Novel Competition test for food rewards reveals stable dominance status in rats

Diana F Costa (1), Marta A Moita (2) and Cristina Márquez* (1)

(1) Neural Circuits of Social Behavior Laboratory. Instituto de Neurociencias (CSIC-UMH), Alicante, Spain
(2) Behavioral Neuroscience Laboratory. Champalimaud Research. Champalimaud Centre for the Unknow, Lisbon, Portugal.

* Corresponding author: cmarquez@umh.es

ABSTRACT

Social hierarchy is a potent modulator of behavior in many species, including humans, that is typically established through overt agonistic interactions between individuals in the group. Once established, social ranks are maintained through subtler interactions allowing the redirection of energy away from agonistic interactions towards other needs. Most of the available tasks for assessing social rank in rodents allow the study of the mechanisms by which social hierarches are formed but fail to assess the maintenance of established hierarchies between stable pairs of animals, which might rely on distinct neurobiological mechanisms. Here we present and validate a novel trial-based dominancy assay, the modified Food Competition test, where established social hierarchies can be identified in the home cage of non-food deprived pairs of male rats. In this task, we introduce a small conflict in the home cage, where access to a new feeder containing palatable pellets can only be gained by one animal at a time. We found that this subtle conflict triggered asymmetric social interactions and resulted in higher consumption of food by one of the animals in the pair. To assess the reliability of the observed asymmetries as reflecting dominance relationships we investigated the behavior of same rat dyads in multiple social tasks adapted from social hierarchy studies. We found a positive correlation in dominance indices across most tests used. Our findings reveal stable dominance status in pair housed rats and provide a novel tool for the evaluation of established social hierarchies, the modified Food Competition test, that is robust and easy to implement, thus expanding the set of tasks available to study dominance in the lab.

INTRODUCTION

Integrative cross-species approaches are crucial in the understanding of the roots of social behavior and in the identification of the proximal and neural mechanisms of how we perceive, compute and react upon social information (Keysers and Gazzola, 2016; Schweinfurth, 2020). Norway rats, used as a model system amenable to monitoring, mapping and perturbation of neuronal circuits, live in complex social groups in the wild (Schweinfurth, 2020). This has motivated a wave of recent laboratory studies, that uncover the diversity and sophistication of rat's social skills (Knapska et al. 2006; Atsak et al. 2011; Knapska et al. 2010; Pereira et al. 2012; Cruz et al. 2020; Han et al. 2019; Hernandez-Lallement et al. 2020; Kashtelyan et al. 2014; Daniel 1942; Conde-Moro et al. 2019; Viana et al. 2010; Bartal, Decety, and Mason 2011; Ben-Ami Bartal et al. 2014; Márquez et al. 2015; Kentrop et al. 2020; Hillman and Bilkey 2012; Rutte and Taborsky 2007; 2008; Schuster and Perelberg 2004; Schneeberger, Dietz, and Taborsky 2012 see Schweinfurth 2020 for review), the neuronal basis of which are starting to be dissected (Hillman and Bilkey, 2012; Twining *et al.*, 2017; Carrillo *et al.*, 2019; Pereira, Farias and Moita, 2020).

Social hierarchy is a multidimensional trait that has a profound impact on emotion and cognition, not only for humans (Sapolsky, 2004) but also other social species (Schjelderup-Ebbe, 1922; Robert M. Sapolsky, 2005), having important consequences for social organization, survival, reproductive success, and health of animals in a group (Robert M Sapolsky, 2005). Adapting behavioral responses depending on the social status of the interacting partner can be not only cost-effective, but in some cases, a crucial survival strategy. Since the seminal observations of the peck-order effect in the early 20's (Schjelderup-Ebbe, 1922) describing reliable patterns of agonistic behaviors in fowls that consistently favored access to resources of one animal of the group, research on the ethology and neural basis of dominance has provided key insights on the regulation of this fascinating behavior.

The most established view is that social hierarchy is built upon aggressive interactions (see Drews 1993 for a conceptual review), and serve as a mechanism of resources management and minimization of energy expenditure by groups of animals; once a hierarchy is established, priority access to resources is organized allowing the reduction of aggressive levels between the interacting animals (Vessey, 1981). Following this view, most of the behavioral paradigms available for measuring social hierarchy in laboratory animals are based in the nature of agonistic interactions while defending access to resources, whether a sexual partner,food or water when they are scarce, or the defense of a territory (Cordero and Sandi, 2007; Timmer and Sandi, 2010;

Wang *et al.*, 2011; Wang, Kessels and Hu, 2014; Hollis *et al.*, 2015; Jupp *et al.*, 2016; Zhou *et al.*, 2017; Zhou, Sandi and Hu, 2018; Pallé *et al.*, 2019, 2020).

In rats, the visible burrow system (VBS) has been widely used to study formation of hierarchies in large groups of animals (Blanchard and Blanchard, 1989; Blanchard *et al.*, 1995). In this task, mixed-sex rat groups living in a complex environment where food and water are difficult to access compete chronically for territory and resources, facilitating the emergence and maintenance of social hierarchy, where body weight was a strong predictor of dominance. This system generates very rich behavioral data sets but is difficult to implement in most laboratories. Hence, other behavioral tasks are commonly used, where animals compete for food or water under deprivation states or for palatable rewards (e.g. Nutella) (Cordero and Sandi, 2007; Timmer and Sandi, 2010; Timmer *et al.*, 2011; Lozano-Montes *et al.*, 2019) in either neutral arenas or contexts that induce conflict between the pairs of animals. In these tests, social isolation of variable durations is performed prior to testing as a means to increase territoriality, favoring strong agonistic interactions during the establishment of new hierarchies. Therefore, these tasks evaluate how a new hierarchy is established between pairs of unfamiliar, frequently isolated animals, in neutral arenas to increase territoriality, where agonist behaviors performed to establish dominance are very evident. However, the establishment of social hierarchy might not rely on the same mechanisms as the expression of dominance when a hierarchy is already established. Recent reports in mice indicate that this is indeed the case (Pallé *et al.*, 2019, 2020).

To our knowledge, the study of the possible differences between de novo and already established social hierarchies in rats has been virtually unexplored. To this end new behavioral paradigms that evaluate social status of familiar animals living in stable dyads are needed. Preferably, the evaluation of the hierarchy would not imply manipulations that could alter internal state in a long-term manner and affect how social interactions of interest are displayed. This is a challenge, as once hierarchies are established agonistic behaviors are minimized and the opportunities to observe dominance interactions are reduced and likely subtle.

With this aim, we developed the modified Food Competition task, where a small conflict for the access to a discrete number of palatable pellets was introduced in the home cage of pairs of non-deprived cage mate rats. In order to validate this new tool, we performed a fine-grained quantification of the behavior of the animals during the task and compared them to those observed in other competitions for positive reinforcers (sucrose 1% solution) and to modifications of standard tests used in the field: food and water competition, both under deprivation, and the Tube Test. Although no aggressive interactions were observed in this task, our results indicate that

stable hierarchies in rats are indeed detectable by the modified Food Competition test. Moreover, these hierarchies are stable and identifiable using different parameters from the other tests used, indicating reliability. Finally, we provide data that indicates that body weight is not a predictor of stable hierarchies in non-deprived rats, except in the case of the Tube Test, where indeed bigger animals have a higher probability to win in the first encounters.

METHODS

Animals

40 adult male Sprague-Dawley rats (Charles-River, France) weighing 325-410 g at the beginning of the experiment were used. Upon arrival, rats were pair-housed and maintained with *ad libitum* access to food and water under a reversed light cycle (12 hours dark/light cycle; lights off at 10 AM) in controlled temperature conditions. Animals were left undisturbed in their home-cages for approximately three weeks, allowing rats to habituate to our Vivarium Facility and routines, and to reverse their circadian rhythm. After this period, animals were handled four times every other day during one week. Body weight was controlled weekly and prior to each testing session. Experiments were performed during the dark cycle, waiting at least 2 hours after the lights were off to start with behavioural procedures. Animal husbandry and all experimental procedures were performed following Portuguese Guidelines, which are in strict compliance with the European Directive 86/6097EEC of the European Council.

Experimental Procedures

Twenty pairs of animals were tested in the different behavioral paradigms (Sucrose Competition, Food Competition with and without food deprivation, Tube test and Water Competition, see below for description of each task) in order to identify social rank and study reliability of social status between them. Each pair of animals consisted of cage mates living in the same cage for 4 weeks before starting the behavioral procedures. The interacting animals were thus familiar and the same pairs were maintained throughout the entire duration of the experiment. In order to control for possible influences of the order of behavioral testing on the evaluation of social status, we divided the animals in two independent groups (n=10 pairs each) where the order of the tests that involved competition for positive reinforcers was counterbalanced. Those tests that required food or water deprivation, and thus were more stressful and/or could induce strong aggressive

behaviors, were performed at the end of the experiment. All pairs were tested in all the tasks with 2 to 4 days of interval between testing sessions.

At the beginning of the experiment, we randomly identified each rat of a cage as 'Animal A' and 'Animal B' and quantified their behaviour and consumption of the resources for each of the behavioral tests. Before each habituation or test session the fur of the animals was marked using a black pen in order to enable clear identification of each animal for post hoc video-annotation analysis. All the tasks, except for the Tube Test, were performed in the animal's home cage with small modifications to the lid to accommodate a customized feeder/water bottle.

Modified Food Competition test

Food competition for palatable pellets (Dustless Precision Pellets, 45mg, Rodent Purified Diet, Bio-Serv) was performed in the home-cage of non-food restricted pairs of animals. For this test, the home-cage lid was replaced by a modified laser-cut acrylic one that accommodated a fully transparent feeder (**Figure S1***)*. The feeder was designed so only one animal could access the food pellets at a time, promoting conflict and competition for the reward. Moreover, the feeder accommodated a sliding door that prevented access to food pellets during the inter-trial interval, and an opening on the top to facilitate delivery of food pellets in each trial with minor interference from the experimenter. This customized lid was used during habituation and test sessions.

Animals were exposed to palatable pellets in their home-cages during the handling period for four days in order to reduce neophobic responses to the food. Then, during three consecutive days, all the animals went through a habituation period to the modified lid, where they were allowed to explore and consume the pellets individually without competition, while the partner would be kept in a separate cage. Specifically, during habituation days, the new lid holding the feeder was placed on the home cage containing 10 palatable pellets. The sliding door was closed, preventing access to the pellets. Two minutes after, the door was opened, allowing the rat to access the pellets for 2 minutes, after which the door was closed again and 10 new pellets were placed. In total, the animal was given 4 minutes access to 20 pellets in a total session of 10 minutes. Next, food competition in a social context was performed for two consecutive days. Pairs of animals were re-marked, and the home-cage lid was replaced by the modified one with the feeder and 10 palatable pellets. 1 minute after, the sliding door was opened allowing the rats to have access to the pellets for 2 minutes, after which the door was closed again for a 1-minute inter-trial interval and 10 new pellets were delivered. We repeated this procedure for 5 trials and a total session of

15 minutes and 50 pellets. After the session, the customized lid was replaced by their home-cage lid.

Modified Food Competition with food deprivation

The modified Food Competition test was performed in the home cage of familiar animals as described above, but in this case animals underwent only one session of social competition after a 24h period of food deprivation. After the test, the modified lid was replaced by the standard one and rats were allowed to eat and drink *ad libitum* for the rest of the cycle.

Sucrose Competition

Pairs of non-water-restricted cagemates competed for access to a bottle containing 1% sucrose solution placed in a modified lid on their home-cages. The lid was designed so the bottle holders were prolonged with a transparent acrylic tube, in a manner that the tip of the bottle was surrounded by an extension that would allow the head of only one animal to drink at a time (**Figure S0B**). We performed three habituation sessions, where no competition was assessed as the modified lid was holding two bottles and each animal could access the reward independently. In the first habituation day, animals were exposed to the new lid for 20 min where no bottle was available. In the two following days, the new lid was holding two bottles of 1% sucrose solution and animals were given free access to the sucrose solution for 20 minutes. Then, animals were re-marked and tested for sucrose competition in two consecutive days for 10 minutes, where the modified lid presented only one bottle of sucrose this time.

Sucrose Competition with intermittent access to the resources

In order to decrease the time that sucrose solution was available and promote competition for the resource, we made a modification in our Sucrose Competition protocol and replaced the 10 minutes free access to 1% sucrose solution by an intermittent access schedule. One minute after the beginning of the session, a bottle with sucrose solution was placed in the dispenser allowing the animals to access the solution for 2 minutes. After this time, the bottle was removed for 1 minute and put back again for 2 more minutes, performing a total of 5 trials. The SCI was performed over two consecutive days. After each session, the customized lid was replaced by their home-cage lid.

Water Competition

Animals were water deprived for 24h and tested for competition for water in their home cage. At the moment of the test, the cage lid was replaced by a modified one where access to the bottle was only possible for one animal at a time. The duration of the test was 10 minutes, after which the standard lid was replaced and both rats had *ad libitum* water access.

Tube Test

The Tube Test is a widely used behavioural assay for identification of social status in mice (Lindzey, Winston and Manosevitz, 1961; Wang *et al.*, 2011) and we performed minor adaptations for the evaluation of established social rank between pairs of male rats. We used a transparent Plexiglas tube with 60 cm length and 8 cm diameter, a size that allows an adult rat to pass through without reversing its direction, and, when two rats are placed in the tube, prevents one rat from crossing the tube by passing the other. We performed one habituation session where animals were allowed to individually explore the apparatus, allowing spontaneous entering and crossing of the tube for 5 minutes, after which animals were returned to their home-cages. During test days, each pair of cagemates rats was simultaneously placed into opposite ends of the tube and met in the middle. At this time, a partition placed at the center of the tube was removed allowing the animals to interact with each other. The rat that first retreated from the tube was designated as the 'loser' and the other as the 'winner'. After each trial, both rats were placed back into their home-cages until the beginning of the next trial. From trial to trial, animals were released at either end of the tube alternately. We performed one testing session of the Tube Test with 10 trials at the beginning of the experiment and assessed stability of social rank within this test with another testing session at the end of the experiment. We quantified the amount of time that one of the animals took to push the other out of the tube and annotated the winner and loser of each interaction.

Video acquisition and Behavioral Quantification

Experiments were performed under the dark cycle of the animals and video recordings were obtained by a high resolution infra-red camera (PointGrey Flea3 -U3-13S2M CS, Canada) under infra-red illumination, capturing frames at 30Hz at 1280x960 pixel resolution. Supervised offline frame by frame video annotation of behaviors of interest was performed by a trained blind experimenter (DFC) after confirmation of highly reliable quantifications. For each animal of the dyad we quantified frequency, latency, and duration of (1) consumption of resources (number of

pellets eaten or time spent drinking water or sucrose); (2) exploration of the feeder (sniffing behavior inside and outside the feeders or bottle holders); (3) self-grooming; and (4) pushing the other animal to gain access to the resource. In those tests with a trial structure, behaviors were aligned to time 0, i.e. the moment where the sliding door that gave access to food pellets was opened for food competition tests with or without food deprivation, or when the bottle was placed in the modified lid in the case of the Sucrose Competition Intermittent, and behaviors were quantified 40 sec before and 80 seconds after this moment. Exploration of the feeder was measured during the whole session, and in those tests with a trial structure, we differentiated between exploration of feeder when reward was accessible and before that, when the sliding door was closed or there was no bottle there yet, as a proxy for anticipatory exploration. Pushing behavior was divided in two distinct categories depending on the outcome: Successful Pushing, if the animal managed to displace the partner from having access to the resource and Unsuccessful Pushing, when animals would attempt to get access to the resources but were unsuccessful to displace their partner from the reward area. In the Tube Test, we quantified the number of wins for each animal and the duration of each trial as a proxy for the time the animals used to solve the territorial conflict.

Bonsai (Lopes *et al.*, 2015) and Python Video Annotator (Ribeiro et al.,2011), both open source computer vision software available online, were used to perform behavioral quantification. First, digitally assigned behaviors were quantified with Bonsai, which created timestamps for the beginning and ending of each behavioral event. Then, the start and end of each behavioral bout was curated with frame-by-frame investigation using Python Video Annotator, which allows fine modification of the timeframes with subsecond resolution. Moreover, Python Video Annotator allowed easy post hoc categorisation of the two types of exploration (anticipatory or during the presence of the resource) and pushing behavior (successful or unsuccessful) which can only be identified once the bouts of pushing behaviour are finished and is thus not possible to analyse with online video analysis.

Statistical Analysis

Data was parsed and processed with Python programming language (Python Software Foundation, v.2.7). The statistical analysis was performed using IBM SPSS Statistics version 24.0 for Windows. The normality of the data was tested using Kolmogorov-Smirnov normality test, and when normality was not observed non-parametric tests were applied. Paired T-test were performed to study differences in the weights between dominant and submissive animals from each pair. One-Way ANOVA followed by post-hoc test Student-Newman-Keuls was performed to

compare behaviors of interest across tasks. When normality was not observed, a Kruskal-Wallis test with post-hoc Dunn-Bonferroni correction was performed. Wilcoxon signed-rank tests with Bonferroni correction were performed to study differences between contra balanced groups in each protocol and of each behavior across the tasks. Wilcoxon signed-rank tests were also performed to study differences in each quantified behavior between dominant and submissive animals. Bivariate Pearson Correlation was performed to measure the strength and direction of association between the Dominance Index of all the tasks and linear regressions for assessing predictive value of Food Competition and Tube Tests controlling for body weight. Statistical significance was set at $p<0.05$.

RESULTS

Characterization of behavioral profiles for the different social competition tests

To identify social status within pairs of cage mates we performed different behavioral tasks in which the animals needed to compete for resources. The resources were palatable pellets, sucrose solution, water or tube occupation. All the tasks, except for the Tube Test, were performed in the animals' home cage (**Figure 1A-E**; left panels show cartoons depicting the design for each behavioral test). Animals displayed different behavioral profiles depending on the configuration of the test, whether it had a trial structure, the amount of reward available, and their internal state (satiated vs deprived) (**Figure 1** and **Figure S2**). In order to control for possible effects of winning history we created two independent groups where we counterbalanced the order of the tests. No differences were observed between the groups, suggesting that hierarchy was already established. Data from both groups was thus merged for the rest of the analysis (Kruskal-Wallis test comparing the duration of consumption in the two counterbalanced groups : mFC Day1: $X^2(2)=0.026$, $p=0.871$; mFC Day2: $X^2(2)=0.007$, $p=0.935$; SC Day1: $X^2(2)=1.516$, $p=0.218$; SC Day2: $X^2(2)=1.904$, $p=0.168$; SCI Day1: $X^2(2)=0.457$, $p=0.499$; SCI Day2: $X^2(2)=2.055$, $p=0.152$; mFCD: $X^2(2)=0.00$, $p=0.989$; WC: $X^2(2)=0.293$, $p=0.589$; TT Day1: $X^2(2)=0.00$, $p=1.0$; TT Day2: $X^2(2)=0.00$, $p=1.0$).

In the Modified Food Competition task (**Figure 1A**), the limited number of available food pellets (10 per trial) led to a very fast consumption of resources which lasted a few seconds (**Figure S2B-C**). When considering the behavior of the pairs of animals they spent 52.3% of the time exploring the feeder, suggesting high expectation of reward. Moreover, animals displayed notable, although variable, amounts of pushing compared to other tasks (**Figure S2A**). On the contrary, during the Sucrose Competition task, although animals spent 10.9% of the time consuming the reward, they

explored the reward location significantly less compared to other tests and virtually never pushed their partners (**Figure 1 B** and **Figure S2A).** However, when access to the sucrose bottle was presented in an intermittent manner, time spent consuming and exploring the bottle location increased compared to when they were given 10 minutes *ad libitum* access (**Figure 1B-C** and **Figure S2A**). As the total time that animals were allowed to drink the sucrose solution was 10 min in both tests, this suggests that only modifying the pattern of presentation of the resource (intermittent compared to continued), made the animals increase their motivation to seek and gain access to the reward when available.

As expected, modulation of internal state (food or water deprivation) modified the behavior of the animals. In the Modified Food Competition task with deprivation (**Figure 1D**), animals consumed the pellets faster ($p<0.001$ compared to each of the two days of non-deprived Food Competition) (**Figure S2B**) and spent significantly more time investigating the feeder (**Figure S2A**), although the amount of pushing did not differ from that displayed in non-deprived animals in the modified Food Competition test (**Figure S2A**). When competing for access to water under deprivation (**Figure 1E**), animals dramatically increased the time they spent drinking to a median of 63.2% of the total time when behavior of both animals of a pair were considered, performing long bouts of drinking and alternating between animals (**Figure 1E**, middle panel for example pair). Levels of exploration of the water bottle were low compared to the rest of the tests (**Figure S2A**), suggesting that animals would mainly approach the water to reduce their thirst. Surprisingly, although the consumption time was high indicating a strong motivation to access the bottle after 24h deprivation, pushing levels did not increase proportionally (**Figure S2A**).

Finally, we conducted the Tube Test (**Figure 1F**) where the animals competed for the occupation of a tube. We performed two evaluations of the hierarchy in this test, one at the beginning of the experiment and one at end. Overall, the dyads established a very stable winner/loser relationship between them, where most of the animals that would start winning in the first trials would continue doing so over the rest. This winner/loser structure was maintained across both test days (**Figure 1F middle panel,** raster plot of winning history for all pairs). Interestingly, on the second day of testing, the loser partner of some pairs showed reluctance to enter in the tube. These incomplete trials (9% of the total trials), suggest a strong subordination towards the partner (**Figure 1F**, “resistant trials” white squares in the middle and right panel). We also quantified the time the dyad needed to solve the conflict, from the moment the partition at the center of the tube was removed until one of them was pushed out from the tube, with the idea that faster trials would indicate a stronger hierarchy. This trial duration was rapidly reduced after the first trial, reaching fast and stable latencies of around 3.5 seconds on average (**Figure 1F,** right panel).

Although we initially planned to quantify aggressive behaviours in all tests, there were virtually no episodes of agonistic behaviour in our conditions (boxing, biting, keep down, nor lateral threat) maybe as a reflection that hierarchy was already stablished. We only observed some bouts of aggressive interaction in three pairs of animals, in independent days and tasks (on pair in Sucrose Competition day 1, another pair in Sucrose Competition day 2, and a third different pair in the Water Competition.

Social hierarchy as priority access to resources

We categorized the animals of each pair as dominant (D) or submissive (S) according to the amount of resources they would consume within each test (pellets eaten in the Food Competition tests, time spent drinking in the Sucrose or Water Competition tests, and the number of wins in the case of the Tube Test). According to this criterion, animals categorized as dominant consumed significantly more resources than their partners in every task (**Figure 2A-E**) (Wilcoxon signed-ranks for consumption in mFC: $z=-3.92$, $p<0.0001$; SC: $z=-3.82$, $p<0.0001$; SCI: $z=-3.92$, $p<0.0001$; mFCD: $z=-3.83$, $p<0.0001$; WC: $z=-3.92$, $p<0.0001$). In the same line, one animal always won more encounters than the other in the Tube Test **(Figure 2F**, Wilcoxon signed-ranks for TT Day1: $z=-4.06$, $p<0.0001$ and for TT Day2: $z=-4.12$, $p<0.0001$), with the exception of one pair of animals in Day 1 and another in Day 2, where both animals of the pair won the same number of trials, thus no categorization as dominant or submissive was possible in this cases.

We then asked whether hierarchy following this criterion would also translate to differences in other parameters within each test. Grooming or general exploration of the resource location during the whole session did not differ between dominant or submissive animals when consumption in the same test was taken as criterion. However, we did observe that general pushing behavior was modulated in some tests. Dominant animals displayed more pushing in the Sucrose Competition with intermittent access ($z=-2.576$, $p=0.010$) and surprisingly, submissive animals were the ones that pushed more in the Sucrose Competition with continued access to the bottle and the Food Competition under deprivation (SC: $z=-2.548$, $p=0.011$ and FD $z=-3.40$, $p=0.001$).

Finally, we decided to investigate how body weight would relate to dominance in established hierarchies of rats. Intriguingly, we did not observe differences in the body weight between dominant and submissive animals in any of the behavioral tests where animals would compete for food, sucrose or water (**Figure 2 A-F**; Paired Sample T-test for weights in mFC: $t=1.208$, $p=0.242$; SC: $t=-0.309$, $p=0.761$; SCI: $t=-0.843$, $p=0.410$; mFCD: $t=0.522$, $p=0.608$; WC: $t=0.067$, $p=0.947$). In contrast when they had to compete for territory for the first time in the Tube Test, we observed a

significant relationship between dominance and body weight (**Figure 2F**; Paired Sample T-test for weights in TTDay1: t=2.529, p=0.021 and TTDay2: t=1.107, p=0.283). In conclusion, although no relation was observed between social hierarchy and body weight in the rest of the tasks, this was not the case in the Tube Test, where bigger rats had a higher probability of winning in the first encounters.

Social hierarchy as a stable trait between tests

To examine whether social hierarchy is a stable trait in familiar animals, we analyzed reliability between the performed tests. For this, we computed the *Dominance Index* for each test following this formula:

$$DI = \frac{Consumption\ of\ Animal\ A - Consumption\ of\ Animal\ B}{Total\ Consumption}$$

where consumption corresponded to the number of pellets in the Modified Food Competition tests, the duration of drinking in the Sucrose and Water Competition tests, and the number of wins displayed by an animal in the case of the Tube Test. A DI close to 0 would mean that animals did not have a strong hierarchy. Positive values indicate that animal A consumed more, while negative values indicate that animal B was the one having priority access to resources or won more trials in the Tube Test. DI for positive reinforcers led to a highly variable distribution, where some animals would strongly differ in their consumption and others where differences were subtle (**Figure 2G**). This was not the case in the Water Competition, where DI was mostly around 0 for all animals, indicating that both animals drank very similar amounts of water during the test. The Tube Test gave very polarized DI, where most of the pairs had one animal winning almost 100% of the trials. To refine our understanding on the hierarchy in the Tube Test, we computed a *Conflict Resolution Index (CRI)*, which would take into account not only who won a trial, but also how long it took for the conflict to be resolved (i.e., the latency for one of the animals to be pushed out of the tube):

$$CRI = \frac{DI}{Time\ to\ solve\ the\ conflict}$$

In this manner, variability increased, giving a more continuous indication of how strong the hierarchy was in each pair in this test (**Figure 2G** right panel).

If social hierarchy was a stable trait, then we should observe correlations between the DI of the different tests performed. Interestingly, the DI from tests with competition for positive reinforcers was positively correlated, and marginally correlated with the DI index in the Tube Test when conflict

resolution time was taken into account (**Figure 2H**). Water competition DI was not correlated with any of the other tests. We then hypothesized that our water deprivation protocol could have challenged the homeostasis of the interacting animals bringing them to a totally different internal state that would not correlate with the more physiological conditions of the other tests. However, it could be also possible that the measure we were using was not sensitive enough to provide significant correlations, as in 10 min animals would have ample time to decrease their thirst and satiate, as suggested by the DI close to zero, indicating that both animals drank for similar durations at the end of the session. In this direction, we wondered if conflict could be only detected in the first moments of the test. Therefore, we set out to perform a finer analysis of the behavior of the animals in this task, taking into account evolution of competition over time and information provided by other behavioral parameters simultaneously.

Dominant rats are more efficient displacing their partners to gain access to water

We identified the epochs with highest conflict in the Water Competition test, i.e., those where the most drinking and pushing behavior was observed for each pair of animals (**Figure S3**), and quantified the amount of pushing displayed by either dominant or subordinates. Surprisingly, subordinates spent more time pushing their partners compared to their dominants (**Figure 3A,** Wilcoxon signed-ranks: $z=-3.920$, $p<0.0001$). However, when we investigated how successful this pushing was in displacing the opponent to gain access to the water, we saw that 100% of the pushing displayed by the dominant was successful (**Figure 3B**, Wilcoxon signed-ranks: $z=-3.743$, $p<0.0001$) while the subordinates were trying to remove the partner from the resource area but without success (**Figure 3C**, Wilcoxon signed-ranks: $z=-3.920$, $p<0.0001$). This highlights the importance of considering multiple behaviors simultaneously, as although animals did not differ strongly in the amount of water consumed in the task, they did consume it in qualitatively different manners, suggesting that social status in the Water Competition task could be better assessed by finer behaviors rather than by pure consumption.

Modified Food Competition tests as a tool to measure stable hierarchies

Last, we calculated for each task a *Conflict Index* as a measure of the degree of conflict that our manipulations introduced in the home-cage. We calculated this index by dividing the time animals spent pushing in one test by the time spent consuming in the Sucrose and Water Competitions, and latency to eat all rewards in the modified Food Competitions. Food competition with and without deprivation were the tasks with higher conflict, as animals displayed high amounts of

pushing and the time available to eat the resources in each trial was very short, and this conflict index was highest when animals were food deprived (**Figure S4**). We selected the Modified Food Competition test as the criterion to classify rats as dominant or submissive, in order to validate this test as a new paradigm to identify established social hierarchies in pairs of rats, as considerable conflict index was observing without having to modify the internal state (deprivation) of the animals.

Animals were then classified as Dominant or Submissive according to their DI in this task (day 2), and those pairs where the DI ranged between -20 and +20 were considered to have an unstable or unclear hierarchy (n=5) and were not included in this last analysis. As expected, dominant animals consumed more pellets in the Food Competition test (**Figure 4A,** Wilcoxon signed-ranks of average Consumption in mFC of both days z=-3.409, p=0.001). They also successfully push their partner away from the feeder more (**Figure 4A,** Wilcoxon signed-ranks of Successful Pushing in mFC of both days z=-2.215, p=0.027) and tended to explore the feeder more during inter the trial interval, where the pellets were present but not accessible (**Figure 4A,** Wilcoxon signed-ranks of Anticipatory feeder exploration in mFC of both days z=-1.874, p=0.061). Moreover, dominant animals according to the Modified Food Competition did consumed more sucrose in both sucrose tests, and that effect was most apparent in those moments when animals actually drunk the most: the second day of Sucrose Competition (**Figure 4B**, Wilcoxon signed-ranks of Consumption in SC Day2 z=-2.760, p=0.006) and the first trial of the second day of intermittent competition (**Figure 4C**, Wilcoxon signed-ranks of Consumption in the First trial of SIC Day2 z=-2.499, p=0.012). The results of the Modified Food Competition under food deprivation replicated the same differences observed in non-deprived animals (**Figure 4D,** Wilcoxon signed-ranks of Consumption in mFCD z=-2.549, p=0.011; Successful Pushing in mFCD z=-2.613, p=0.009; Anticipatory feeder exploration in mFCD z=-1.988, p=0.047). Moreover, dominant animals also successfully pushed away their partner more in the Water Competition test (**Figure 4E** Wilcoxon signed-ranks of Successful Pushing in WC z=-2.158, p=0.031). The relation to the Tube Test was not direct, as the amount of wins in the Tube Test did not differ between dominant and submissive animals. We previously described that differences in body weight were affecting the probability of winning in the Tube Test, especially on the first day. Therefore, we decided to examine the relationship between the hierarchy in the Modified Food Competition and the Tube Test by controlling for the effect of the body weight. For this, we first regressed the amount of pellets eaten against the body weight of the animals at the time the test was performed and calculated the residuals. In the same manner, the residuals correcting for the body weight against the wins and duration from the first trial of the Tube Test

were calculated. Interestingly, the linear regression of these residuals was statistically significant (p=0.036) indicating that indeed, when correcting for the effect of body weight, consumption in the modified Food Competition predicts who will win in the first interaction of the Tube Test (**Figure 4F**). Thus, dominant animal in the Modified Food Competition test also won more trials in the Tube Test when the influence of the body weight was controlled

DISCUSSION

Here, we developed and validated the modified Food Competition task as a new tool to identify stable hierarchies in non-food deprived familiar dyads of rats. While most of the available tests for the evaluation of social hierarchy in rats are based on the identification of dominant animals during the establishment of a new hierarchy (Timmer and Sandi, 2010; Timmer *et al.*, 2011; Wang, Kessels and Hu, 2014; Hollis *et al.*, 2015; Zhou, Sandi and Hu, 2018) it is uncertain whether becoming the dominant in a first encounter will translate into keeping the same rank when the hierarchy becomes stable. In this direction, previous reports indicate that repeated encounters are needed for two animals to establish a stable social rank (Cordero and Sandi, 2007; Timmer and Sandi, 2010; Timmer *et al.*, 2011). The differences between the establishment of a hierarchy and its maintenance are starting to be explored, and the field would vastly benefit from new behavioral tools to address this fascinating question. Recent reports indicate that indeed these mechanisms are different in mice (Pallé *et al.*, 2019, 2020) and our new behavioral task opens the possibility for the study of such differences in rats.

In our task, cage mates with a stable hierarchy (with at least 4 weeks of cohabitation before the start of experimental procedures) competed in their home cages for access to positive reinforcers. The introduction of a small conflict in the home cage, where consumption of appetitive food is only possible for one animal at a time, led to subtle competition which translated into increased consumption by the dominant rat. This measure reliably predicted differences in other behavioral parameters observed in the different social tasks evaluated, such as competition for sucrose solution or food and water competition under deprivation. Importantly, testing was performed in the home cage of the animals, thus minimizing the influence of the experimenter in the social interactions displayed, and importantly, the interference of possible differences in other behavioral traits when assessing hierarchy, such as anxiety or explorative responses usually displayed in novel environments.

Interestingly, although competition was observed and hierarchy could be crystalized, no agonistic behaviors (biting, boxing, keep down, lateral threat) were observed between the cage mates in our task. This is in accordance with previous reports that indicate that once social hierarchies are established, the number of agonistic encounters decreases (Vessey, 1981). Moreover, the fact that no food deprivation was required, nor aggressive behaviors were observed, can be considered as an added value of our task. Aggressive behaviors induce robust stress reactions in the animals (Márquez *et al.*, 2013), and food deprivation, although widely used in neuroscience to increase motivational salience during behavioral testing, modifies internal state (Kennedy and Shapiro, 2009) and social behavior (Chen and Hong, 2018; Reppucci *et al.*, 2020). Minimizing the possible long term effects of these manipulations on the internal state of the animals was an important factor that we seriously considered while designing the present behavioral task, as they could strongly affect later social interactions while assessing the modulatory power of social hierarchy on other behavioral paradigms or brain function.

The fine behavioral characterization across the different social tasks used, allowed us to identify very interesting social patterns, that to our knowledge have not been reported before. In this direction, we describe that social hierarchy tasks with a trial structure, where access to resources was presented in a repeated and intermittent manner, promote competition. This was the structure in the Modified Food Competition Task, and was confirmed by results in the competition for sucrose solution, where animals increased their drinking time and exploration of the location of the sucrose bottle, when it was presented in an intermittent manner. Importantly, the total amount of time that animals could drink in both tests (continued vs intermittent tasks) was the same, thus differences in behavioral profiles were related to the trial structure. Regarding the Sucrose Competition Tests, although animals did drink from the 1% sucrose solution, conflict was very low, as measured by the amount of pushing or exploration of the bottle holder. It is probable that increasing the rewarding properties of the solution (using 10% sucrose) could promote competition between the animals. Whether titering the reward value of the resources affects the degree of competition needs to be assessed in the future.

Although the magnitude of social hierarchy between pairs could be nicely observed in the dominance index of tasks that involved competition for positive reinforcers, this was not the case in the Water Competition test under deprivation, where the animals drank around the same amount of water (dominance index around 0), nor in the case of the Tube Test, where very polarized results were observed. In this last case, we showed that taking into account multilayered behavioral measures, such as not only who wins a trial, but also, how long the pair of animals

takes to solve the conflict (latency for one of the animals to be pushed out of the tube) revealed a more granular view of the strength of social hierarchy in this test, which then tended to correlate to the social hierarchy observed in the modified Food competition test. When taking into account the body weight of the animals, this relationship was significant, as shown by the regression of the number of pellets eaten in the modified Food Competition test and the how an animal would win in the first encounter in the Tube Test when regressing out body weight. Body weight has been largely assumed to be a good indicator of social hierarchy in rats (Macdonald, Berdoy and Smith, 1995), although this has not been replicated for mice (Wang, Kessels and Hu, 2014; Pallé *et al.*, 2020). This view was inspired by classic works (Hoyenga and Rowe, 1969) and the seminal contributions in the field upon the development of the visible burrow system (Blanchard and Blanchard, 1989; Blanchard *et al.*, 1995). However, our data did not support this observation, as dominant and submissive animals did not show differences in body weight, with the exception of the Tube Test. It is important to take into account the difference in body weight between the animals within a pair in our experiment was lower than 10%, as they were age-matched. It is possible that larger body mass differences would indeed influence social rank in these tests, as previously shown in mice (Bartos and Brain, 1994; Kim *et al.*, 2015) and rats (Macdonald, Berdoy and Smith, 1995). In our conditions, only the Tube Test results were affected by body weight, where bigger animals had indeed more chances to win in the first encounters in the tube, probably indicating that in rats, as opposed to mice (Zhou *et al.*, 2017) body mass does affect the pushing behavior and the output in this task. On the other hand, the Tube Test has also recently started to be used in rats to identify hierarchy in large groups or unfamiliar animals, with conflicting results (Jupp *et al.*, 2016; Cao *et al.*, 2017; Saxena *et al.*, 2018). Ecological differences between species should be taken into account when studying social behavior, as rats and mice clearly differ in their social structure in the wild, where rats live in larger groups and better tolerate the presence of other in-group males, compared to mice (Schweinfurth, 2020).

Surprisingly, we did not observe differences in hierarchy-related consumption in the Water Competition task. Here, animals tended to perform long bouts of drinking, alternating consumption until both animals were satiated, which gave very similar final consumption levels in both animals. We asked whether a finer analysis of the temporal dynamics in this task would reveal important bouts of conflicts where correlations with other social competition tests would be observed, however, to the extent that we could quantify, we did not observe any pattern. Strikingly, we found that although consumption between animals was very similar, the qualitative behaviors that they displayed while accessing the water bottle were clearly different. Although submissive animals in

this task spent more time pushing their cage mate, they were rarely successful to access the water if the dominant was already drinking. However, when the inverse situation was observed, dominant animals successfully displaced their submissive practically every time. Indeed, the ability to successfully displace their partners from the resource location was not limited to the Water Competition, but was reliable among those tests where more conflict (pushing) was observed. These results thus confirm the necessity of including multilayered analysis of behavior and the importance of taking into account qualitative measurements when describing social interactions.

Very little is known of how social status affects different aspects of an individual's cognitive functioning upon different social interactions. Intense efforts in the last years have highlighted the Norway rat as a very interesting animal model to identify the proximate mechanisms and neural circuits of highly sophisticated social function. (Knapska *et al.*, 2006, 2010; Atsak *et al.*, 2011; Pereira *et al.*, 2012; Cruz *et al.*, 2020; Han *et al.*, 2019; Hernandez-Lallement *et al.*, 2020; Kashtelyan *et al.*, 2014; Daniel, 1942; Conde-Moro *et al.*, 2019; Schuster and Perelberg, 2004; Rutte and Taborsky, 2007, 2008; Schneeberger, Dietz and Taborsky, 2012; Viana *et al.*, 2010; Bartal, Decety and Mason, 2011; Ben-Ami Bartal *et al.*, 2014; Márquez *et al.*, 2015; Kentrop *et al.*, 2020; Hillman and Bilkey, 2012). It is conceivable that each of these behaviors is modulated in some way by hierarchy (subtly or not), and therefore a more complete understanding of rat social behavior and its underlying neural mechanisms should consistently take hierarchy into account. Our modified Food Competition task provides a simple, robust, and unintrusive means of assessing established social hierarchy that can be readily incorporated into many future studies.

In summary, here we provide and validate a novel trial-based dominancy assay to be performed in the home cage of familiar non-deprived rats, the modified Food Competition test, which is easy to adapt and implement in any behavioral laboratory. Our work adds on recent efforts to provide ecologically relevant paradigms (Puścian *et al.*, 2016), where extremely rich information of the social interactions of rodents can be obtained with minimal intervention of the experimenters.

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FIGURE LEGENDS

Figure 1 – Behavioral profiles change according to the specifics of each social competition test. (**A**) modified Food Competition test, (**B**) Sucrose Competition with continuous access to the bottle, (**C)** Sucrose Competition with intermittent access to the solution, where animals could only drink during 2 minutes and the bottle was absent during 1 min inter-trial interval; (**D**) modified Food Competition test with deprived animals, (**E)** Water Competition test, (**F)**: Tube test. For all tests, **left panel** shows the schematic representation of the task. Cartoons with a shaded circle as background indicate that tests were performed under deprivation. **A-F middle panel**: raster plots showing frequency and duration of behaviours of interest in an example pair of animals (animal A in blue, animal B in grey), except for **F** where the animal wining each trial is depicted for the 20 pairs of animals in all trials. White coloured trials in the Tube Test correspond to "resistant trials" where the looser of the pair resisted to enter the tube and trial could not be completed. In those tasks with a trial structure, (**A**, **C**, **D**) grey shaded areas indicate the moments where access to the resources where available, while intertrial interval during which animals could explore the feeders, but not access the food or sucrose bottle are indicated with white background. **A-E right panel**: Boxplot representation of the duration of each behaviour of interest for each individual animal. Median, quartile 1 and 3 are represented, whiskers indicate minimum and maximum values and extreme values are signalled as a dot. Consumption in red, pushing in dark green, Feeder Exploration in light green, Grooming in yellow. **F right**: Trial duration in the Tube Tests in the two testing days. Black line represents the median duration that animals took to push their partner out of the tube, and grey shadows depicts 95% confidence interval. As an insert in each day, pie charts represents the percentage of trials that were completed. Note that 9% of trials in the second day of the Tube Tests were not completed as one animal (the looser) refused to enter the tube.

Figure 2: Categorization of animals as dominant and submissive according to behaviour within each task. (**A-E)** Behavioural profiles when consumption of the resources within each test is used to define dominant and submissive animals. For all panels, a schematic cartoon with experimental design is provided. Cartoons with shaded background indicate that tests were performed under deprivation. For each test, differences between dominant and submissive animals regarding body weight, time spent consuming the resource, duration of pushing, exploration of the feeder and grooming is provided, where median and 95% interval confidence

is represented and individual values are showed with light lines. Colour coded raster plots of behaviors of interests display raw data in a testing session, where pairs of animals are sorted according to the stronger differences in hierarchy for each test. When more than one day of testing was performed, data represents average of the two days and rasters display data of day 2. In those cases were the animals of one pair had identical values in the categorizing criteria (same amount of pellets eaten, duration of consumption or trials won in the Tube Test), no hierarchy was assumed for this specific task, and these pairs were removed from this analysis (one pair for the Sucrose Test, one for the Tube Test and one for the Food Competition under deprivation). For all graphs and rasters, consumption is represented in red, pushing in dark green, feeder exploration in light green and Grooming in yellow. Time of consumption in each test was significantly different between animals defined as dominant or submissive, and in some cases, these differences also were translated to a differential amount of pushing behaviour. Interestingly, no differences in body weight were found in these tests, indicating that priority access to resources in stablished hierarchies is not influenced by the size of the animals. (**F)** In the Tube Test, the amount of winnings was clearly different between dominant and submissive rats, and was related to differences in body weight, which reached significance in the first days of testing, being bigger animals those more likely to win. (**G)** Dominance index (DI) based in consumption of resources for each behavioural test is represented being each pair of animals colour coded. Competition for positive reinforcers produced highly variable DI indicating detectable differences in the strength of social hierarchy between the pairs. Water Competition, however produced DI close to 0 indicating that the animals of most of the dyads drank very similar amounts during this test. The Tube Test produced a very polarized distribution of DI, where one animal of each pair would win most of the trials. These differences in hierarchy became less polarized when taking into account the time the animals took to solve the conflict (DI in the Tube Test /latency to finish the trial). (**H)** Correlation matrix between DI from all tests. Competition for positive reinforcers DI were correlated between tests, and these correlations were stronger when animals were not food deprived. Hierarchy in these tests tended to be in the same direction as the one measured with the Tube Test, when conflict resolution time was taken into account. However, no significant correlations were observed with the Water Competition tests. #$p<0.10$, *$p<0.05$, **$p<0.01$, *** $p<0.001$

Figure 3: Dominant animals are more successful in displacing their subordinate partners to gain access to water. (**A)** Surprisingly, when classifying animals according to their consumption in the Water Competition test, submissive animals spent more time pushing, in the moments with higher conflict, defined as the bout where intense drinking was displayed and high levels of pushing behaviour were observed. However, when we studied the outcome of these episodes, (**B**) dominant animals were almost always successful to displace their partners in every bout of pushing and (**C**) virtually all the unsuccessful pushing epochs, i.e. those that did not manage to remove the partner from the water access, were performed by submissive animals. In each graph, bars represent the median and dispersion can be observed by individual values of each pair. D: dominant, S: submissive according to the consumption in the Water Competition tests. *** $p<0.001$

Figure 4: Food competition in the home cage is a simple and reliable measure of stablished social hierarchies in rats. Animals displaying differences in the amount of pellets eaten in the modified Food Competition test were classified as dominant or submissive animals, and their behavioural profiles studied in the rest of the tests. (**A**) When considering average behaviors of both testing days in the modified Food Competition test, dominant animals significantly ate more pellets, were more successful to displace their partners from the food magasin during the competition and tended to explored more the location of the resource when access was still prevented. Moreover, they (**B**) drank more in the Sucrose Competition test, and (**C**) in the first trial of the intermittent Sucrose Competition test, where higher consumption levels were observed. (**D**) Similar behavioural profiles were displayed in the Food Competition under food-deprivation, where differences in consumption, successful pushing and anticipatory exploration were also observed. (**E**) Dominant animals according to the modified Food Competition test, were also more successful to displace their submissive in the Water Competition test, indicating that although no differences in the amount of water drank were observed, the quality of the interaction was significantly different. (**F**) The amount of pellets eaten in the modified Food Competition test significantly predicted the probability of wining in the first interaction in the Tube Test, when latency to win the trial was taken into account, and values were regressed out of the influence of body weight. Dashed line around panel A indicates that consumption in the modified Food Competition test was the criteria to evaluate differences in all the rest of the tasks. Median, 95% CI and individual values for all animals are represented. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Supplementary Figure 1 – Design and measurements of the behavioral tests used for identification of established dominance status. (A) Schematic illustration of the transparent lid and feeder in the modified Food Competition apparatus. **(B)** Detailed schematic illustration of the feeder used in Food Competition protocols (with and without deprivation) including the measurements to fit the bottom part of a Rat IVC cage (Sealsafe PLUS Green Line ventilated cages, Techniplast). The sliding door can be opened leaving a 5 cm high access which only allows one animal to eat at a time, and allows a trial structure for the task. A small opening on the top of the feeder allows to refill new pellets during inter trial interval. If adaptation of measurements to another type of home cage is needed, we advise to leave 3-4 cm from the end of the feeder and the bottom of the home cage. This prevents bedding to go into the feeder, which difficults the visibility of the pellets while animals are consuming. **(C)** Two cage mates can be observed at the feeder area, where one is consuming the pellets while the other is pushing to get access to the food. **(D)** Schematic illustration of the lid and bottle holder for the Water and Sucrose Competitions protocols (with continuous or intermittent access) protocols **(E)** Detailed schematic illustration of the bottle holder. A 5x5 cm restraining tube around the lick spout was created to prevent simultaneous access of both animals to the resource. **(F)** Two rats behaving in the Water Competition task, where one of the rats is drinking while the other is pushing to have access to the bottle **(G)** Schematic of the Tube Test with measurements used in this task, the transparent partition in the middle of the tube is removed at the beginning of a trial once both animals reach this area. Laser-cut acrylic holders were used to give stability to the set up **(H)** Two rats interacting inside the tube during the initial moments of a trial.

Supplementary Figure 2: Descriptives of the behavioural analysis for all social tasks involving competition for resources (**A**) Comparison of the behaviors of interest displayed by each animal across all behavioural tests. Boxplots depict the median and quartile 1 and 3. whiskers indicate minimum and maximum values and extreme values are signalled as a dot. Consumption in red, pushing in dark green, Feeder Exploration in light green, Grooming in yellow. Letters denote statistically significant differences between behavioral tests after one-way ANOVA with SNK posthoc comparisons. Time spent grooming was not normally distributed, thus non-parametric analysis was performed. (**B**) Latency to eat all pellets for each trial is shown in the Food Competition tests, with or without deprivation where median and 95% CI for each trial are represented. Animals only took around 20 seconds to eat all pellets available, decreasing this latency when food deprived. Shaded background indicates session performed under food-

deprivation. (**C**) Boxplots representing the average time to eat all pellets over testing days. Letters denote statistically significant differences between behavioural tests.

Supplementary Figure 3: Consumption in the Water Competition test does not correlate with Dominance Index (DI) in any other test. (**A**) No significant correlation was observed between Dominance Indexes (DI) of Water Competition when calculated with the drinking duration in the whole session, nor in the first min or the two first minutes, nor when taking into account the moment when animals started drinking. We then identified the epochs with longest drinking, as they could be not necessarily in the early moments of the test, but no correlation was observed with other behavioural tasks either. We then selected the epochs with higher conflict (see B for representative examples of the identification of the epochs), but again, no correlation was observed. Values in the correlation matrix correspond to Pearson correlation r values. (**B**) Graphical representation of dynamics of consumption (red, left axis) and pushing behaviors (green, right axis) and selection of the bout with highest conflict (shaded red rectangle) in three example pairs of animals. Drinking and pushing raw data was transformed into modified cumulative plots and smoothed using convolution with a Gaussian filter of 30ms standard deviation. In this plots the direction of the cumulative graph indicated which animal was drinking or pushing over the session, either animal A or B. In this way, increases in this cumulative plot would indicate that animal A would be drinking, decreases that animal B would drink, and flat stable lines that no animal was drinking. The same applied for pushing data (in green). For example, for the pair in the left panel animal B would start drinking while animal A was pushing for around 100 seconds, then they would alternate for a brief period of time, followed by another alternation, where a long bout of drinking was performed again by animal B while animal A continued pushing. After that, no significant pushing was performed by any of the animals, and although some alternations in drinking would be observed, now animal A would take over and drink more. In the different example graphs we can observe that dynamics between pairs are different over time but that animals mostly alternate in their drinking times, and pushing behaviour decreases around half of the session. In order to select the bouts of highest conflict, we first defined epochs of consumption and pushing displayed by the pair by identifying the turning points that marks the moments when significant changes in the behaviour occurs ($x[n+1] - x[n] < 0$). For each epoch we calculated the duration of both consumption and pushing behaviours and selected the epoch with the highest value of both consumption and time pushing the partner from the water dispenser.

Supplementary Figure 4: Food Competition tests reflect higher conflict. We calculate a conflict index for each test that involved consumption of resources by dividing the duration of pushing performed by the time the spent consuming (in the case of the Food Competition tests, we considered the latency to eat the 10 pellets available each trial). Due to the short duration of pellet availability and high pushing levels observed in the Food Competition tests, which was more marked when animals were under deprivation. Average and SEM are represented, and letters denote statistically significant differences between behavioural tests after one-way ANOVA with SNK posthoc comparisons.

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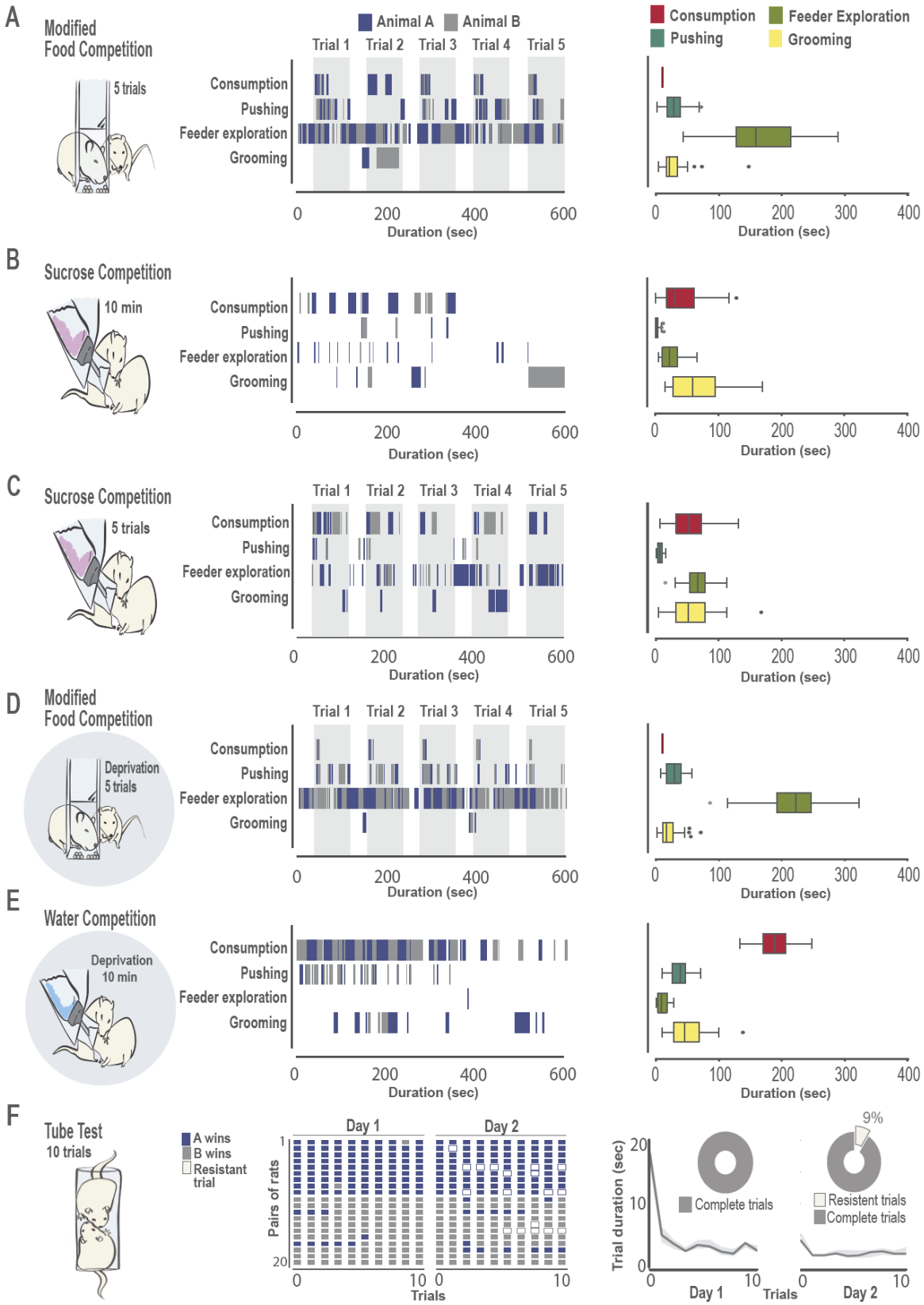

A
Modified Food Competition
5 trials
Animal A
Animal B
Trial 1
Trial 2
Trial 3
Trial 4
Trial 5
Consumption
Pushing
Feeder exploration
Grooming
Duration (sec)
Consumption
Feeder Exploration
Pushing
Grooming
B
Sucrose Competition
10 min
C
Sucrose Competition
5 trials
D
Modified Food Competition
Deprivation 5 trials
E
Water Competition
Deprivation 10 min
F
Tube Test
10 trials
A wins
B wins
Resistant trial
Day 1
Day 2
Pairs of rats
Trials
Trial duration (sec)
Complete trials
9%
Resistent trials
Complete trials

Figure 1

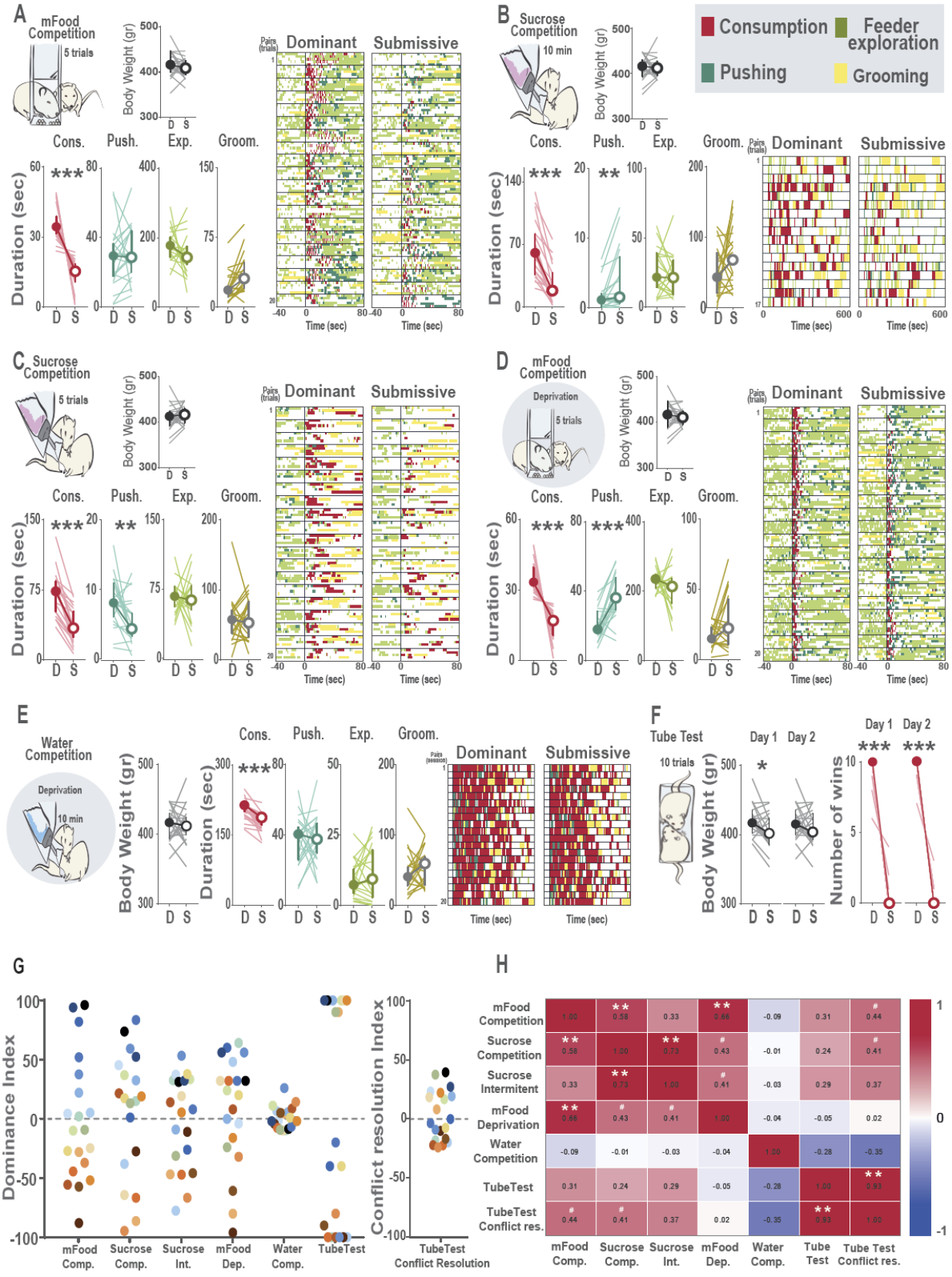

A
mFood Competition
5 trials
Body Weight (gr)
Cons.
Push.
Exp.
Groom.
Duration (sec)
Dominant
Submissive
Time (sec)
B
Sucrose Competition
10 min
Consumption
Feeder exploration
Pushing
Grooming
C
Sucrose Competition
5 trials
D
mFood Competition
Deprivation
5 trials
E
Water Competition
Deprivation
10 min
F
Tube Test
10 trials
Day 1
Day 2
Number of wins
G
Dominance Index
mFood Comp.
Sucrose Comp.
Sucrose Int.
mFood Dep.
Water Comp.
TubeTest
Conflict resolution Index
TubeTest Conflict Resolution
H
mFood Competition
Sucrose Competition
Sucrose Intermitent
mFood Deprivation
Water Competition
TubeTest
TubeTest Conflict res.
Tube Test

Figure 2

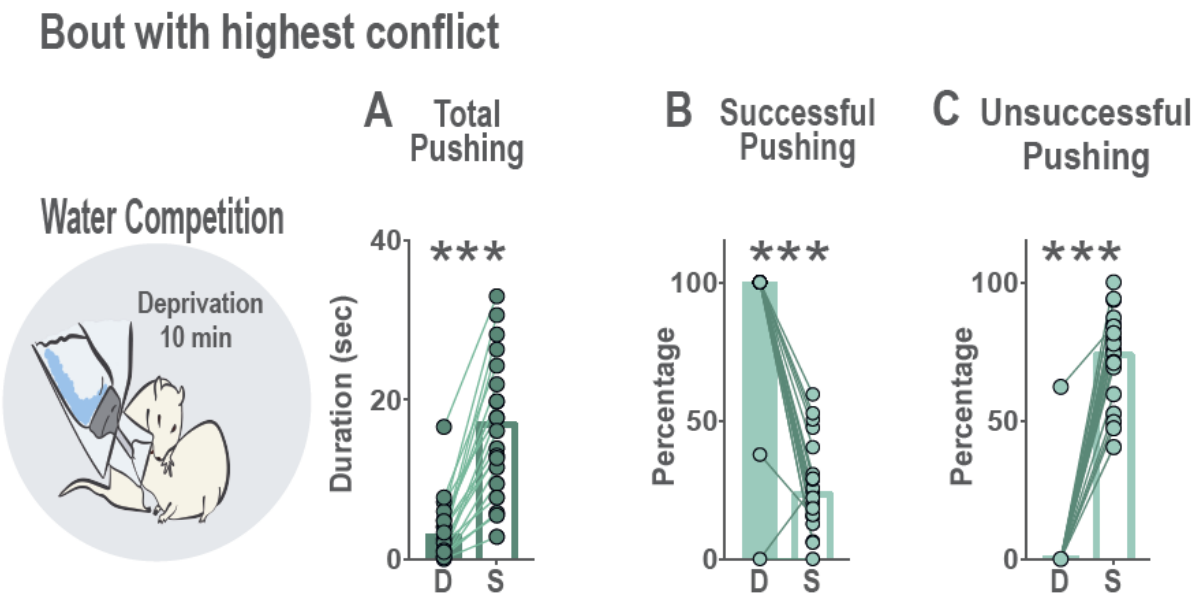

Bout with highest conflict
Water Competition
Deprivation
10 min
A Total Pushing
B Successful Pushing
C Unsuccessful Pushing
Duration (sec)
Percentage
Percentage
***
***
***
40
20
0
100
50
0
100
50
0
D
S
D
S
D
S

Figure 3

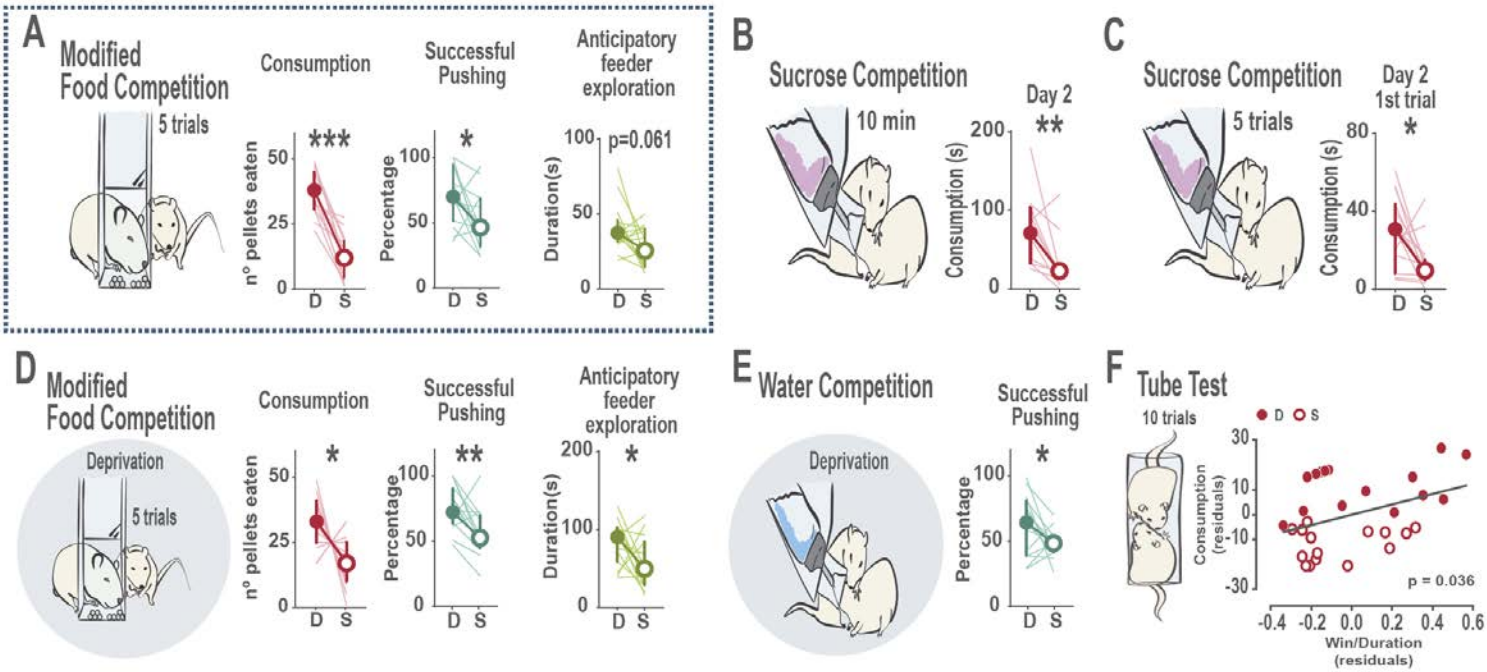
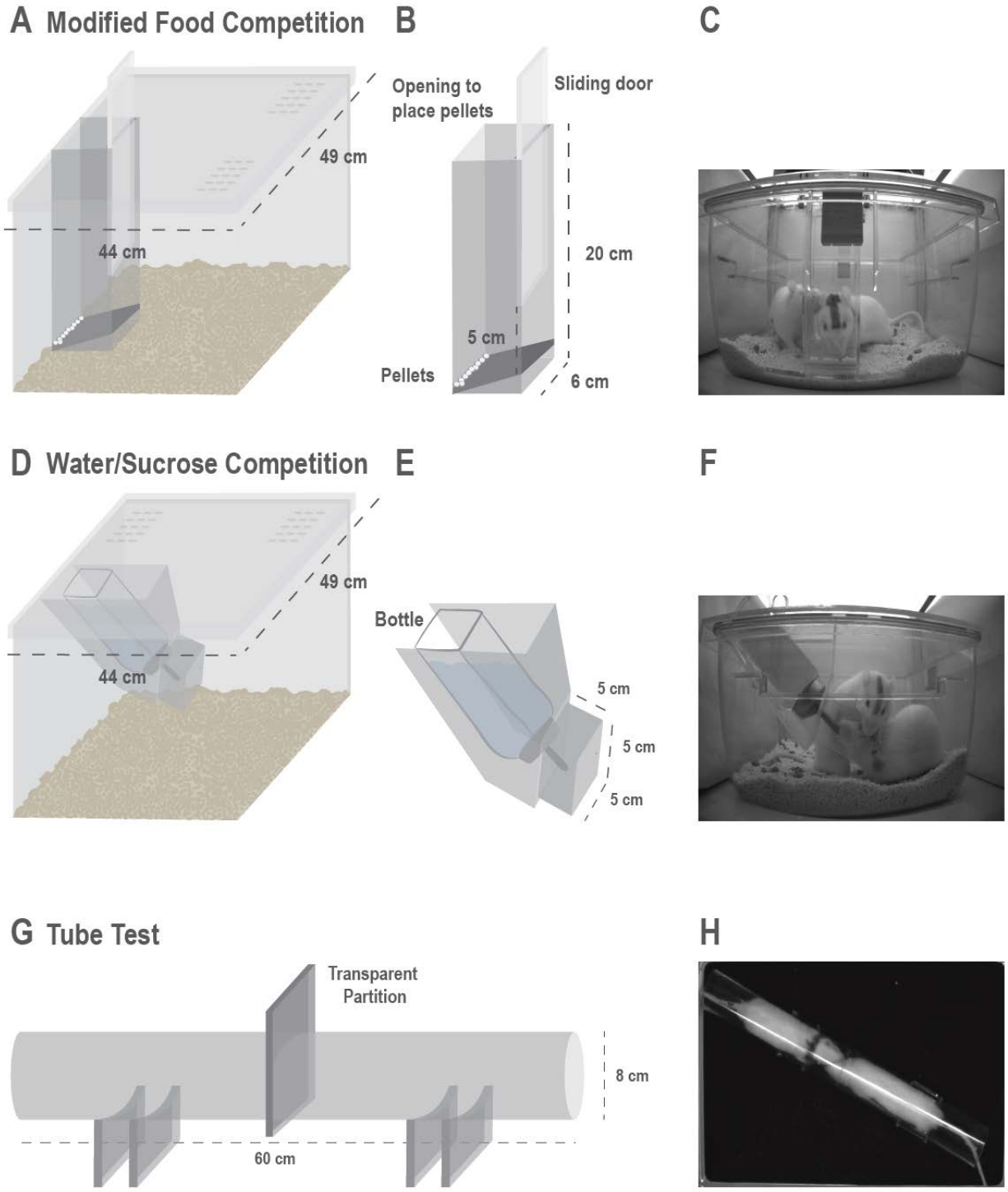
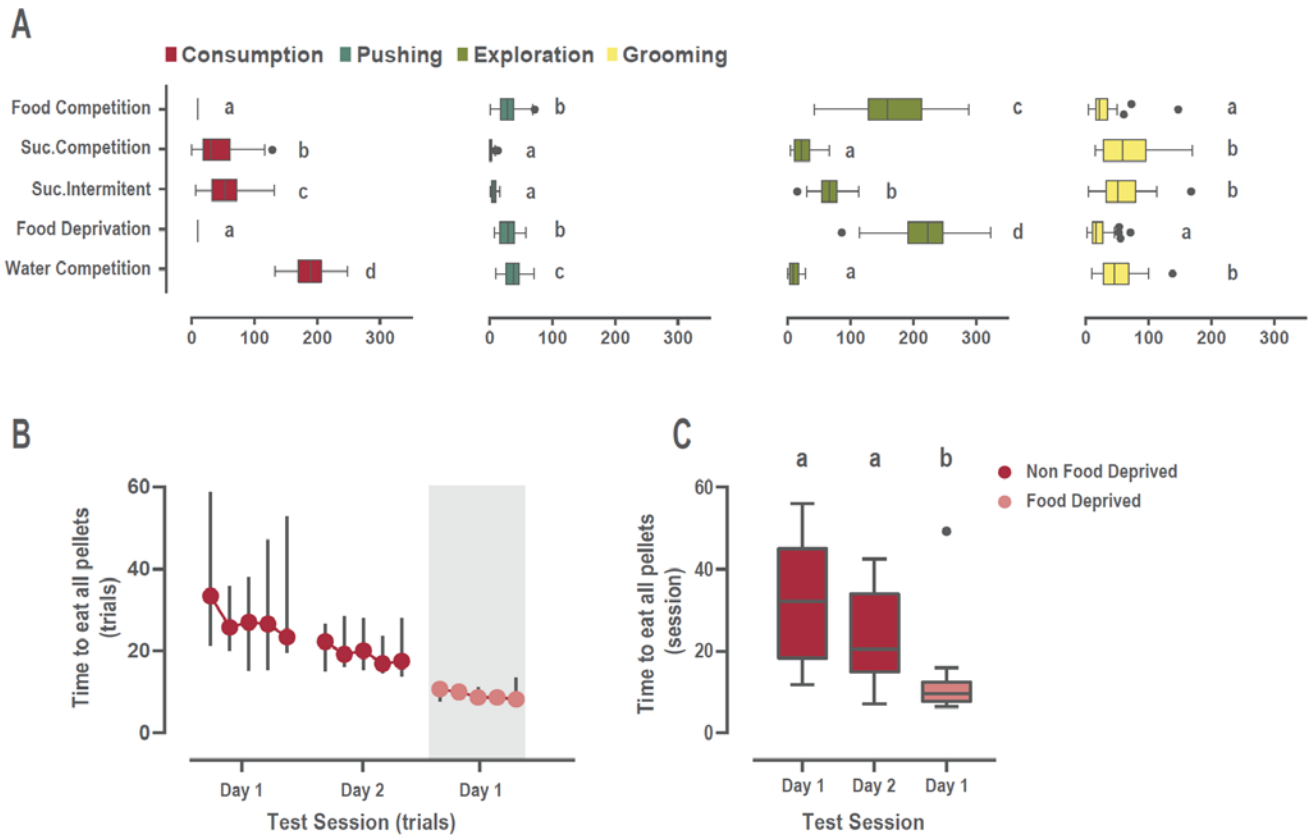

A
Modified Food Competition
5 trials
Consumption
Successful Pushing
Anticipatory feeder exploration
n° pellets eaten
Percentage
Duration(s)
p=0.061
B
Sucrose Competition
10 min
Day 2
Consumption (s)
C
Sucrose Competition
5 trials
Day 2 1st trial
Consumption (s)
D
Modified Food Competition
Deprivation
5 trials
Consumption
Successful Pushing
Anticipatory feeder exploration
n° pellets eaten
Percentage
Duration(s)
E
Water Competition
Deprivation
Successful Pushing
Percentage
F
Tube Test
10 trials
D S
Consumption (residuals)
p = 0.036
Win/Duration (residuals)

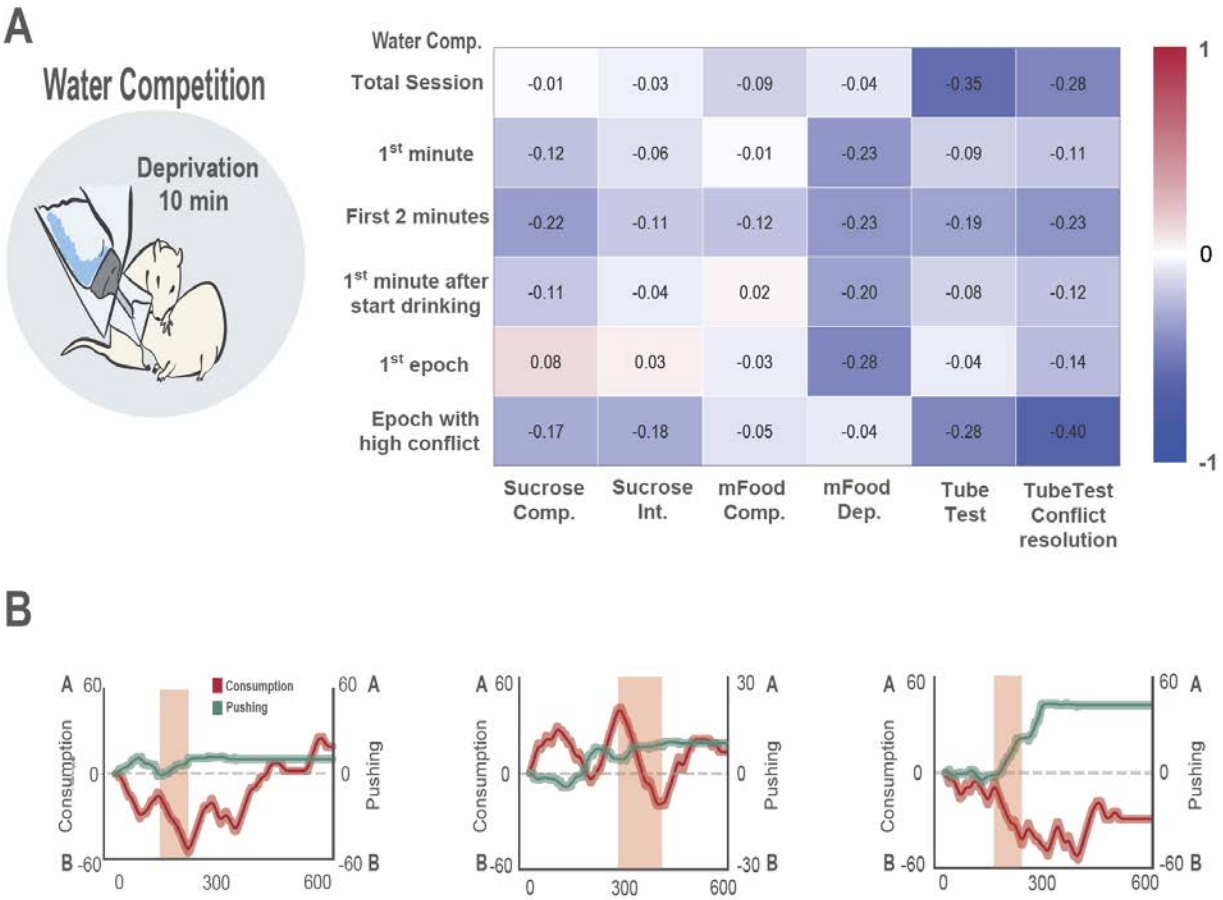
Figure 4



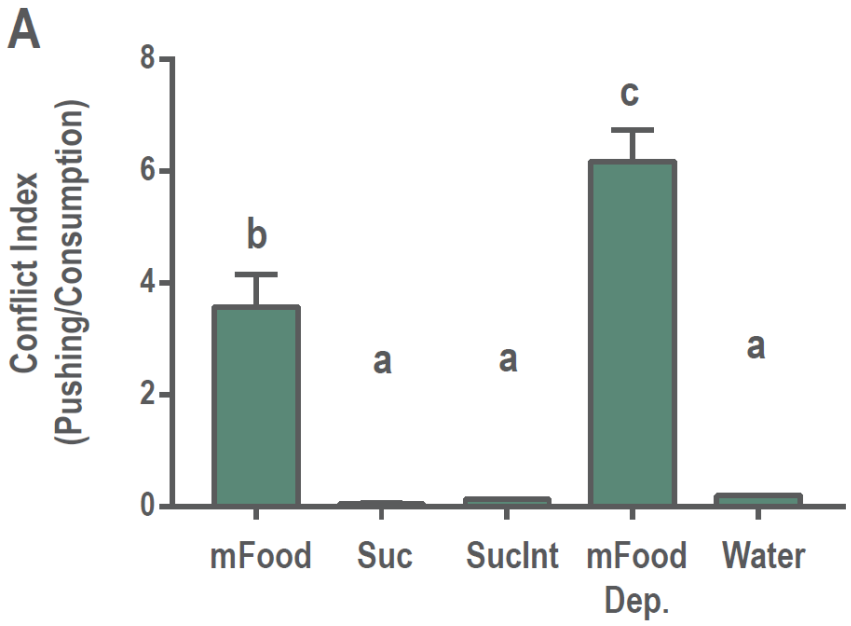
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4