



Research report

Rats exhibit reference-dependent choice behavior

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HIGHLIGHTS

- The origin of reference dependence will help delineate the neural mechanism of choice.
- We developed 'a novel paradigm' based on a modification of the "T" maze.
- Rats showed reference-dependent behavior by avoiding the option framed as a loss.
- Reference dependence is likely a conserved from the emergence of mammals.

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ABSTRACT

Human preferences depend on whether a chosen outcome appears to be a loss or a gain compared with what had been expected, *i.e.*, in comparison to a reference point. Because reference dependence has such a strong influence on human decision-making, it is important to uncover its origins, which will in turn help delineate the underlying mechanisms. It remains unknown whether rats use reference points in decision-making, and yet, the study of rats could help address the question of whether reference dependence is evolutionarily conserved among mammals and could provide a nonhuman animal model to investigate the neural mechanisms underlying this important cognitive process. The aim of the current study was to determine whether rats show reference-dependent choice behavior. We developed a novel paradigm by modifying the "T" maze by installing "pockets" to the left and right of the "T" stem that held reward pellets so rats would potentially develop reference values for each option prior to choice. We found that the rats were indeed sensitive to the way alternatives were presented. That is, they exhibited reference-dependent choice behavior by avoiding the choice option framed as a loss (*e.g.*, having four reward pellets in the pocket, but receiving only one), at least under conditions with certain outcomes and clear differences between the reference and outcome quantities. Despite the small number of rats in this study, this species-level capacity suggests that reference dependence in general and loss aversion in particular may be conserved traits that evolved at or before the emergence of mammals.

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1. Introduction

According to expected utility theory [1], the preference between choice options should depend only on the utility received from each option; however, substantial evidence suggests that human preferences are affected by other factors. For example, our preferences depend on whether outcomes appear to be losses or gains

compared with what had been expected, *i.e.*, in comparison to a reference point [2]. Moreover, people avoid loss, even in cases when the actual outcome is better than other alternatives. Because decision-making based on reference points can lead to suboptimal and thus apparently irrational choices, and because such behavior can have a wide-ranging impact on human economic and social behavior, it is important to understand the underlying causes of this cognitive strategy.

On the one hand, the use of reference points in decision-making could result from the complexities of human society, culture, and language; on the other hand, it could instead reflect an evolved cognitive architecture that conferred a selective advantage to our evolutionary ancestors, *i.e.*, it could be an adaptation sculpted by natural selection. To delineate its origins, it is important to conduct a comparative analysis that can test these general possibilities by

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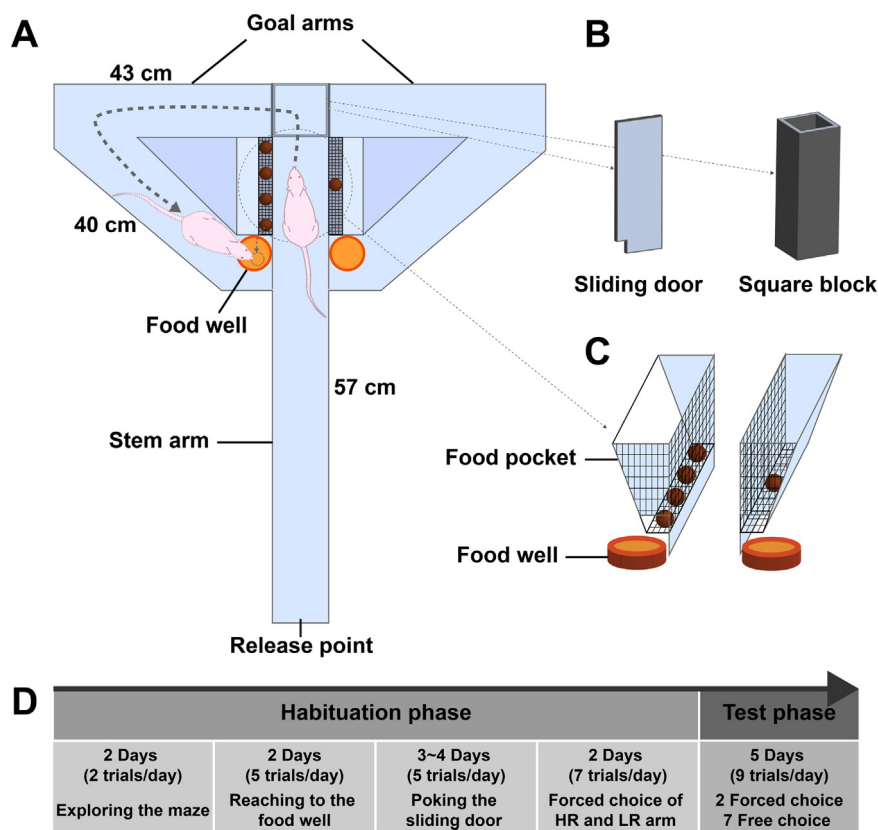


Fig. 1. Schematic of the apparatus and behavioral experiment design. (A) Schematic top view of the modified T-maze. The rats were allowed to explore the reward presented in the food pockets located in the stem arm just before the junction. After exposure to the presented reward, the rats could then choose one arm. (B) Rectangular removable block (right) and the rectangular-piece removable sliding door (left). The two components were designed to provide the rats with more opportunity to explore the presented reward before an actual decision was made and to prevent impulsive choices. (C) Magnified view of the food pocket. Food rewards were presented in meshed food pockets to provide potential reference points for each arm option. Each food pocket had an exit hole (3 × 3) for providing the selected food when the rats arrived at the food well. (D) Experimental procedure. The habituation and test phases of the experimental procedure are depicted.

determining whether the ability is shared with other species that do not share our complex society, culture and language. Recent nonhuman animal studies have found behavioral biases similar to those in humans [3–8]. Most of these species are relatively large-brained, however, and we know that their last common ancestor had a relatively small brain [9,10]. Thus, it remains unclear whether the behavioral biases in question are homologous (*i.e.*, inherited from a common ancestor) or result instead from parallel evolution under similar selection pressures. Further comparative work is therefore necessary to clarify the origins of these heuristics and biases such as reference dependence in decision-making. To this end, it is useful to study a species that closely resembles a common ancestor of humans to determine when the trait may have arisen in the human lineage. With respect to our mammalian lineage, the rodent appears to be a reasonable extant species that resembles an early mammalian ancestor that could help address the question of whether reference dependence is shared among mammals and thus evolved at or before the emergence of mammals.

Several laboratory studies have shown that rats exhibit economic behaviors similar to humans' in terms of consumer demand, value theory and labor supply [11–13]. These results, together with recent findings in decision-making under risk [14,15] and intertemporal choice [16] in rats, suggest the potential presence of behavioral biases that reflect some comparable cognitive strategies to those of humans. However, whether the rat actually exhibits reference dependence remains unknown. In fact, it has been suggested that rats may not be able to learn a task that can test for such higher-cognitive phenomena [11–13].

Therefore, the aim of the current study was to investigate whether rats exhibit behavioral biases in their economic decisions that depend on the problem setting (*i.e.*, how the problem is framed) and resultant reference points that are formed. To do so, we developed a novel paradigm to study framing effects based on the problem structure that may lead to reference points in rodent decision-making. We modified a conventional T-maze that controlled for impulsivity and provided a means to display different amounts of food items on either wall of the "T" stem to allow the potential development of reference points prior to choice. We then examined whether the rats' preferences reflected the influence of reference points based on whether the choice options were perceived as gains or losses as in humans and other select nonhuman animals [17]. A further motivation for developing this paradigm to study rats is that they provide an excellent nonhuman animal model that can be used to delineate the specific behavioral, cognitive and neural mechanisms underlying important and ubiquitous heuristics and biases [18,19].

2. Methods

2.1. Subjects

Nine seven-week-old male Sprague–Dawley rats weighing 250–350 g were used in this study. All procedures were approved by the KAIST Institutional Animal Care and Use Committee. After arrival from the supplier (Koatec, Pyeongtaek, South Korea), the rats were housed in transparent Plexiglas cages at 22 °C with 50–60% humidity. They were maintained on a 12-h light/dark cycle. Food

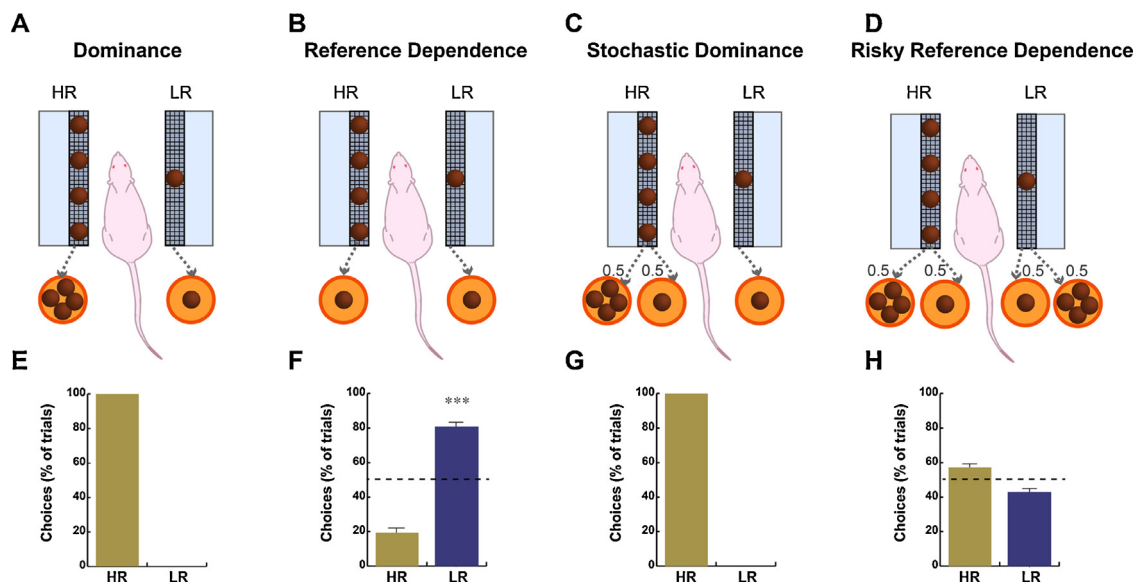


Fig. 2. Experimental design and behavioral results. (A) Schematic representing the Dominance Experiment. The rats obtained the reward that was presented in the food pocket. In all figures, HR, high reward option; LR, low reward option. (B) Schematic representing the riskless Reference Dependence Experiment. Choosing the HR arm always resulted in the removal of three pellets and a reward of one pellet. Thus, both arms earned the same pellet amount (one). (C) Schematic of the Stochastic Dominance Experiment. Choosing the HR arm might result in four pellets (probability of 0.5) or one pellet (probability of 0.5). (D) Schematic representing the Risky Reference Dependence Experiment. Outcomes for both the HR and LR arms were randomized, yielding four pellets (probability of 0.5) or one pellet (probability of 0.5). (E)–(H) The behavioral results of choosing between the HR and LR arms (*i.e.*, percent of trials chosen) in the four respective experiments. Error bars are the standard error of the mean (SEM) across subjects.

and water were freely accessible, except for the duration of the experiment. A predetermined amount of standard laboratory chow (3 pellets for each rat) was given at the end of the session. All experimental testing was performed during the dark cycle (9:00 am–6:00 pm).

2.2. Apparatus

A modified T-maze was used in this study. At the beginning of the experiment, the rats were released at the end of the stem arm. The major difference between our T-maze and a conventional one is the introduction of food ‘pockets’, in which food pellets were displayed on each side of the stem of the ‘T’ (see Fig. 1). As shown in Fig. 1, the two goal arms at the top of the ‘T’ were also modified to turn back to the front of the pockets where the rats would receive the food reward in a food well. The reward pellets were either rolled from the food pocket into the food well by the experimenter or placed in if additional pellets were needed according to the requirements of each experiment, which are described below. The maze consisted of a 0.8 cm thick transparent acrylic board. The 75 cm long stem arm was forked into two goal arms, both of which extended perpendicularly from the stem arm at a length of 43 cm and then bent back to the food well at a length of 40 cm (Fig. 1A, left). The food well was a plastic cap 4.5 cm in diameter and 2.5 cm high. Food pockets (15 cm in length) were installed on both sides of the maze (Fig. 1C) before the stem arm joined the goal arm. The pocket was made from mesh to allow the rats to explore the smell, sight, and texture of the presented food. Thus, the pellets were used as a discriminative stimulus to enable the rats to develop a *reference point*. The amount of pellets in the pocket was adjusted according to the experimental paradigm. The junction immediately following the food pocket was blocked by a removable square block to ensure sufficient time for the rats to experience the amount of reward before the choice was made and to prevent impulsive choices (Fig. 1B, right). The arms were further blocked by sliding doors (Fig. 1B, left). Each sliding door had a square hole (3 cm × 3 cm) in the bottom left (for left goal arm) or right (for right goal arm). Poking this

hole was the behavior we used as the indicator of the rat’s direction preference (only the poked door was subsequently opened). Between trials, the apparatus was cleaned with a mini vacuum and 70% ethanol. The location of the maze was not moved throughout the experiment.

2.3. Behavioral procedure

The behavioral study consisted of a habituation phase and then the test phase. The latter consisted of four experiments (Fig. 1D). Our design is a modified version of the procedure used in effort-based decision-making experiments [20].

2.3.1. Habituation phase

Before the habituation phase started, the rats were handled and exposed to the food (chocolate ball cereal pellets) used throughout the study. The habituation phase began by encouraging the rats to explore the maze for 10 min using 14 food pellets placed throughout the maze, without any blocks or doors in the arms. This was conducted for two days, and two trials were performed on each day. In the next stage, conducted over the next two days, pellets were presented in the food pockets and then delivered to the food wells. Five such trials were performed on each day. The next stage was conducted over the next 3–4 days. The sliding door was introduced, and the rats were allowed to enter the arm only when they poked the hole on either door. Five such trials were conducted each day.

The final stage of the habituation phase was a *forced-choice task* with a different amount of reward in the pockets. One arm was a high reward arm (HR) with four pellets in the associated pocket and four received as reward, and the other was a low reward arm (LR) with one pellet in the associated pocket and one received as reward. In the forced choice trials, the rats had the chance to visit both arms and consume the reward from each arm. Thus, it was technically a forced-choice task composed of two trials (visiting both arms, the first as free choice, the second as forced—but together considered as a forced choice task to sample both options). This task was designed to establish an association between the presented

food and its payoff. Before the rats chose the arm in either direction, they were permitted to examine the pellets in the pockets on both sides with the square block keeping them in the stem arm. Exploration was deemed successful when the rats examined the food pockets at least three times on each side; the block was then removed. This procedure improved the ability of the rat to discriminate the choice options and helped create a reference point for each option. We continued this exploration policy in the test phases. The rats entered the selected arm by poking and were then allowed to consume the presented amount of reward in the selected side. A given rat was then moved to the stem arm where it was required to select the alternate arm and experience the outcome. However, during the *free-choice task* (discussed in the next section), a second opportunity to experience the alternative arm and obtain its outcome was not provided. The location of the HR and LR options was counterbalanced between trials and subjects. Seven forced-choice trials were performed each day for two days.

2.3.2. Test phase

After the habituation phase was completed, the test phase began. During the test phase, we conducted a series of four experiments. All experiments included a free-choice task, which was a simple discrimination task that was similar to the forced-choice task during habituation except that the rats could only enter the first selected arm and consume pellets on the selected side. The pellet(s) assigned to the arm that was not chosen were removed. In addition, in all experiments, the HR pocket always contained four pellets, and the LR pocket always contained one pellet. The HR arm and LR arm were randomly assigned between the two goal arms, and each session consisted of two forced-choice trials (*i.e.*, the same as the forced-choice task in habituation) followed by seven free-choice trials. All experiments continued until the rats' choices stabilized, defined as the rate of change in choice between the HR and LR arms being less than 20% in five consecutive sessions. Fig. 2A–D depicts the general experimental contingencies for each experiment.

2.3.2.1. Experiment 1, Dominance test. In Experiment 1, Dominance (D), the reward for the high and low reward arms matched the pellets in the associated pockets, *i.e.*, four and one, respectively. Furthermore, in this experiment, the sessions continued until the rat could discriminate between the HR and LR arm—and thus choose the HR arm—with 100% accuracy.

2.3.2.2. Experiment 2, riskless Reference Dependence test. In Experiment 2, riskless Reference Dependence (RD), the food earned was always one pellet, regardless of whether the rat chose the HR arm or LR arm. In other words, when a rat chose the HR arm, the experimenter always removed three pellets and delivered the remaining one to the rat. Thus, the HR option was framed as a loss, whereas the LR arm offered the same single pellet that was initially in the food pocket.

2.3.2.3. Experiment 3, Stochastic Dominance test. In Experiment 3, Stochastic Dominance (SD), choosing the HR arm offered a 50% chance of receiving four pellets or one pellet. The reward was decided randomly, but the total earning was counterbalanced and adjusted so that it did not exceed an average payoff of two and a half pellets upon choosing the HR arm. The LR arm delivered the same amount of food initially offered (*i.e.*, one pellet).

2.3.2.4. Experiment 4, Risky Reference Dependence test. Finally, Experiment 4, Risky Reference Dependence (RRD), provided a gamble on the LR and HR arms that was the same as that on the HR arm in the SD paradigm. That is, both arms had a 50% chance of earning four pellets and a 50% of earning one pellet, so the expected value

of either arm was equal to two and a half pellets. A random number generator determined beforehand whether any given trade would result in a payoff of four or one. Thus, the LR arm was framed as a gain (one pellet presented, two and a half received on average), and the HR arm was framed as a loss (four pellets presented, two and a half received on average).

2.4. Experimental design

To establish a baseline performance, all nine rats first performed Experiment 1, the basic dominance test (D), prior to any other experiment. Four rats then performed Experiment 2 (RD), were given two weeks rest, were re-tested on Experiment 1 (to again obtain a baseline performance), and then were tested in Experiment 3 (SD). The other five rats were tested only on Experiment 1 followed by Experiment 4 (RRD).

2.5. Data analysis

For the choice analysis in each experiment, the last five sessions (7 trials per session) of the free-choice trials (35 per rat) were pooled across subjects, and two-tailed binomial tests were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA) to determine whether arm choice could be due to chance. Additionally, the data of each individual rat were assessed with a two-tailed binomial test to determine if individuals showed a significant preference between the HR arm and LR arm.

3. Results

The rats showed high inter-subject variability in the learning time required to reach stable choice behavior in each experiment. It required between 21 sessions (147 trials) and 35 sessions (245 trials) in Experiments 1 and 3, and between 14 sessions (98 trials) to 28 sessions (196 trials) in Experiments 2 and 4. However, all rats achieved stable performance in all experiments.

In Experiment 1, Dominance (D), all rats were able to discriminate between the HR and LR arms with 100% accuracy over five consecutive sessions (our imposed criterion) (Fig. 2A and E). During acquisition, the rats gradually began to choose the HR arm and successfully achieved stable behavior criteria within four weeks, on average (range: 3–5 weeks). Because the HR arm was changed randomly between the two arms across trials, to maximize reward, the rats needed to associate the amount of food in the pocket with the delivered food after arm choice. The results confirm that the rats were able to develop this association at a very high level.

In Experiment 2, riskless Reference Dependence (RD), the rats exhibited a preference for the LR arm over the HR arm (Fig. 2B and F). Overall, 81% of choices were for the LR arm (two-tailed binomial test, $N = 140$, $p < 0.00001$). Individually, all rats preferred the LR arm to the HR arm (two-tailed binomial tests, $N = 35$, $p < 0.01$, Fig. 3A). Thus, the preference of the rats changed based on a reference point effect, with their preference for the HR arm reversing because it was now framed as a loss (*i.e.*, four pellets in the pocket, followed by one pellet reward).

In Experiment 3, Stochastic Dominance (SD), all of the rats showed a strong preference for the HR arm, which was stochastically dominant in the SD test (Fig. 2C and G). In the five consecutive sessions it took to reach our criterion for stable behavior, all four subjects selected the HR arm in 100% of the trials. This result shows that the rats remained sensitive to the option with the higher average outcome and that this behavior was as strong as the selection of the option with the guaranteed higher outcome in Experiment 1 (Fig. 2A and C), even though the outcome now was (a) stochastic, providing one or four pellets 50% of the time, and (b) lower than in Experiment 1 (two and a half pellets *versus* four, on average). We

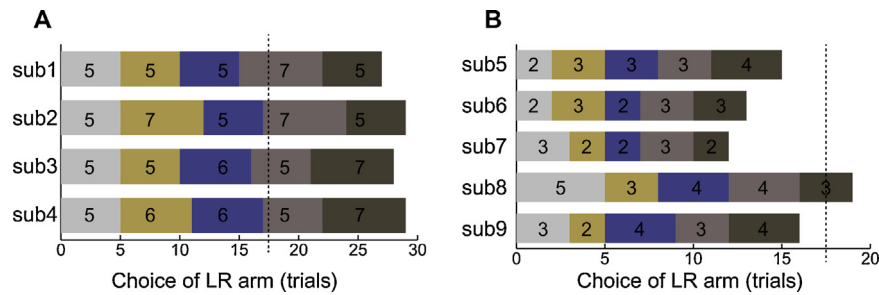


Fig. 3. Choice of the LR arm in RD and RRD in the final five sessions (35 total trials) per experiment. (A) The choice trials for the LR arm of individual subjects in the RD experiment. Different colors represent different days and the length of the bar denotes the number of trials in which the LR arm was chosen. (B) The choice trials for the LR arm of individual subjects in the risky RD experiment. The dashed line indicates the point where the LR arm would be chosen on half of the choice trials (out of 35 total trials), and thus represents chance level performance.

also note that the rats progressively learned to select the HR arm in this SD Experiment. Thus, the HR arm preference in this experiment was not a side effect of the initial dominance test (Experiment 1). Future work will be required to determine exactly which components of the HR arm outcome drove preference, including the higher overall expected value, outcome variance, and the potential for the large four pellet 'jackpot'.

Experiment 4, Risky Reference Dependence (RRD), had the same framing structure as the other experiments (HR=4; LR=1), and in addition, identical average outcomes for each arm (2.5 pellets). In this experiment neither arm was preferred (two-tailed binomial test, $N = 175$, $p = 0.067$) (Fig. 2D and H). This result was confirmed for all five subjects individually: no subject exhibited a preference for either arm (two-tailed binomial tests, $N = 35$, $p > 0.09$, Fig. 3B).

Finally, Table 1 displays the experimental contingencies, gains, losses, expected values and results for each choice option in all four experiments. From the table, it is clear that the results of Experiment 1 (D) reveal a strong overall effect of expected value on choice behavior. The results of experiment 2 (RD) reveal a clear reference dependence effect and, more specifically, the effect of losses on choice behavior. Experiment 3 (SD) pitted the reference effect of loss against expected value, *i.e.*, the HR option had, on average, a loss of 1.5 more than the LR option as well as an expected value of 1.5 more than the LR option. Given the very strong preference for the HR option (chosen 100% of trials), Experiment 3 suggests that expected value had a greater influence on choice behavior than reference dependence. Finally, in Experiment 4 (RRD), neither the 1.5 pellet gain with the LR option (which should drive behavior toward this option) nor the 1.5 pellet loss with the HR option (which should drive behavior away from this option) appeared to be great enough to produce a preference for the LR option. Thus, no significant preference was found between the two options. We discuss the possible influences on preference in Experiment 4 in the Section 4.

4. Discussion

To determine the possible presence of reference dependence in rats, we made several modifications to the standard "T" maze. In instrumental learning paradigms, arbitrary stimuli are typically used to signify that a reinforcement contingency is in place. That is, they are used as discriminative stimuli to signal that a particular action will lead to a reward. In our paradigm, particular amounts of the reward item itself (chocolate ball cereal pellets) acted as the discriminative stimulus. We used 'pockets' on either side of the "T" stem to house these discriminative stimuli and used blockers in the maze to increase the exposure to the pellets and prevent the rats from making relatively impulsive choices. The fact that all rats attained the 100% correct criterion in the Dominance (Experiment 1) test and reached this same perfect

level in the Stochastic Dominance (Experiment 3) test shows that the experimental paradigm achieved its purpose in successfully controlling their behavior.

In fact, this 100% discrimination in the D and SD tests by the rats is a higher level of performance than that demonstrated by capuchin monkeys (*Cebus apella*) in an SD test (100% versus 87%) by Chen et al. [3], as well as other T-maze dominance tests in rats [20,21]. Possible reasons for the high performance in our study include extended exposure to the discriminative stimuli before choice, blocking impulsive choice behavior, greater motivational impact per unit reward (using chocolate ball cereal pellets), a higher reward amount in the HR option than in the capuchin SD experiment (on average, 2.5 versus 1.5), a greater difference in the reward amount between the two options (on average, 1.5) than in the capuchin SD experiment (on average, 0.5), and direct presentation of the reward without requiring any token exchange (which was used in Chen et al.'s [3] capuchin monkey study, in which the monkeys were required to hand a token to one of two experimenters to signify choice).

Our main test for reference dependence was Experiment 2, the riskless Reference Dependence (RD) task, in which the rats were initially exposed to one (LR) and four (HR) reward pellets in the food pockets and received one reward pellet for either choice. Thus, three of the four pellets were always removed, and therefore, the HR option was framed as a loss. If there was no reference dependence effect, choice of either arm should be random or based on a location bias. We found no evidence for either of these possibilities. If, instead, the rats were using the initial food pellets as reference points, the HR arm would appear as a loss and should be avoided. This latter result was what we found, with the rats on average and individually preferring the LR arm and thus generally avoiding the HR arm. Thus, although the number of subjects is quite limited, Experiment 2 provides clear evidence that rats can exhibit reference dependence. Furthermore, we found that a loss, in particular, affected choice behavior; thus, we also found evidence for loss aversive behavior in rats. This same general effect was observed with capuchin monkeys by Chen et al. [3]. In this experiment, the monkeys were offered (a) one choice option that presented one piece of food and always resulted in receiving the same piece versus (b) a second option that presented two pieces of food and resulted in receiving either the two pieces or one piece (with one removed and thus framed as a loss). With this offer, the capuchins exhibited a 79% preference for the LR option, which is quite similar to what we obtained here (80.7% preference for the LR option). Thus, like capuchin monkeys and humans, rat decision-making is affected by reference points, and more specifically, rats also exhibit loss aversive features.

In Experiment 3, the rats strongly preferred the (HR) option with the higher expected value. This option also had higher outcome variance and the potential for the large four pellet 'jackpot'. Future

Table 1

Experimental contingencies, gains, losses, expected values, and percent of trials chosen for each choice option. HR, high reward option; LR, low reward option.

Experiment	Dominance (D)		Reference dependence (RD)		Stochastic dominance (SD)		Risky reference dependence (RRD)	
	HR	LR	HR	LR	HR	LR	HR	LR
Gamble (presented:received)	(4:4)	(1:1)	(4:1)	(1:1)	(4:1,4)	(1:1)	(4:1,4)	(1:1,4)
Gain	0	0	0	0	0	0	0	1.5
Loss	0	0	3	0	1.5	0	1.5	0
Expected value	4	1	1	1	2.5	1	2.5	2.5
% of trials chosen	100%	0%	19.3%	80.7%	100%	0%	57.1%	42.9%

work is therefore needed to determine the main factors underlying the HR arm preference. In any case, given that we found both reference dependence and effects related to expected outcome on behavior, one can potentially compare their relative influences on choice behavior. For example, Experiment 3 (SD), in fact, pitted the reference effect of loss against expected value (the HR option had, on average, a loss of 1.5 more than the LR option as well as an expected value that was 1.5 higher than the LR option). The strong preference for the HR option (chosen in 100% of trials) suggests that although both sets of factors influenced behavior, the influence of expected value related factors was greater than that of reference dependence, as was found with capuchin monkeys [3].

Finally, in Experiment 4 (RRD), the HR option was framed as a loss while the LR option was framed as a gain. Thus, both reference dependence influences should have driven the subjects toward the LR option. However, the rats exhibited no significant preference. Considering the choice behavior in the RRD experiment with capuchin monkeys [3], in which the monkeys avoided risky losses, these results differed. Although these differential findings could represent species differences, there are other possible factors that could also explain the differences. First, we used one and four pellets for our HR and LR options, respectively, whereas the capuchin study used one and two. The greater difference between the options (as well as a potentially heightened motivational impact per unit reward) could have led to an increased inherent preference for the HR over the LR option in rats. A great deal of work has shown that many nonhuman animals, including chimpanzees, as well as young children and autistic children have difficulty inhibiting a prepotent tendency to select the larger of two food quantities, even under reverse-reward contingencies in which they need to select the smaller quantity to receive the larger one [22–30]. In addition, besides having a smaller difference between the smaller and larger quantities in the capuchin study, the use of tokens to make their choices (*i.e.*, handing a token to one of two experimenters to signify choice) might also have reduced any prepotent response tendency to reach for the larger quantity [3].

However, note that we did not find a significant prepotent influence in the riskless Reference Dependence Experiment (Experiment 2). That is, all rats preferred the LR rather than the HR option. Our RRD experiment resulted in a smaller loss (loss in HR arm: -1.5 pellets) than the RD experiment (loss in HR arm: -3 pellets). Thus, loss aversion in rats might be sensitive to the degree of loss, and perhaps more so than in capuchin monkeys. In addition, the *risky* component of Experiment 4 may have been a critical factor that interacted with the presented pellets in the food pockets. In risky decision-making generally, there is evidence that nonhuman animals, such as rhesus monkeys (*Macaca mulatta*), focus inordinately on the higher payoff [31]. If this were so in the current study, it would mitigate the framing effect of a loss. That is, in the extreme, if the rats only focused on the outcome of four pellets, there would be no sense of loss from the HR option because the discriminative stimulus (*i.e.*, four pellets in the HR pocket) would match the outcome. Why there was no clear *gain*

effect on the LR option is yet to be determined. To be sure, there likely are multiple possible influences on choice behavior, including reference dependence, expected value, and inherent response tendencies (whether innate or from extensive experience), and their relative influences need to be fleshed out in future work.

It was previously unknown whether rats used reference points in decision-making, and it had in fact been speculated that they may not. Here, however, we found that they do, at least for some individuals (in our case, four out of four), under at least some circumstances, such as in environments with certain outcomes and clear differences between the reference and outcome values. Thus, reference dependence in general and loss aversive behavior in particular are shared among rats, some nonhuman primates and humans. Our findings support the possibility that reference-dependent decision-making is a shared trait, at least among some mammals. Whether this ability, which is shared with certain bird species, is conserved (*i.e.*, homologous) or convergent (*i.e.*, due to common selection pressures but not common ancestry) awaits further comparative analysis [7,9].

Our results thus suggest that, once appropriate paradigms are developed, rats can be used as a model to investigate the specific mechanisms underlying cognitive heuristics and biases [18,19]. The importance of the rat model is at least twofold. First, using rats allows for rigorous control of independent variables and possible confounding factors such as experience that are likely to affect the long-term decision-making process. This is virtually impossible to do with human subjects. Second, we can utilize invasive methodological approaches with rats to provide higher resolution and causal analyses of the neural and neurochemical mechanisms underlying cognition and behavior [32].

With respect to the underlying neural mechanisms of reference dependence and loss aversive behavior, the amygdala is a clear candidate for avoiding alternatives framed as losses, especially given its role as a general interface between emotion and goal-directed action control. The amygdala is also consistently associated with loss aversion in human fMRI studies [33–35]. However, the involvement of the amygdala and other related neural substrates in reference-dependent decision-making in rats remains to be determined. We hope that the present study offers a valuable approach to undertaking these investigations in the future.

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