continue to retrieve food at the feeding site. Our observations goes along with it as every day the food made available was almost, if not all retrieved from the feeding sites (but a portion of food was found outside the feeding sites indicating dragging (in the future we aim to distinguish the activity in the feeders when there is food, from the activity when there is no more food). Thus even if not all activity recorded corresponds to feeding, this method can give us an insight on the rats' food related behavior in all five days.

Le Magnen & Devos (1980) registered the food intake of free feeding animals during a twenty-four hours period and registered an intake of about two grams per hour during the dark period and half a gram during the light period. We didn't measured the amount of food eaten but the activity at feeders matches their findings in the sense that during the dark period, animals spent on average around seventeen minutes per hour with their heads inside the feeding sites and in the light period the average time spent feeding was around four minutes. Just like Le Magnen & Devos observations, the light period feeding activity corresponds roughly to one quarter of the dark period activity.

The number of times that both rats visited the feeding sites confirms once again that rats with *ad libitum* access to food are grazers rather than true meal eaters (Goulding et al., 2008; Rowland, 2012). During the dark period the mean number of visits per hour is around 87, and 21 for the light period.

To our knowledge there are no reports on the duration of grazing of free-behaving animals with *ad libitum* access to food, our data demonstrates that during the dark period the average duration of visits is 32 seconds, and 21 seconds for the light period. The variability of the duration of feeding between the first day and the remaining ones is in accordance with a previous report suggesting that when exposed to a new environment rats adapt their feeding behavior within twenty-four hours (Raslear et al., 1988).

The experimental cages were kept in an animals' holding room during the experimental days. On Wednesday, the animals house in that holding room (not the experimental ones) were changed to clean cages. This cage changing was done during the third dark period, which could explain the variations between cages observed during that period. The variations on the fifth dark period were not caused by cage changing, but most likely caused by some other external disturbance.

Female rats when in groups isolated from males often suppress their estrous cycle, this is known as the Lee-Boot effect, when exposed to males pheromones their estrous cycle are restored (Gleason & Reynierse, 1969). Our animals before the experiments were pair-housed, with a conspecific from the same gender, and kept in a Tecniplast® Isolated Ventilated Cages (IVC) racks. When in the experimental boxes females may have been exposed to males pheromones as we tested pairs of both genders side by side. Kennedy & Mitra (1963) showed that female rats almost double their food consumption upon entering estrous cycle. It is possible that the females used in our experiments were exposed to the males’ pheromones, thus restoring their estrous cycle and increasing their food intake. Additionally, the males' visits duration were also suggestively shorter than females, if males eat faster than females that can explains the differences observed between the feeding activity of males and females. Since we were not able to quantify the amount of food eaten, further investigation on this matter is necessary.

Regarding the preferences for the feeder types, the differences observed shows that rats rather eat on the social competition feeder than in the non-social feeders. Despite the lack of statistical significance of the difference between the social no-competition and the social competition feeder, the last one showed higher levels of activity when compared with the first one. Rats when motivated, actively compete attempting to achieve the same goal, that frequently happens when foraging for food (Hillman & Bilkey, 2012) this behavior was often observed when resources are limited. Although this was not our case, when planning the experiment we decided to add a social competition feeder to verify if that behavior was also displayed by animals with *ad libitum* resources and maybe could drive social feeding. Competitive behavior was not a prevalent factor modulating preference between social feeders, as animals fed mostly alone.

Additionally olfactory cues of conspecifics play a role in orienting the feeding behavior of a hungry naive rat (Gerrish & Alberts, 1995), however this social facilitation is not important on orienting individuals' behavior of well-fed rats (Barnett & Spencer, 1951). In our case both rats were naive to the environment and never had food restrictions, therefore social facilitation may not be enough to explain the preference for the social competition feeder, especially because they were experimented together.

Two aspects that may underline this preference is the actual format of the feeder, with a twelve centimeters entrance (two times bigger than the others) this feeder may be the most comfortable one to eat (the average duration was significantly higher in this feeder than others) and also the amount of food available in the space is twice as much in this feeder than the others.

Rats are capable of modulating their feeding pattern according to particular circumstances. With *ad libitum* access to food, rats display grazers' like behavior eating small portions multiple times throughout the day, but as the access becomes more restricted rats start to increase the portions size and decrease the number of meals. Even small alterations in the food availability seems to have drastic results in the meal size and the intervals between meals (Collier et al., 1999; Houston & McNamara, 1989; Kaufman & Collier, 1983).

Barnett & Spencer (1951) were one of the first to report observations of rats feeding together. In their report, many social aspects of the feeding behavior were described. Rats from different species shared the same feeding site without aggressive contact between the individuals. When restricting the rats' access to food, rats were seen taking food from the paws of another hungry rat without evoking agnostic behaviors. They even hypothesized that it is possible in some conditions that hearing or seeing rats feeding stimulates others (i.e. c to feed, although they claim that social facilitation does not seems to be a important factor in the feeding behavior of adults rats that are not hungry.

In general, the above authors, observed that rats tend to ignore each other except "when mating or sleeping". Nowadays we know that is not true as the rats modulate their behavior for different social contexts, in terms of feeding behavior. Kashtelyan, Lichtenberg, Chen, Cheer, & Roesch (2014) recorded dopamine release in the ventral striatum of hungry rats observing reward delivery to a conspecific, showing a early increase followed by a decrease in the dopamine release. Márquez, Rennie, Costa, & Moita (2015) recently shown that rats can be prosocial, providing access to food to a conspecific that displays food-seeking behavior.

Additionally, Thompson (1948) observed a major difference in hungry rats and well-fed rats, the first condition leads rats to concentrate their behavior in feeding and drinking whilst the second contemplates other normal behaviors such as exploration. Barnett & Spencer fed their rats in a defined time schedule, and as we know by now rats can modulate their feeding frequency according to their access to food (Collier et al., 1999; Houston & McNamara, 1989; Kaufman & Collier, 1983), therefore it is probable that what they observed was a meal behavior which does not correspond to the feeding behavior of animals with *ad libitum* access to food.

Our data regards free-behaving animals with *ad libitum* access to food. In such conditions animals appear to lack preferences for social feeding as time spent feeding alone corresponds to roughly 90% of all feeding activity observed.

The available space in our experimental boxes was 0,16 square meters (40cm x 40cm), with this reduced size we are concern that a rat may perceive the presence of the other as indication of company when feeding, even thought the other is not engaged in the same behavior. To address this question (in the future, due time constrains), we already designed a one square meter box with the same configuration in feeding sites, giving the animals more than double of the space as the previous ones.

Additionally, in the future, we aim to use idTracker (a animal recognition software that tracks each animal's position and trajectories in a video (x and y coordinates) (Pérez-escudero, Vicente-page, Hinz, Arganda, & Polavieja, 2014)), to distinguish the activity originated from each animal, what can give us individual preferences.

On average both rats spent 29% of their time feeding in the dark period and 7% during the light period. Our data corresponds to feeding by pairs of rats. Here we report the pooled data from two rats, but Inglis et al. (1996) & Whishaw (1992) compared the individual variation on food intake and found a high level of consistency between individuals. Although we cannot differentiate the feeding behavior of both animals, if we consider that it may be evenly distributed the expected proportion of seeing both animals feeding at the same time in dark period is 2% and 0,1% in light period ($P_{2Rats}=P_{1Rat} \cap P_{1Rat}$; Dark - $P_{2Rats}=14{,}5\%\cap 14{,}5\%$; Light - $P_{2Rats}=3{,}5\%\cap 3{,}5\%$). Our results from the dark period shows that rats feed at the same time roughly 10% and 5% in the light time, although higher than expected out of chance, is still significantly lower than the activity of rats feeding alone. Which lead us to believe that rats tolerate rather than prefer enjoy, the presence of others when feeding.

Attachment1: Extended introduction

The social environment and its influences on behavior

Rowland (2012) stated that "(...) the animal and its behavior are a complex interaction among many environmental variables", the social environment offers many variables of use to others which are known as social information, i.e. information provided by the activities of other individuals (Lindeyer, Meaney, & Reader, 2013).

Social behavior is a prominent ethological feature of animals living in groups, which involves any activity that directly influences the behavior of other individuals (Barnett & Spencer, 1951; Young, Liu, & Wang, 2008). Animals are capable of modulating their behavior as a function of the current social environment and previous social interactions. This behavioral trait, known as social plasticity, like any other trait, impacts the animal's Darwinian fitness. Darwin's (1859) theory of evolution on the struggle for life and the survival of the fittest by means of Natural Selection, implies that the most adaptable individuals should thrive, therefore this plasticity on behavior that optimizes animals' social interactions seems to be a excellent survival tactic of animals living in heterogeneous environments, but it can slow down evolution as this changes on the phenotype can happen without changes in the genome (Oliveira, 2012).

Animals in their daily routines provide information of use to others, that could be actively (e.g. vocalizations) or passively (e.g. pheromones) (Williams, 1966). This social information can influence the behavior of animals either subtly or prominently. The usage of such information can lead to synchrony in the performance of a task (i.e. **contagious behavior**), disinhibition (i.e. **social facilitation**) and may even offer the animals a learning opportunity (i.e. **social learning**) (Nicol, 1995). The nuances between the first two parameters are hard to define and are often combined and referred to as social facilitation.

- **Contagious behavior** refers to occasions where the behavior of a animal releases the performance of the same behavior by an observer (Galef, 1988). Examples of that can be seen in many animals, pigs for example, were observed to show preferences for social feeding, eating at the same time in the same feeding site with the company of others (B. Nielsen, Lawrence, & Whittemore, 1996) that is also true for grazing sheep (Rook & Penning, 1991). It was shown that well-satiated chickens may be stimulated to begin feeding again upon seeing a conspecific pecking (Fischel 1927; Bayer 1929 (cyted by Harlow (1932)). *C. elegans* on the other hand, displays natural variations at the feeding behavior, where some animals displays preferences for social feeding while others don't (Bono et al 2002). Contagious behaviors can also be observed in humans, where observation of a conspecific yawning is a strong stimulus for the observer to perform the same behavior (Provine, 1989).

- **Social facilitation** describes the effects of presence of a conspecific on the individuals behavior, the conspecific doesn't need to be engaged in such behavior. Tolman (1968) found that just the companion of an active non-feeding chicken evoked pecking behavior in some subjects. Interestingly just the sight of a chicken feeding in a visual distinctive site trough television images caused observing birds to feed from similar feeding sites when tested two days later (McQuoid & Galef, 1993).
- **Social learning** is the acquisition of a novel behavior by social information, which can happens trough either contagious behavior or social facilitation. Such information are the basis of almost all known cases of animals social learning about when (1), where (2), what (3) and how to eat (4) (Valone, 1989).
    1. Galef & White (1997) trained a colony of rats to eat at a fixed time schedule. When introducing naive animals into the colony they observed that the naive rats would feed on average five minutes after the trained animals. If the colony were not trained the latency to feed was on average forty minutes.
    2. There are extensive reports on the influence of the social information on guiding the animals where to feed. Karl von Frisch received a Nobel Prize for his decoding of the honeybees' "waggle dance", where the dancer communicates to others the direction and distance of a food source (von Frisch, 1967; Riley, Greggers, Smith, Reynolds, & Menzel, 2005).
    3. The flavor of foods that mothers ingests when nursing are detected by the pups and when weaned, they show preferences for foods with those flavors (Galef & Sherry, 1973). Animals also learn what not to eat trough social information, for example the presence of urine from a stressed pig around the feeding site results in long lasting aversion of that area by other female pigs (Vieuille-Thomas & Signoret, 1992).
    4. Terkel (1994, 1995) observed back rats natives of pine forests in Israel that are able to retrieve pine seeds from the pine cones. Rats from elsewhere in Israel were

unable to execute this task, but when reared in a foster family capable of performing it, this rats were also success in retrieving the seeds.

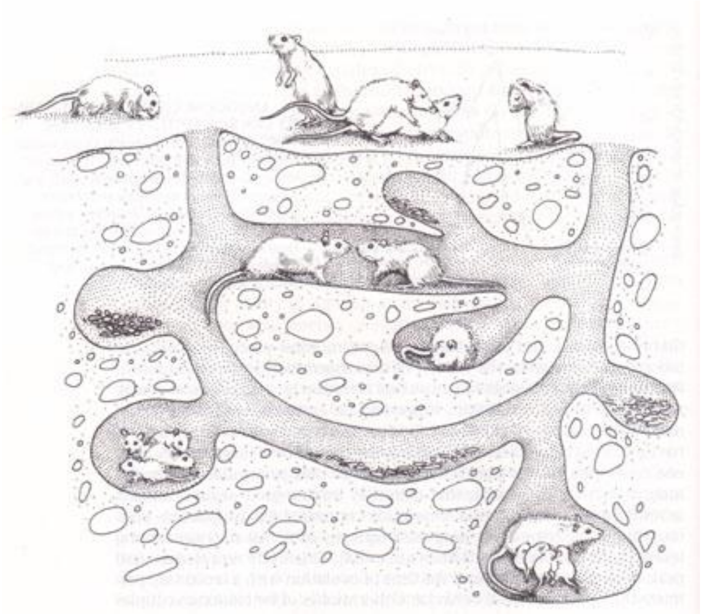
In the last decades we saw a increase in the number of reports on the subject of social foraging. Such behavior can offers animals many advantages, as each member of the group can spend less time being vigilant and more time eating, this is known as the detection effect (Pulliam, 1973). Each member also has diminished probability of becoming a prey when compared to solitary foraging (a.k.a. dilution effect) (Hamilton, 1971; Powell, 1974). But it can also bring disadvantages, as the group can be easier detected by a predator than just one individual, and when resources are scarce group foraging can lead to competition between individuals (Hillman & Bilkey, 2012; Sasvári, 1992).

Social interactions are essential for the well-being of social animals, individuals' impairments on social interactions are used as diagnostic tools for various psychological disorders - that affect humans - as autism (Pletnikov, 2002), schizophrenia (Jones, Watson, & Fone, 2011) and bipolar disorder (Malatynska & Knapp, 2005). These interactions are so important that social isolation is considered a risk factor for morbidity and mortality due its pronounced effects on the animals' physiology, neurochemistry and behavior (Cruces, Venero, Pereda-Péeez, & De la Fuente, 2014; Lopak & Eikelboom, 2004; van Leeuwen, Bonne, Avraham, & Berry, 1997).

Rats are social animals

Early studies done with groups of rats living together revealed an amazing social system between them (Barnett & Spencer, 1951; Calhoun, 1949; Chitty & Shorten, 1946; Pisano & Storer, 1948; Thompson, 1948). In naturalistic (or semi-naturalistic) environments rats cave burrows in the dirt and establish nests in them (Calhoun, 1963; Chu & Ågmo, 2015). These burrows usually house many females and few males (Fig. 1). The number of males tends to vary positively with variations in the population densities (Moore, 1999).

In the laboratory, studies of behavior in semi-naturalistic environments are often done with the Visible Burrow System (VBS). This technique was designed to mimic the burrow systems that rats create for themselves when in dirt subtract. Rats were reported to quickly populate the tunnel-chamber system, sleeping in the chambers and remaining in the burrows for most of the diurnal period (Blanchard, Blanchard, & Flannelly, 1985), what is not preoccupant once the VBS allows for visualization and recording of the behavior of animals inside the burrows as well as outside them (Blanchard & Blanchard, 1989).



Supplementary figure 1 - Illustration of a typical burrow system of wild Norway rats. These burrow systems house a few females, a small number of males and their many subadult offspring (McClintock, 1987).

Males and females have different roles in the colony. Females frequently rear the offspring alone in individual chambers inside the same burrow system (Schultz & Lore, 1993; Telle, 1966), however some cases of kin females sharing the same nest were observed, in this communal nests they often rear each other's offspring (Barnett, 1975; Calhoun, 1963; Steiniger, 1950). In low population densities male rats often exhibit territorial behavior for the nesting site and the females by displaying agnostic behavior towards a intruder male. In high densities, defending a territory may be too costly, therefore rats often opt for a different strategy, a hierarchical one, where one rat becomes socially dominant and the remaining ones become subordinates, within the subordinates there is a social scale as well with different levels of hierarchy. This hierarchical relationships revealed to last for long periods of time demonstrating individual recognition between them (Barnett, 1958; Lott, 1984).

Although displays of agnostic behaviors were observed in both high and low densities colonies, they were rarely aggressive (Barnett, 1956; Calhoun, 1949) (in a assessment made by Blanchard et al (1988) aggressive behaviors corresponded to 0,5% of the observed time (cited by Blanchard et al., 1995). Aggressive encounters were often caused by special stressors stimuli (e.g. placement of one intruder male (Telle, 1966) or between males fighting over a receptive female (Thor & Carr, 1979)). Often even upon stressful events no aggressive behaviors were observed: Barnett & Spencer (1951) introduced a colony of different species of rats into an already settled colony and their interactions seemed serene, some of the newcomers being absorbed into the existing colony. Individuals from the two species were seen feeding at the same food source without aggression between them.

Social information, specially olfactory clues, are an important factor for food orientation in rats. Norway rats when traveling from their burrows to forage for food and water create trails that influences the movements of conspecifics that come after them (Calhoun, 1963; Telle, 1966). The breath of rats from recent eaten foods is used as cues by naive animals when selecting what to eat, this preferences were shown to last for weeks (Galef, 1989), and can even reverse learned aversions to food (Galef, 1986; Heyes & Durlach, 1990; Yoerg, 1991), but when reared in social isolation, rats do not exhibit signs of this kind of social learning (Galef, 1981).

Juvenile rats tend to approach feeding sites where an adult conspecific is present rather than feeding sites with others juvenile rats (Gerrish & Alberts, 1995) suggesting discrimination regarding the reliability of social information. Passive transmission of feeding preferences were shown between generations trough nursing, as the flavor of foods that mothers ingests while nurturing are detected by the pups in the milk and when weaned, they show preferences for foods with those flavors (Galef & Sherry, 1973). This effects are so robust that permit cultural traditions to be formed that can last for generations (Galef & Allen, 1995; Laland & Plotkin, 1993; Laland, 1999).

Surprisingly this social facilitation doesn't inform rats about foods to be avoided. Galef (1985) and Galef, McQuoid, & Whiskin (1990) when studying this subject, observed that interactions with conspecifics that have eaten unfamiliar foods and become sick or unconscious leads to enhanced preference rather than aversion to that food. Showing that social information is not always reliable and the usage of it can negatively impacts for the individual.

Rats have been shown to be sensitive to the conspecifics display of food-seeking behavior. Márquez, Rennie, Costa, & Moita (2015) when studying prosocial behavior in rats that had to decide between two-choices, they demonstrated that rats significantly prefer the option where both rats receive food rewards and that this preference depends upon displays of food-seeking behavior by the conspecific. Dopamine (DA) has been implicated in the mediation of a variety of natural rewards (Wise & Rompre, 1989; Bressan & Cripa, 2005). Kashtelyan, Lichtenberg, Chen, Cheer, & Roesch, (2014) recorded DA release in the ventral striatum, and saw that when rats are hungry, the observation of a food reward delivery to a conspecific initially increases the DA release but soon it decreases to levels lower than basal. Which suggests that rats are also sensitive to the consumption of food by a conspecific

The usage of social information is not restricted to food foraging. A example of that is the social transmission of fear (Atasak et al., 2011; Bruchey, Jones, & Monfils, 2010; Kim, Kim, Covey, & Kim, 2010): a normal behavior of rats in fear situations is to freeze, the silence resulting from the cessation of movement by one individual (due freezing) is perceived by others as indication of danger, leading them to freeze, even if themselves haven't felt threatened by the situation (Pereira, Cruz, Lima, & Moita, 2012).

These reports show that rats are highly social animals. When living in colonies they share the same nesting and feeding sites, being capable of adapting their behavior for different social contexts, with few or, even in some cases no, aggressive behavior being observed between individuals living together.

The feeding behavior of rats

To this day the feeding behavior of rats remains vastly understudied, the main reason is that this behavior is very difficult to quantify accurately and objectively (B. L. Nielsen, 1999). Rats' feeding patterns seems to be highly affected by exogenous (e.g. resources availability, social environment) and endogenous stimuli (e.g. hunger, previous experience) (Clifton, Popplewell, & Burton, 1984; Collier, Hirsch, & Hamlin, 1972).

Despite scarce, there is information on the rats' feeding behavior dating back to the mid twenty century (see below). In these early studies, colonies of rats were kept in semi-naturalistic environments, the feeding behavior, unfortunately, constitutes only a small portion of these reports.

In all colonies the majority of activity registered was during the night period, showing that rats are nocturnal animals. Feeding orientated behavior arose shortly before sunset, being almost always the first orientated behavior after a resting period (Chitty & Shorten, 1946; Thompson, 1948). Individual differences in the feeding behavior were observed, some rats eat at the feeding site whilst others rely on scavenging fragments left by other rats on the floor around the feeding point (Barnett & Spencer, 1951). Small sized foods were usually eaten at the feeding point whilst large ones were carried to the nest where it could be hoarded.

The interesting part of the hoarding behavior is that rats do not rely on the horded food thus continue to visit the feeding site to retrieve food (Pisano & Storer, 1948). Hording often occurs due lack of security on the availability of food (Bindra, 1948a, 1948b), that can happens when the resources are limited (Morgan et al., 1943) or in the presence of a predator where the risk to forage is increased (Wernecke, Brüggemann, & Fendt, 2015).

With *ad libitum* access to food, rats' feeding behavior occurs in bouts, around ten meals per day (Le Magnen & Devos, 1980), but when food restrictions were imposed, animals modulated the meal size and frequency of feeding according the cost of feeding (Collier, Johnson, & Mitchell, 1999; Houston & McNamara, 1989; Kaufman & Collier, 1983). Even the colonies' social organization was modulated by food availability, which when restricted gave rise to individual behavioral differences when food is difficult to obtain: carrier rats that bring food to the nest, and non-carrier rats that steal food from other members of the group (Grasmuck & Desor, 2002; Thuller, Desor, Mos, & Krafft, 1992)

The problem with those reports is that the researchers organize feeding in meals by restring the availability of food (Collier et al., 1999; Houston & McNamara, 1989; Kaufman & Collier, 1983) or the way the organize the data (Le Magnen & Devos, 1980), stating that two to ten meals are normal behavior for rats, but rats are grazers rather than true meal eaters, therefore forcing the analysis of data into meals may not be a true reflection of the organizational units of the feeding behavior (Rowland, 2012). Goulding et al. (2008) home-cage observations of mice concluded that they are almost continuously active for several hours during the night and feeding occurs in short bouts throughout that prolonged activity period.

Due to increasing number of reports on the influences of the social environment in the behavior, including on feeding (Barnett & Spencer, 1951; Calhoun, 1963; Calhoun, 1963; Calhoun, 1966; Galef, 1981, 1986, 1989; Galef & Sherry, 1973; Gerrish & Alberts, 1995; Heyes & Durlach, 1990; Moore, 1999; Telle, 1966; Yoerg, 1991, and others), it is commonly accepted that rats enjoy feeding together since they were observed eating together in some circumstances. However these reports do not test directly whether rats show preference for social feeding, therefore further investigation on this matter is still to be done.

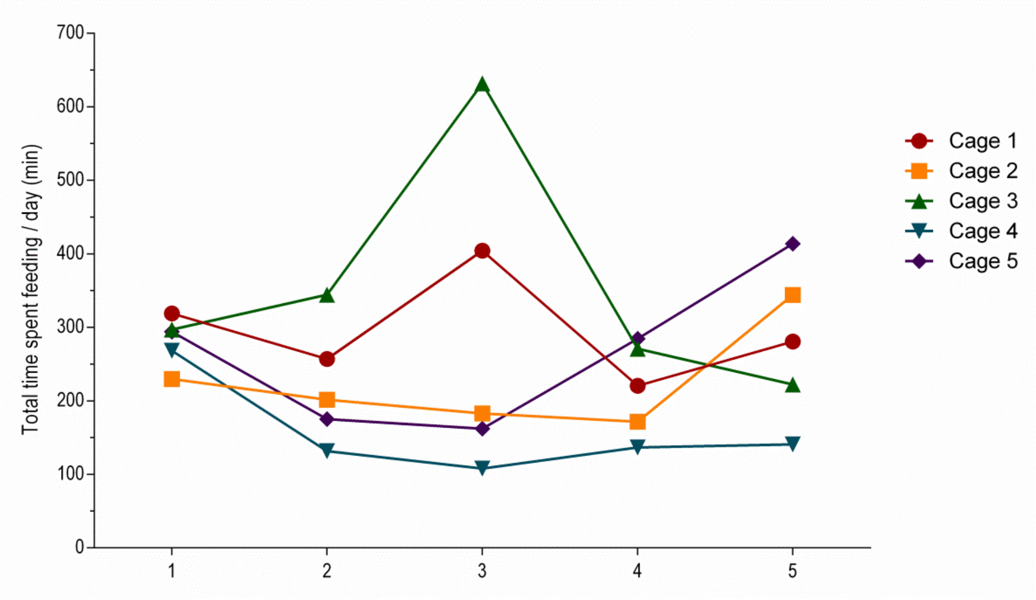
The present project aimed to address some of those questions above discussed: 1) how is the pattern of feeding of rats with *ad libitum* access to food without forcing meals? 2) Do rats show preferences for specific feeding sites? 3) Do rats have a preference for social feeding? To do so, we developed a new behavioral paradigm with three different feeder types, that allows us to constantly monitor the feeding behavior of a pair of rats for five consecutive days, at the same time contemplates three different feeding scenarios: feeding alone, simultaneous at different feeding sites or feeding together at the same feeding site.

Attachment 2: Supplementary data

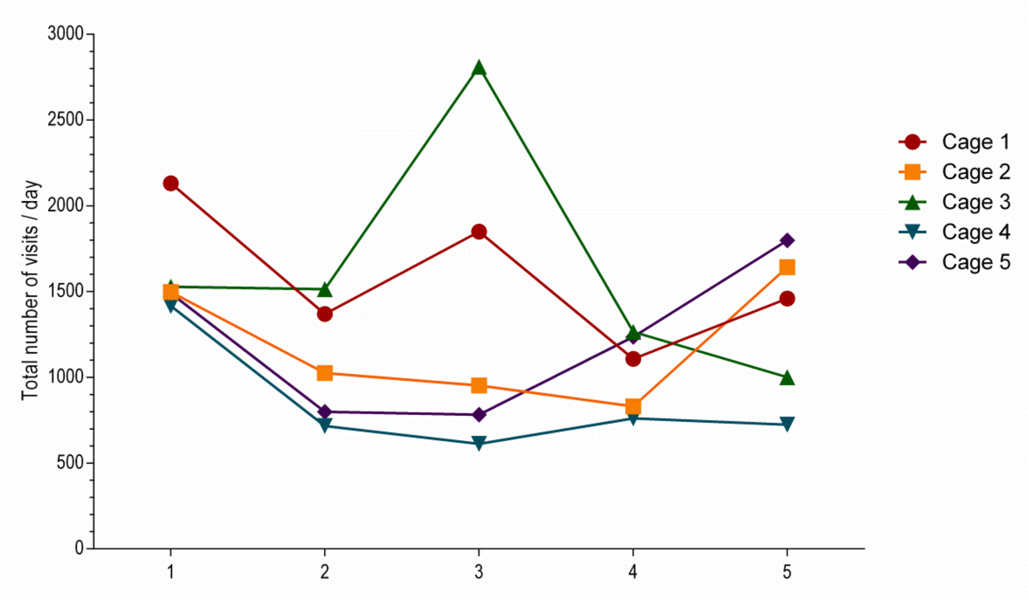
Supplementary data 1

Comparisons between cages and days

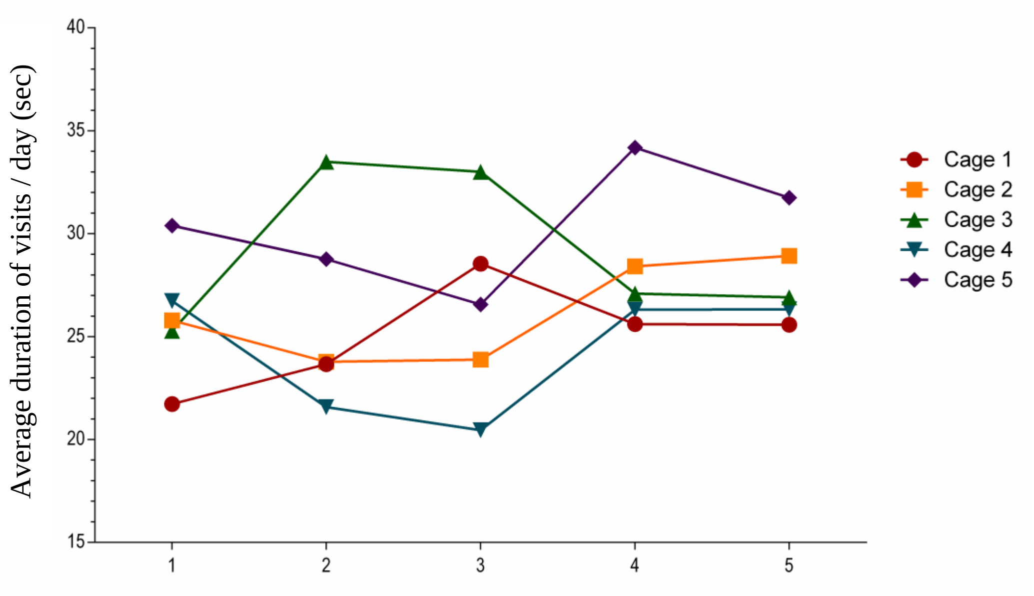
Despite the variance between days, no significant statistical differences were found between days within the parameters: time spent feeding - $X^2_{KW}(4)=2{,}932$; p=0,569; N=25 (supplementary fig. 2); number of visits- $X^2_{KW}(4)=5{,}191$; p=0,268; N=25 (supplementary fig. 3); and average duration of visits per hour - $X^2_{KW}(4)=2{,}031$; p=0,730; N=25 (supplementary fig. 4). But significant statistical differences were found between the cages within the five days: time spent feeding - $X^2_{KW}(4)=10{,}398$; p=0,034; N=25 (post-hoc pair-wise comparisons reveled a significant difference between cage 3 and cage 4, p=0,035); number of visits - $X^2_{KW}(4)=9{,}526$; p=0,049; N=25 (post-hoc pair-wise comparisons reveled marginal differences between cage 1 & cage 4, p=0,063); and average duration of visits per hour - $X^2_{KW}(4)=10{,}442$; p=0,034; N=25 (post-hoc pair-wise comparisons reveled marginal differences between cage 4 & cage 5, p=0,099).



Supplementary figure 2 - Distribution of the total time spent feeding per day (in minutes) for each cage, throughout the five days. Each dot corresponds to the cumulative value of one day.



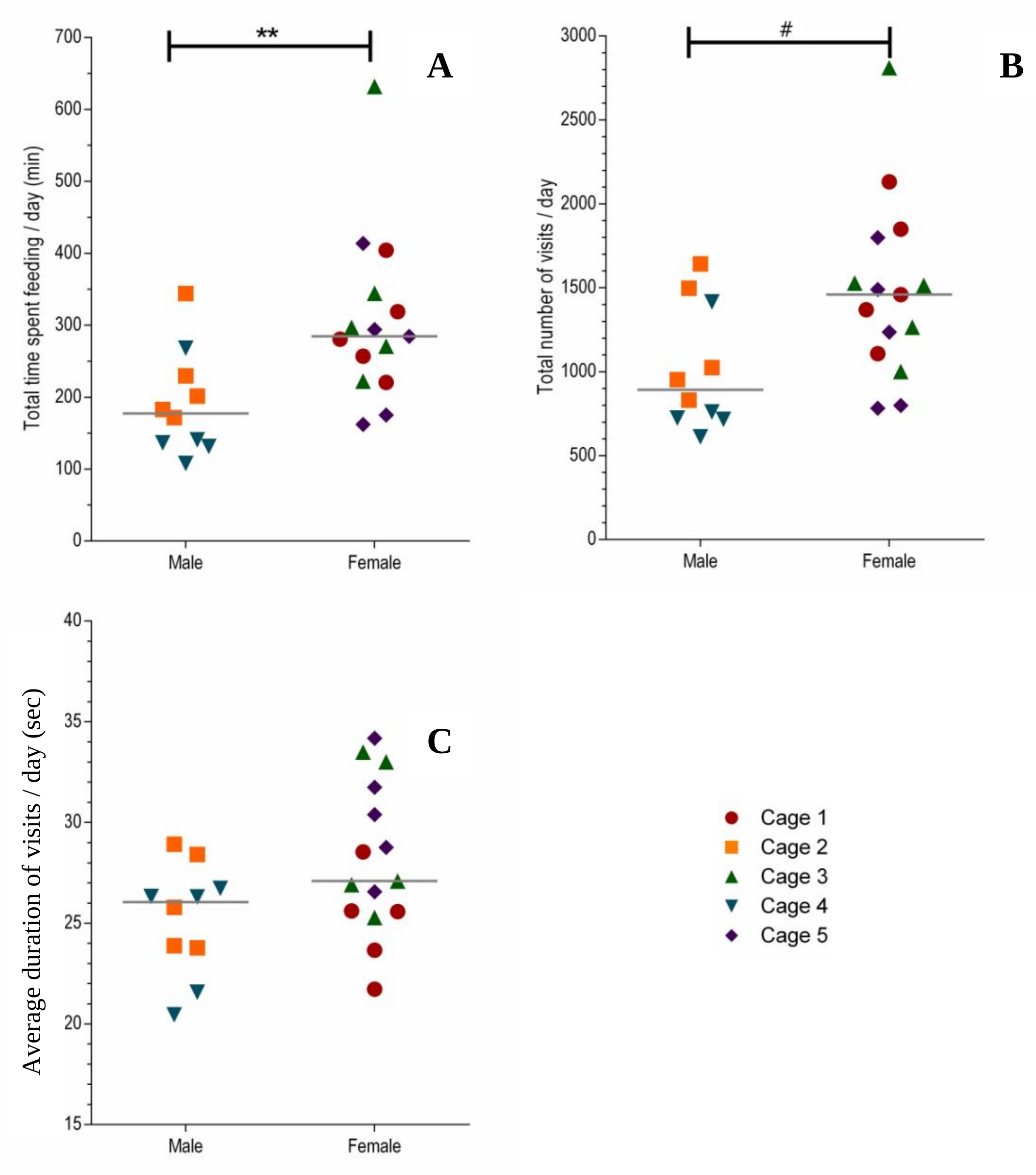
Supplementary figure 3 - Distribution of the total number of visits per day for each cage, throughout the five days. Each dot corresponds to the cumulative value of one day.



Supplementary figure 4 - Distribution of average duration of visits per day (in seconds) for each cage, throughout the five days. Each dot corresponds to the average value of one day for each cage.

Comparisons between males and females cages

Comparisons in the feeding behavior between genders (male and female) reveled statistical significant differences on the time spent feeding showing that females have higher feeding activity than males: time spent feeding - U=125, W=245; p=0,012; N=25; marginal differences on the number of visits - U=116, W=236; p=0,069; N=25; in the average duration of visits per hour no significant differences were found - U=107, W=227; p=0,24; N=25. P-values presented with Bonferroni correction (p-value multiplied by three) (Supplementary fig. 5).

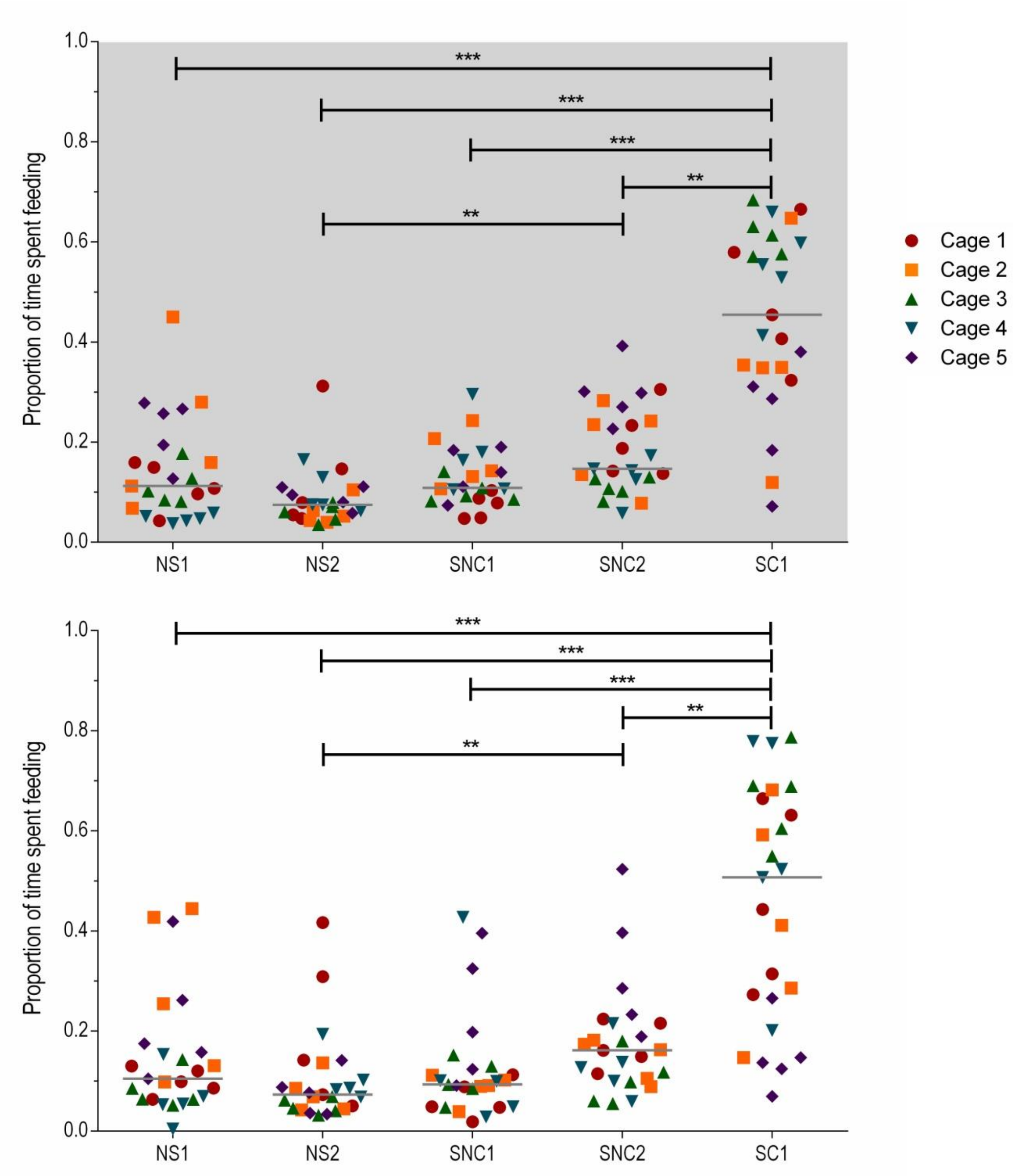


Supplementary figure 5 - A - Total time spent feeding per day (in minutes) for each cage, throughout the five days; B - total number of visits per day for each cage, throughout the five days; C - Average duration of visits per day (in seconds) for each cage, throughout the five days. Each dot corresponds to the value of one day for each cage.

Supplementary data 2

Comparisons between feeding sites

When comparing the time spent feeding on each individual feeding site instead of feeder types we can see a clear preference for the social competition feeder. Dark period - $X^2_{KW}(4)=59,617$; $p<0,001$; N=125. Light period - $X^2_{KW}(4)=47,641$; $p<0,001$; N=125 (Supplementary fig. 6).



Supplementary figure 6 - Proportion of time spent feeding in each feeding site for both dark (grey background) and light (white background) periods. NS1 & NS2 - Non-social feeders; SNC1 & SNC2 - Social no-competition feeders; SC1 - Social competition feeder.

Attachment 3: Previous dissertation project: A new task for the study of prosocial behavior

Methodology used in the study of prosocial behavior

Paradigms used in studies on prosocial behavior may vary, but usually consist of two animals (one focal and one recipient) separated, but with possibility for contact between them, where one animal (the focal) have a two-choice task, where one choice corresponds to a prosocial choice i.e. a choice that benefits both animals, and a selfish choice where the only one that beneficiates from that is himself (Brosnan et al., 2009; Burkart et al., 2007; Di Lascio et al., 2013).

Rats have been shown to be sensitive to the conspecifics food-seeking behavior. Márquez, Rennie, Costa, & Moita (2015) When studying prosocial behavior in rats that had to decide between two-choices, they demonstrated that rats significantly prefer the option where both rats are receive food rewards upon displays of food-seeking behavior by the conspecific.

In this work we attempt to develop a new prosocial task, that follows the common paradigms used in the field, it consists of an open box with two choice points where the focal animal (the one that can choose) press a lever and it can either be a prosocial choice i.e. a pellet of food drops for both animals or a selfish choice i.e. a pellet of food drops only for himself. Both choices present the same level of effort and the same outcome for the focal animal.

The aim of this project was to see if the focal animal modulates its behavior if the other (recipient) does not eat the reward that he made available. In this task we would be able to independently manipulate what the animal sees and smells and what the animal receives. For that goal we built a small feeding site with four compartments, two available for each animal. The outer compartments has grids that allow animals to smell and lick the reward (Supplementary fig. 9 - B), but the reward they receive is in the inner compartment (Supplementary fig. 9 - A). With this method we would be able train the recipient animals to display food-seeking behavior to a determinate type of food reward, stimulating the focal to choose that side and beneficiate both. Once that training was done, we would replace the reward of the inner compartment of the recipient animal by some type of aversive food that he wouldn't eat, and see if the focal is sensitive to the recipient eating or not the reward he made available by changes in its behavior.

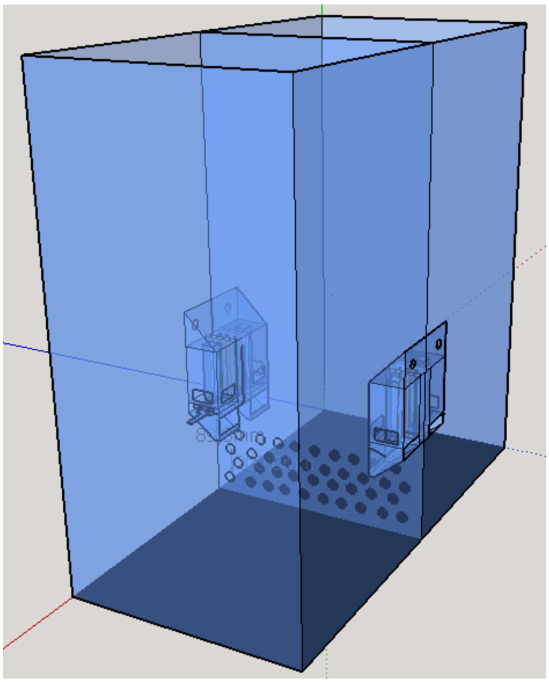
Methods & Results

Animals: Ten adult males Sprague-Dawley rats obtained from Charles-River, France and Germany, weighting between 400g-485g were used in the experiments.

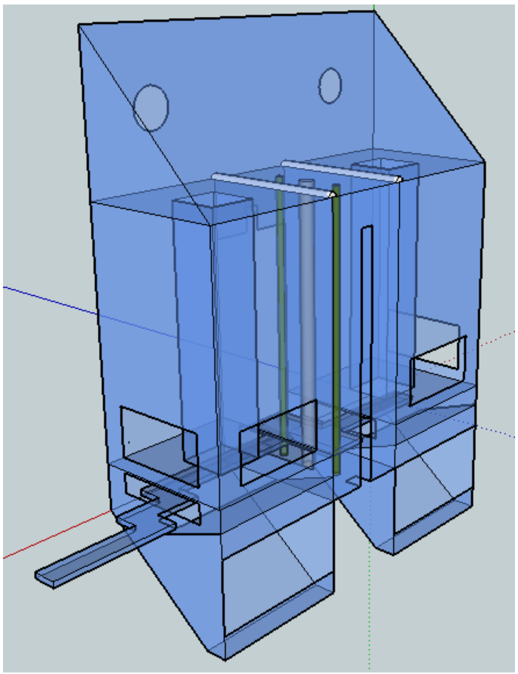
Five of those animals were trained to press a lever (described below), these ones had the power of decision - **focal rats**. The remaining five animals were trained to display active behavior to the side were it would get reward - **receiver rats.**

Experimental box: We designed a 20 cm x 40 cm x 40 cm box with a middle divider that has 1 cm diameter holes, allowing animals to interact with each other (i.e. smell, touch, ...) (Supplementary fig. 7).

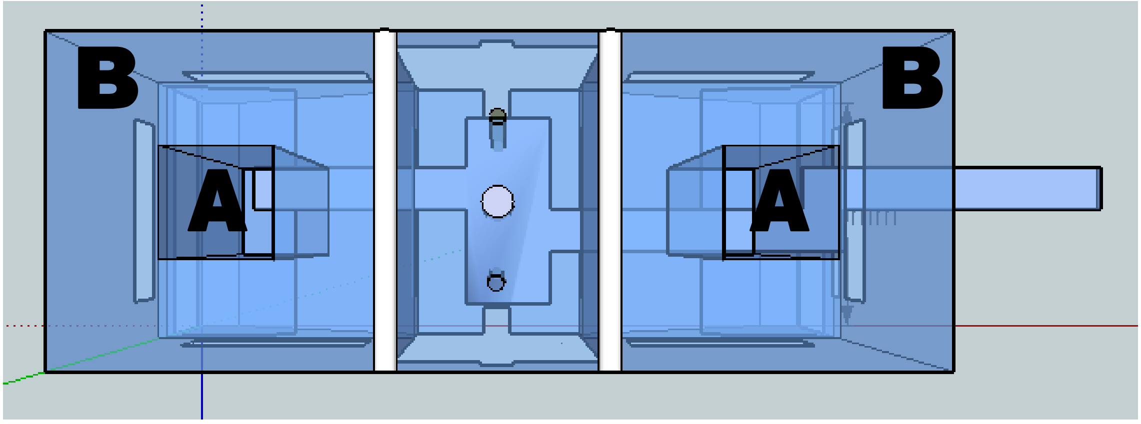
The divider has two small feeding boxes, each one with four compartments (two in each side of the divider) (Supplementary fig. 8). The outer compartment has grid windows that allow animals to smell and lick the reward (Bio-serv® 45mg sucrose pellets) without retrieving it. The inner compartments are sealed with a lever that when the animal pushes it releases a pellet to the feeding site of both sides (Supplementary fig. 9).



Supplementary figure 7 - 3D of the experimental box with the two small feeding boxes, one in each side.



Supplementary figure 8 - 3D model of the small feeding box.



Supplementary figure 9 - Top view of the small feeding box. A - inner compartment, B - Outer compartment.

Training box: We designed a 25 cm x 30 cm x 15 cm box that allows us to attach one feeder small box (described above).

First round of trainings: Three days before training started, the animals (four) were mildly food deprived and everyday fifteen pellets (Bio-serv® 45mg sucrose pellets) were placed in their home-cages for habituation.

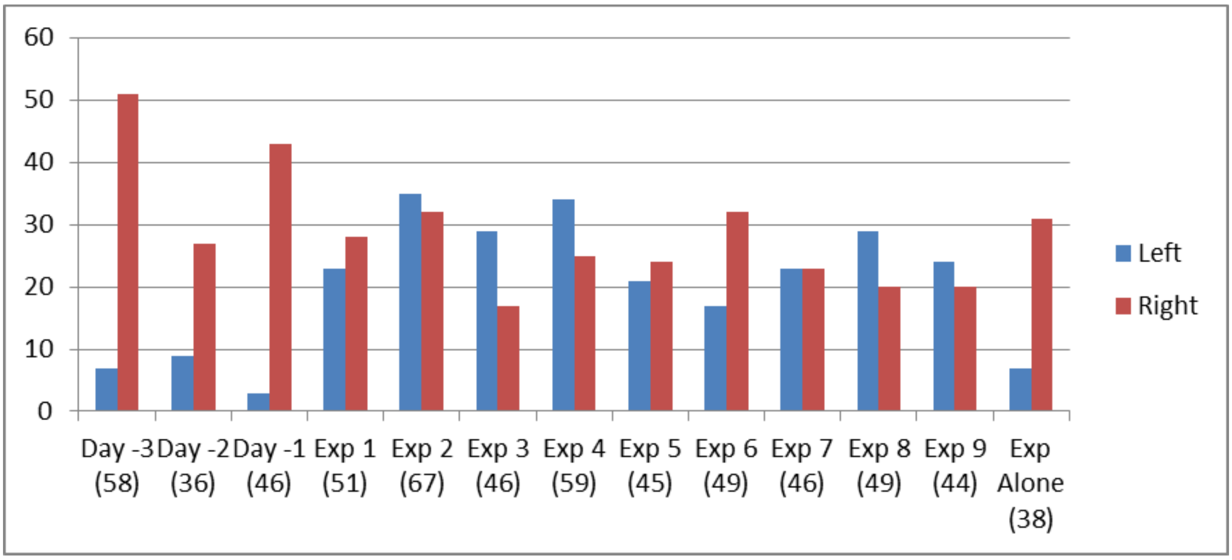
The animals were trained by placing one rat alone in the experimental box, in the focal side (the one with the levers), for thirty minutes, at the beginning three pellets were available (both in the left and right side). The lever was involved with a gaze soaked in sucrose 1%.

We considered the animal trained once he could reliably perform the task i.e. push the lever and eat without delay for consecutive times. After all four animals were trained, the cage with the best performing animals (cage 1) was used as focals and the other (cage 2) as receivers.

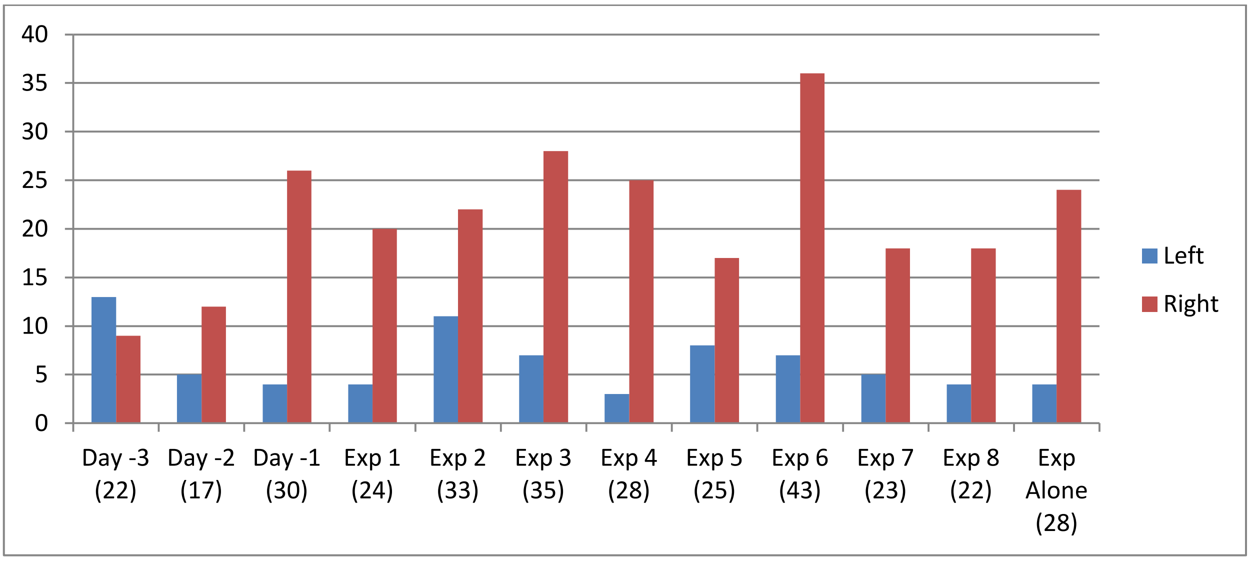
First round of experiments: The animals from the two cages were matched pair-wise in day one of experiments and remained with that pair throughout all the experimental sessions. Each session lasted for twenty minutes.

During the trainings both animals from cage 1 showed a strong bias to the right feeding site (animal 1 – left 14%, right 86%; animal 2 – left 32%, right 68%**)**, so we placed the prosocial choice (the one that beneficiates both animals, i.e. a pellet drops for both animals) in the left side.

Animal 1 actually changed its behavior pressing more on the left side when matched with a pair then when alone, and returning to its original bias when experimented alone on the final day (Supplementary fig. 10). Animal number two just kept its bias throughout all sessions (Supplementary fig. 11).



Supplementary figure 10 - Number of lever presses of animal 1 for each session, from three days prior to experiments, nine experimental sessions and a control session.



Supplementary figure 11 - Number of lever presses of animal 2 for each session, from experimental day two to nine and a control session.

Second round of trainings: Three days before training started, the animals (six) were mildly food deprived and everyday fifteen pellets (Bio-serv® 45mg sucrose pellets) were placed in their home-cages for habituation.

The animals were trained by placing one rat alone in the training box, for thirty minutes, at the beginning three pellets were available at the feeding site. The lever was involved with a gaze soaked in sucrose 1%. We considered the animal trained once he could reliably perform the task i.e. push the lever and eat without delay for consecutive times.

After all six animals were trained they were placed in the experimental box and noticed once again a strong bias in all animals, but now to the left side.

Comments

I started working in this project in September 2014, since then I developed the boxes and trained the animals. After the second round of trainings we observed the strong bias once again and from that point on I focused my efforts in trying to correct the bias by:

1. Changing the box placement and orientation to exclude outside clues that may underline this bias;

2. Removing food from the left side, forcing the animals to eat on the right one. But once food was again available on the left side, the preference was again restored;
3. If the animal systematically pressed the left side lever, I would introduce a mechanical block disrupting the behavior. The introduction of the mechanical block agitated the animals therefore their performance dropped;
4. We changed the lever mechanism to remove suspicions in the difference of the pressing resistance between both sides but then in June 2015, due to time limitations and unsuccessful attempts we decided to change project to the one presented in this dissertation.

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