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Rats Benefit from Winner and Loser Effects

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[1] We dedicate this article to the eminent scientist, prudent mentor and inspiring example Wolfgang Wickler on the occasion of his 80th birthday.

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Abstract

Prior fighting experience of opponents can influence the outcome of conflicts. After a victory, animals are more likely to win subsequent contests, whereas after a defeat animals are more likely to lose, regardless of the identity of opponents. The underlying mechanisms and the adaptive significance of these winner and loser effects are as yet unknown. Here, we tested experimentally whether agonistic behavior of male wild-type Norway rats is influenced by social experience, and we investigated whether this might reduce fighting costs (duration of contest, risk of injury) in subsequent encounters. Rats were randomly assigned to receive either a losing or a winning experience and subsequently tested with unfamiliar, naïve opponents. We found that most rats with a winning experience won the subsequent encounter, and all rats with a losing experience lost the next contest. Previous winners attacked more rapidly in the subsequent encounter and reduced their aggressive behavior sooner; the contests were decided more quickly, which saved time and behavioral effort to the winner. Previous losers received less aggression in the next encounter, despite emitting fewer submissive ultrasonic calls than in the preceding contest, thereby reducing the risk of being injured by the opponent. Thus, anonymous social experience influenced rats' subsequent behavior toward size-matched, naïve, unknown social partners. Furthermore, apparently, they benefit from showing winner and loser effects in intraspecific contests by saving time, energy, and risk.

Introduction

The outcome of contests over resources such as territories, food, and mates is determined by resource holding power (RHP) and resource value (Parker 1974; Huntingford & Turner 1987). In addition, previous fighting experience strongly influences the odds of winning. Winner and loser effects denote the higher probability of a winner to win a subsequent encounter and the higher probability of a loser to lose a subsequent encounter, regardless of the identity of the opponent (Chase et al. 1994; Dugatkin 1997; Hsu & Wolf 1999). Both effects have been demonstrated across a broad range of taxa, including invertebrates and vertebrates (for reviews see Hsu et al. 2006; Rutte et al. 2006). Mammals, however (e.g., Huhman et al. 2003; Oyegbile & Marler 2005; Jennings et al. 2009), seem to have received less attention regarding winner and loser effects than other organisms such as fishes (e.g., Hsu et al. 2009; Oliveira et al. 2009). This is true even for rodents, including the most widespread model organisms for behavioral studies such as rats (c.f. Oyegbile & Marler 2005), even though winner and loser effects are well suited as models to study proximate hormonal and neural mechanisms of interactive behavior and social conflict (Huhman 2006; Fuxjager & Marler 2010).

Adaptive explanations of winner and loser effects are based on the provisioning of information on contestants' fighting ability (Parker 1974), as winner effects may enhance an individual's self-assessment of its RHP (Hsu et al. 2006, 2008; Arnott & Elwood 2009), while loser effects may reduce individual perception of RHP. This could provide a mechanism for altered contest behavior in subsequent encounters. In general, adaptive explanations of winner and loser effects are based on the idea that social experience is used to assess the opponent or oneself relative to others (Whitehouse 1997; Rutte et al. 2006). In this way, its functional significance may resemble generalized reciprocity, where also anonymous social experience decides about the behavior toward new social partners, in this case whether or not to cooperate with anonymous conspecifics (Rutte & Taborsky 2007, 2008; Barta et al. 2011).

In rat species, experiments with albino rats (*Rattus norvegicus*) revealed that aggressive behavior of males is influenced by prior fighting experience (Seward 1945a,b, 1946; van de Poll et al. 1982a,b). However, these rats were not tested for winner and loser effects, i.e., whether the outcome of a future encounter is influenced by prior agonistic experience. Scholtens & van de Poll (1987) found that in male albino rats, losers of contests showed hardly any aggressive behaviors in the following encounter against naïve rats, whereas there was no difference in aggression between winners and naive partners. The purpose of our study was first, to investigate whether the outcome of a conflict is influenced by prior fighting experience in wild-type Norway rats (*Rattus norvegicus*), both for winners and losers, regardless of the identity of the new opponent, and second, to investigate whether acting upon social experience would help rats to reduce costs (duration of fight, risk of injury) in subsequent encounters. We expected that a rat might benefit from a winning experience by bringing about a speedy decision in the subsequent encounter, thereby reducing the investment in time and energy. We predicted that a winner would show its motivation to invest in an escalated fight. Behaviors that decide contests are thus expected to occur earlier during an encounter after a rat won a previous fight than when it had no prior experience, and this should settle the outcome earlier. A loser might benefit from its experience by revealing submissiveness more quickly or more clearly in a subsequent contest, thereby reducing the attacks it receives and thus the risk of injury. We predicted that a loser would quickly assume a subordinate position and would thus receive less aggression from its opponent than during an encounter without previous experience.

Methods

Subjects and Housing

We used Norway rats (*Rattus norvegicus*) as study animals because they live in large groups from which strangers are usually expelled. We only used males because male rats form a dominance hierarchy, whereas in females, rank differences are less pronounced (own unpublished data; Calhoun 1962; Telle 1966).

The rats were 2 yr old and had been bred from eight pairs of wild-type Norway rats (origin: Animal Physiology Department of the University of Groningen, the Netherlands). These rats descended from four pairs of wild-trapped animals that have been outbred in the Groningen laboratory for 25 generations (de Boer et al. 2003). It should be noted that the breeding program of the Groningen physiology laboratory is decidedly aiming for controlled outbreeding. The size of the founder population was limited, but not to an unusual degree when compared to other rodent populations used in many behavioral studies, which are actually often based on inbred strains. Using outbred wild-type rats as focal animals rather than an inbred laboratory strain, we aimed to avoid potential effects caused by artificial selection. Furthermore, 6-mo-old rats of the inbred Wistar line (origin: Institute of Pathology, University of Bern, Switzerland) were solely used as opponents of focal rats in some experiments. The animals were housed with same-sex littermates in groups of three to nine in small (80 × 50 × 38 cm) or large cages (95 × 73 × 50 cm), according to litter size. Male groups could not interact with each other between cages because of the arrangement of cages. The housing room had an average temperature of 22°C and a 12:12 h light/dark cycle with lights on at 20:00 h. Food (conventional rat pellets produced by Provimi Kliba SA, Kaiseraugst, Switzerland) and water were provided *ad libitum*. Two weeks before an experiment started, the food amount was standardized [5–7 (i.e., 15–21 g) food pellets per rat per day] to keep the weight and hunger level of focal rats constant. This food allocation approximately corresponded to the mean amount consumed when the rats had unrestricted access to food.

Experimental Setup

In the experiments, only rats that were unfamiliar with each other and originated from different cages (i.e., were not closely related) were paired. Our aim was to test winner and loser effects in a context that is as close as possible to a natural situation of resource competition. Therefore, we chose wild-type Norway rats and a semi-natural setup. Under semi-natural conditions, Norway rats tend to fight in front of paths leading to a food resource (Calhoun 1962). We, therefore, took advantage of this observation in our experimental setup and connected two cages (80 × 50 × 38 cm) with a transparent Plexiglas tube (length: 64 cm, diameter: 9 cm). The tube was used also by inferior rats to seek protection from attacks by an aggressive rat (thus, it did not represent a resource being defended by dominant rats). In one cage, the experimental rats were released, and in the other cage, a food pellet was provided (Fig. 1). Pilot experiments showed that aggressive behavior and fighting do indeed occur in this setup. Prior to testing, each experimental rat was accustomed to the experimental setup and trained to pass through the tube to have access to the food pellet. Rats are predominantly nocturnal, and we thus performed our experiments during the dark phase in the morning hours with a red light bulb as the only light source. Laboratory rats can hardly perceive red light (Weiss et al. 1996), and we thus assumed that our experimental rats (wild-type Wistar rats) were not disturbed by it.

Experimental Procedure

Two subjects were put into the experimental cage at the same time, and a lid blocking the tube entrance was removed. All encounters were terminated after 45 min. Between subsequent experimental encounters, there was a break of 20 min each, during which the focal rat was put into a third cage and the experimental cages were cleaned.

We randomly selected which focal rat would receive a winning or losing experience (Begin et al. 1996), but controlling that the two groups of focal rats selected for either the winner or the loser experiment did not differ in (1) their absolute weight (i.e., they corresponded to the average size of individuals in our colony of male wild-type rats) or (2) their ranking in weight among group mates within their home cage. We calculated a 'weight-rank-group-ratio' (WRG-ratio) for all focal rats as follows: [number of group mates in home cage heavier than focal rat '*x*']/[number of group mates in home cage − 1]. If a focal rat was the heaviest rat among its group mates in the home cage, its WRG-ratio was '0', whereas a WRG-ratio of '1' identifies the lightest rat in a cage. A WRG-ratio of '0.5' means that the same number of group mates was heavier and lighter than the focal rat.

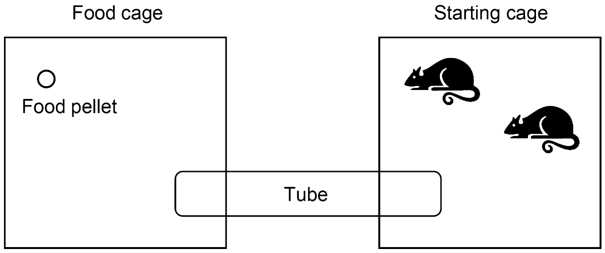

Fig. 1: Experimental setup. Two cages were connected with a transparent tube. After release in the starting cage, rats had to pass the tube to get into the food cage for access to the food pellet.

1. At the start of the winner experiment, the wild-type rats' mean weight was 502 g (SD: ±37 g) and that of the subsample of rats receiving a winning experience was 499 g (±31 g). At the start of the loser experiment, the wild-type rats' mean weight was 507 g (±40 g) and that of the subsample of rats receiving a losing experience was 509 g (±27 g). There was, hence, no consistent weight difference between the groups of focal rats chosen as either winners or losers.

2. Also, the WRG-ratios did not differ consistently between experimentally assigned winners and losers. For focal rats of the loser experiment, the mean WRG-ratio was 0.51, which means that they had on average the same number of heavier and lighter group mates in their home cage. The focal rats of the winner experiment had a WRG-ratio of 0.56, indicating that they were slightly lighter than the majority of their home cage group mates, which is conservative with regard to the predictions of this experiment. Thus, weight cannot be a relevant factor for differences found between winner and loser experiments.

Winner experiment

For the winner experiment, twelve focal wild-type rats were used. The experiment consisted of three phases, separated by a time gap of 20 min: an experience encounter (E_W), a first test encounter ($T1_W$), and a second test encounter ($T2_W$; Table 1). In the experience encounter, focal rats met Wistar rats to obtain a winning experience. Wistar rats were chosen as opponents because they are known to be inferior in fighting ability to the inbred lines S3 (Tryon Maze Dull) and Long Evans (Scholtens & van de Poll 1987). We thus assumed that wild-type rats would also dominate Wistar rats. Indeed, all focal wild-type

Table 1: Experimental procedure and conflict outcomes

	Experience encounter (E_W)	First test encounter ($T1_W$)	Second test encounter ($T2_W$)
Winner experiment (N = 12)			
Test animal	Wild-type rat against	Wild-type rat against	Wild-type rat against
Opponent	Wistar rat	Wild-type rat	Wistar rat
Outcome	12 winners	8 winners, 1 loser, 3 undefined	11 winners (out of 11)
	Experience encounter (E_L)	Test encounter (T_L)	
Loser experiment (N = 10)			
Test animal	Wild-type rat against	Wild-type rat against	
Opponent	Wild-type rat (previous winner)	Wild-type rat	
Outcome	10 losers	10 losers	

The winner experiment consisted of three staged encounters, and the loser experiment consisted of two staged encounters. There was a time gap of 20 min between staged encounters. In all encounters, the opponents were unfamiliar and not closely related to the focal rats. In the loser experiment, we used wild-type winners from the winner experiment as opponents for the focal rats in the experience encounter (E_L).

rats assigned to receive a winning experience won their experience encounter (E_W). In any phase of the winner experiment, weights of opponents were matched. With this procedure, we aimed to exclude the influence of physical body size on fight outcome, as body size is a good surrogate index for fighting ability in many species of animals (Hsu et al. 2006). Furthermore, fish, for example, adopt different contest strategies against differently sized opponents (Hsu et al. 2008). In the first test encounter ($T1_W$), the focal rats were paired with naïve (i.e., without previous fighting experience) and unfamiliar wild-type rats, to test whether a winner effect is shown among wild-type Norway rats. In the second test encounter ($T2_W$), the focal rats were again paired with Wistar rats (each focal rat was paired with a new, unknown Wistar rat). We staged this second test encounter to compare a focal rat's behavior in two situations with similar opponents in which the only difference was whether the test rat had no recent fighting experience (E_W) or had a recent winning experience ($T2_W$). The opponents in both of these encounters (E_W and $T2_W$) were naïve and unfamiliar Wistar rats.

Loser experiment

For the loser experiment, 10 wild-type focal rats were used. We did not succeed in finding a rat strain that would be superior in competition with wild-type rats to matched body size. Therefore, we had to adopt a slightly different design for the loser experiment. Adjusting the procedure described by Oyegbile & Marler (2005), to generate a losing experience for rats determined by the experimenter to become losers, in the experience encounter (E_L), these rats were combined with wild-type winners from the winner experiment weighing 5–10% (15–25 g) more than the designated losers (Table 1). We created only a small weight difference in these experience encounters to minimize the possible influence of the opponent's body size on the focal animals' behavior. All rats assigned to lose in the experience encounter (E_L) indeed did so. In the test encounter (T_L), the focal rats were paired with naïve and unfamiliar wild-type rats that were matched in weight.

Determination of Winner and Loser

During the experiments, the behaviors listed in Table 2 were recorded for both rats with help of the software 'The Observer 3.0' (Noldus Information Technology, Wageningen, the Netherlands). In pilot experiments, these behaviors had been observed frequently and are important to determine winners and losers. The clearest criteria to determine the dominant individual in an encounter were behaviors that had been almost exclusively performed by one rat during the encounter but not by the other. There was no single behavior that occurred in all contests by which rats could be defined unequivocally as a winner or loser. We used five behavioral criteria to determine winners, and of these, a criterion pointing in the opposite direction than the others occurred only 0.02 times of all replicates together. Thus, our determination of winners and losers was unequivocal, as we elaborate in more detail below.

The clearest and most frequent aggressive behavior observed was 'lateral approach'. Additional aggressive behaviors used as winning criteria were 'chase'

Table 2: Behavioral parameters recorded during the experiments

Behavior	Description	Category
Lateral approach	Broadside orientation to the opponent; includes pushing the opponent away with side or rear end	Aggressive
Fight	Rolling over and kicking the opponent	Aggressive
Chase	Chasing (running after) the opponent that is fleeing	Aggressive
Keep down	Keeping the opponent that is lying on its back down with the forepaws	Aggressive
Aggressive groom	Grooming the opponent's fur or genital organs	Aggressive
Autogroom	Grooming oneself	Aggressive
Ultrasonic call	Call at a frequency of 22 kHz, recorded with help of a bat detector	Submissive
Tube	Being in tube; the whole body is inside, the head can be outside	Submissive
Push	Pushing opponent out of the tube while staying hidden in the tube	Submissive
Feed	Feeding	Neutral
Inactive	Sitting or lying	Neutral
Audible call	Call that is audible for the human ear	Undefined

See also Scholtens & van de Poll 1987.

and 'fight'. The strongest losing criterion to determine the outcome of an encounter was ultrasonic calls emitted at a frequency of approx. 22 kHz, which we recorded with help of a bat detector. Rats emitting these 22-kHz ultrasonic calls received significantly more aggressive behaviors (sum of lateral approach, chase, and fight) than they dealt out (Wilcoxon matched-pairs signed-ranks tests: $Z = -4.798$, $N = 33$, $p < 0.001$), which confirms the loser status of these individuals and validates the use of these 22-kHz calls as a losing criterion. An inferior rat emitting ultrasonic calls after receiving aggressive behavior would then often sit in a corner or hide in the tube. By hiding in the tube, a rat was protected from further attacks. The observed rhythmic thoracic movements were consistent with the recorded ultrasonic calls, which match the previous observations (Sales 1972; Brudzynski et al. 1993). Recently, Burgdorf et al. (2008) also found 22-kHz calls to be positively related to withdrawal behaviors of Norway rats during aggressive interactions. Thus, ultrasonic calls and 'time in tube' were used as losing criteria, whereas 'lateral approach', 'chase', and 'fight' were used as winning criteria.

For the evaluation of every single encounter, the following conditions were required: lateral approach, chase, and fight were all used as a winning criteria in an encounter if only one of the two rats showed it or if one rat performed it at least three times more often than its opponent. Ultrasonic calls were used as a losing criterion in an encounter if they were emitted by one of the two rats only. 'Time in tube' was applied as losing criterion if a rat spent more than 75% of the time in the tube or if one rat was at least three times longer in the tube than its opponent. Applying a combination of these five criteria enabled us to define rats unequivocally as winners or losers in most cases: The mean number by which these five behavioral criteria occurred in the 47 encounters where we determined winners and losers was 3.64, whereas the criteria that pointed in the opposite direction occurred only 0.02 times in these replicates. In 42 of these 47 encounters, two or more criteria were met consistently (while no criterion pointing in the opposite direction applied). In one case, four criteria were met consistently, whereas an additional one pointed in the other direction. In four cases, we defined winners and losers based on a single criterion only, which was once the criterion 'ultrasonic calls' and three times the criterion 'time in tube'.

Ethics Statement

Following the guidelines of the Association for the Study of Animal Behavior (ASAB 1991, 2006) for experiments that are potentially detrimental to animals, we used the smallest number of animals possible to accomplish the study goals, which was adjusted to the clear effects we expected. This was requested also by the responsible veterinary authority that gave permission for the study to be conducted. The housing of the rats and the experimental procedure were approved by the Swiss Federal Veterinary Office under license 52/01. In our experiments, no injuries occurred apart from minor scratches and plucked hairs in two instances.

Data Analysis

The data were mostly analyzed with non-parametric statistics using the software packages SPSS 11.0 and

14.0 (SPSS Inc., Chicago, IL, USA). Binomial tests were performed to test the probability of winning after a victory or a defeat, respectively. The null hypothesis about the expected proportion of each outcome direction was 0.5. We further tested whether a winning experience resulted in more aggressive behaviors shown in comparison with the respective opponents and whether a losing experience resulted in less aggressive behaviors initiated than by the respective opponents, using two-tailed Wilcoxon tests.

To compare the behavior of winners in the experience encounter (E_{W}) with the second test encounter ($\mathrm{T2}_{\mathrm{W}}$) in the winner experiment, we used a one-way analysis of variance. The dependent variable was a summed total of aggressive behaviors (i.e., lateral approach, chase, and fight). Treatment (i.e., E_{W}, $\mathrm{T2}_{\mathrm{W}}$) and interval (1, 2, or 3) were fixed factors. For comparing the number of ultrasonic calls emitted by the opponent in the first and last third of the encounter periods in E_{W} and $\mathrm{T2}_{\mathrm{W}}$, a Fisher Exact Probability Test was used. For comparison of losers' behaviors in the experience (E_{L}) and the test encounter (T_{L}) of the loser experiments, we used two-tailed Wilcoxon tests.

Results

The test rats showed both winner and loser effects. In the 47 encounters where we could determine winners and losers, winners showed much more aggressive behaviors (sum of lateral approach, chase, and fight) than their opponents (winners: $\tilde{x} = 10$; range = 0, 40; losers: $\tilde{x} = 0$; range = 0, 1; Wilcoxon test: $Z = -5.646$, $N = 47$, $p < 0.001$), while losers emitted more 22-kHz calls (losers: 8; 0, 366; winners: 0; 0, 2; Wilcoxon test: $Z = -5.013$, $N = 47$, $p < 0.001$).

Winner Experiment

Eight of 12 focal rats that had won the experience encounter (E_{W}) also clearly won the subsequent test encounter ($\mathrm{T1}_{\mathrm{W}}$), while one prior winner lost the test encounter (Table 1; binomial test: $p = 0.040$; $N = 9$). In three additional cases, focal rats clearly did not lose in the test encounter ($\mathrm{T1}_{\mathrm{W}}$) either but could not be unequivocally determined as a winner following our five criteria described earlier. Focal rats with a winning experience showed more aggressive behaviors (sum of lateral approach, chase, and fight) in the test encounter ($\mathrm{T1}_{\mathrm{W}}$) than their opponents (focal rats: $\tilde{x} = 5$; range = 0, 19; opponents: 0; 0, 2; Wilcoxon test: $Z = -2.943$, $N = 12$, $p = 0.003$), while there was a trend that they emitted less 22-kHz calls (focal rats: 0; 0, 6; opponents: 1; 0, 33; Wilcoxon test: $Z = -1.863$, $N = 12$, $p = 0.063$).

To check whether a winner benefits from its winning experience by deciding a future encounter more rapidly, we tested whether behaviors deciding the outcome of contests occurred earlier in the second test encounter ($\mathrm{T2}_{\mathrm{W}}$) than in the experience encounter (E_{W}). Note that in $\mathrm{T2}_{\mathrm{W}}$, eight rats had two winning experiences, and three rats had one winning experience only (from E_{W}). The one rat that had clearly lost in $\mathrm{T1}_{\mathrm{W}}$ after winning in E_{W} was excluded from this test because of its divergent experience. The time pattern of aggressive behaviors (lateral approach, chase, fight) differed significantly between E_{W} and $\mathrm{T2}_{\mathrm{W}}$ (Fig. 2). We compared the first 2 min of experience and second test encounters, because contests can be settled in rats within such a short period (personal observation). The frequency of aggressive behavior in the first 2 min was significantly higher in the second test encounter ($\mathrm{T2}_{\mathrm{W}}$: $\tilde{x} = 2$; range = 0, 10) than in the experience encounter (E_{W}: 0; 0, 3; Wilcoxon test: $Z = -2.354$, $N = 11$, $p = 0.019$). Aggressive behavior decreased immediately afterward in the second test encounter. In contrast, in the experience encounter, aggression increased after the start of the encounter to reach its maximum only after 5 min. Thereafter, aggressive behavior remained on a slightly higher level in the experience encounter than in the second test encounter. To test the time pattern of aggression throughout the entire encounters, 15-min intervals were analyzed (Fig. 2). One rat had to be excluded from this analysis because it was an outlier (outlier test (Dixon 1953): calculated test ratio 0.723 >> threshold ratio 0.577). Univariate analysis of variance showed that both the type of encounter (E_{W} or $\mathrm{T2}_{\mathrm{W}}$) and the time interval had a significant effect on aggression. Aggressive behavior (sum of lateral approach, chase, and fight) declined over the 15-min intervals (One-way ANOVA: $F_{2,60} = 11.515$, $p < 0.001$, $N = 10$), and former winners showed less aggression overall in $\mathrm{T2}_{\mathrm{W}}$ compared to E_{W} ($F_{1,60} = 5.370$, $p = 0.025$, $N = 10$; Fig. 2). This confirmed that the time pattern of aggression differed between the experience and test encounters, with an earlier decline in $\mathrm{T2}_{\mathrm{W}}$ than in E_{W}, and it showed that former winners were able to keep winning in a subsequent contest despite reducing their overall aggression effort.

We also analyzed the number of ultrasonic calls that were emitted by the opponents of focal rats in

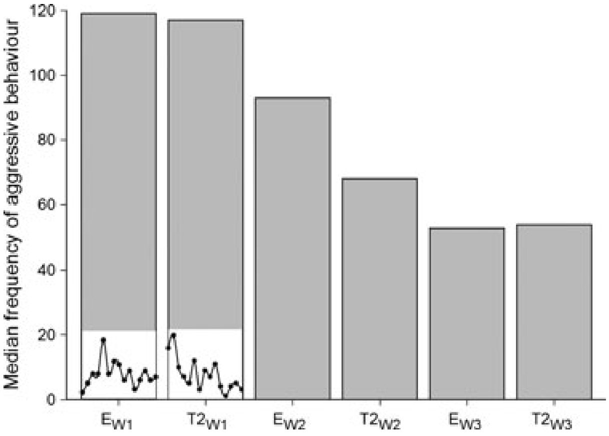

Fig. 2: Winner experiment. The number of aggressive behaviors of focal rats (n = 11) exhibited in the first, second, and third 15-min intervals of the experience encounter ($E_{\mathrm{W1-3}}$) and of the second test encounter ($\mathrm{T2_{W1-3}}$). In the first 15-min interval, 1-min sums of aggressive behavior are shown.

the winner experiment to reveal whether encounters in $\mathrm{T2_W}$ were decided faster than in E_{W}. We found no difference in the overall number of emitted ultrasonic calls between E_{W} and $\mathrm{T2_W}$ (Wilcoxon tests: ultrasonic calls: $Z = -0.840$, N = 11, p = 0.401). However, the temporal distribution of ultrasonic calls differed significantly between $\mathrm{T2_W}$ and E_{W}. More ultrasonic calls were uttered in the first 15 min of $\mathrm{T2_W}$ than in the corresponding time period of $\mathrm{E_W}$, while in the last 15 min of the encounters, this relationship was reversed (minutes 1–15: $\mathrm{T2_W}$: 306, E_{W}: 82; minutes 31–45: $\mathrm{T2_W}$: 172, E_{W}: 381, Fisher Exact Test; $p < 0.001$, N = 11).

Loser Experiment

In the loser experiment, all ten wild-type rats that had lost the experience encounter (E_{L}) also lost the test encounter (T_{L}; Table 1; Binomial test: p = 0.002; N = 10). Focal rats with a losing experience showed fewer aggressive behaviors (sum of lateral approach, chase, and fight) in the test encounter (T_{L}) than their opponents (focal rats: $\tilde{x} = 0$; range = 0, 1; opponents: 0.5; 0, 11; Wilcoxon test: $Z = -2.032$, N = 10, p = 0.042), but they emitted more 22-kHz calls (focal rats: 1.5; 0, 29; opponents: 0; 0, 0; Wilcoxon test: $Z = -2.023$, N = 10, p = 0.043).

We further investigated whether a loser might benefit from its experience by revealing submissiveness more quickly or more clearly in a subsequent contest, thereby reducing the attacks it receives and thus the risk of injury. We tested whether rats with a losing experience would receive less aggressive behavior than in their preceding encounter and whether they would emit more or fewer ultrasonic calls to reach this goal. Indeed, the loser rats received significantly fewer aggressive behaviors in T_{L} than in E_{L} (sum of lateral approach, chase, and fight; $\tilde{x}$ E_{L}: 9, $\mathrm{T_L}$: 0.5; Wilcoxon test: $Z = -2.245$, p = 0.025, N = 10; Fig. 3a). This difference in received aggression was mainly because of lateral approach and chase. Furthermore, losers emitted significantly fewer ultrasonic calls in T_{L} than in E_{L} ($\tilde{x}$ E_{L}: 50.5, T_{L}: 1.5; Wilcoxon test: $Z = -2.073$, p = 0.038, N = 10; Fig. 3b). The frequencies of aggressive behaviors and ultrasonic calls were too low in T_{L} to allow testing for different time patterns between E_{L} and T_{L}.

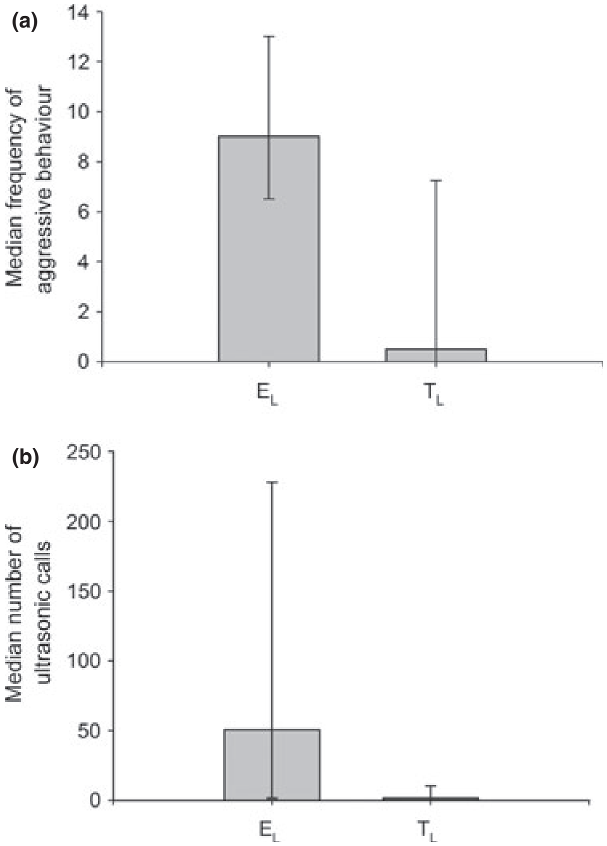

Fig. 3: Loser experiment. (a) The number of aggressive behaviors received by the focal rats (n = 10) in the experience encounter (E_{L}) and test encounter (T_{L}). (b) The number of ultrasonic calls emitted by focal rats (n = 10) in the experience encounter (E_{L}) and the test encounter (T_{L}). Medians and quartiles are shown.

Discussion

Our experiments demonstrated that both winner and loser effects are shown by wild-type Norway rats in a resource competition paradigm. Changes in aggression after agonistic experience have been previously reported in albino rats (Seward 1945b; van de Poll et al. 1982a,b; Scholtens & van de Poll 1987) and mice (Ginsburg & Allee 1942; Bevan et al. 1960; Brain & Poole 1974; Andrade et al. 1989). However, these studies applied different experimental methods that did not involve all procedures required for a conclusive test of winner and loser effects: Namely, (1) a random selection of focal animals determined to receive a winning or losing experience, (2) a standardized time interval between experience and test, and (3) the use of a naïve, size-matched experimental partner of the focal animal in test encounters (cf. Begin et al. 1996; Rutte et al. 2006). Furthermore, these studies had not tested whether losers lost subsequent encounters and winners won subsequent encounters against a new opponent. By demonstrating that subjects with a losing experience are more likely to lose the following encounter, and those with a winning experience show an increased probability to subsequently win – in addition to a change in the frequencies of aggressive and submissive behaviors as revealed in the previous studies, including a recent study on mice (Kloke et al. 2011) – our results, therefore, confirm that winner and loser effects in rodents do conform to the strictest experimental criteria, which may be viewed as an essential merit of our study. The importance of demonstrating winner and loser effects according to these strictest experimental criteria had also been suggested and pursued in an experiment with California mice, where a winning experience increased the probability of winning a subsequent encounter (Oyegbile & Marler 2005). However, in that study, only males with three winning experiences showed a significant winner effect, whereas males with fewer winning experiences were not more likely to win subsequent contests. Furthermore, the partners of the focal test mice in the test phase of that study were not naïve or were they size-matched. In laboratory bred male albino rats [S3 (Tryon Maze Dull)], losers showed hardly any aggressive behavior with naïve partners in contrast to dyads of winners and naives, leading the authors to conclude that the winning experience did not increase aggression whereas the losing experience inhibited it (Scholtens & van de Poll 1987). In a setup where focal test mice in their home cage faced intruders, mice with winning experience showed more offensive aggressive behaviors to defend their territory and attacked their opponent sooner than test mice with losing experience (Kloke et al. 2011); however, data revealing whether focal mice with winning experience were more likely to win their test encounter were not reported.

In other mammals, Jennings et al. (2004) found that a previous fight outcome was related to the penultimate fight outcome in European fallow deer, but this was only the case when a third category for fights that ended in a draw (i.e., encounters without winner nor loser) was included, i.e., there was little evidence for winner and loser effects when only individuals that had either won or lost their first fights were considered. When an animal had experience of drawing a fight, there was an increased probability that its subsequent fight would also be a draw. Thus, in effect, the study demonstrated a 'draw-effect' rather than winner or loser effects. Other studies of mammals have found effects of previous fighting experience but must be interpreted cautiously with regard to winner and loser effects because of deviations from the experimental protocol required to demonstrate winner and loser effects conclusively (cf. Begin et al. 1996; Oyegbile & Marler 2005; Rutte et al. 2006). For example, in some studies, roles were not randomly assigned (Bevan et al. 1960), opponents were not naïve before the start of the experiment (Ginsburg & Allee 1942; Seward 1945a,b), opponents were familiar or closely related to test individuals (Ginsburg & Allee 1942), tests were pseudo-replicated (Ginsburg & Allee 1942; Seward 1945b, 1946), fights and aggressiveness were induced by unnatural means, e.g., by hormone application and specific housing schemes [isolation and presence of females increase aggression in laboratory rats (Ginsburg & Allee 1942; Seward 1945a,b, 1946; Scott & Marston 1953; Bevan et al. 1960; Brain & Poole 1974; van de Poll et al. 1982b; Scholtens & van de Poll 1987; Scholtens et al. 1988; Andrade et al. 1989)] or other invasive manipulations [e.g., electroshocks (Bevan et al. 1960); or physical restraint (Scott & Marston 1953)]. Nevertheless, overall, the results of these studies together with our work suggest that winner and loser effects are probably as widespread in mammals as in other animal taxa (cf. Hsu et al. 2006; Rutte et al. 2006).

Our results suggest that animals that act upon their prior fighting experience benefit by reducing fighting costs in subsequent encounters. A rat with a winning experience benefits from its experience by deciding the following contest faster. Analysis of the timing of aggressive behavior showed that a rat with winning experience attacked its subsequent opponent more quickly and then lowered its level of aggression earlier. In the winner experiment, the opponents of the focal rats emitted submissive ultrasonic calls earlier in the second test encounter than in the experience encounter. This suggests that, after a winning experience, the focal rats influenced their naïve opponents to submit to them earlier, which supports the hypothesis that a prior winner decides a subsequent encounter faster. In other words, acting as a winner might cause the opponent to concede faster. This suggestion is supported by results of Oyegbile & Marler (2005) in California mice, where opponents of individuals with three previous winning experiences showed losing behavior (freezing) earlier than opponents of individuals with fewer winning experiences. Furthermore, males that attacked earlier displayed less aggressive behavior overall than those that attacked later (Oyegbile & Marler 2005). This matches our results, showing that former winners won subsequent encounters even with a reduced aggression effort. Apparently, a previous winner benefits from its winning experience by deciding a subsequent contest quicker, thereby reducing its fighting costs. A rat with a losing experience benefits by causing its opponent to be less aggressive, which reduces the risk of being injured. Ultrasonic calls of 22 kHz have been found to be positively related to withdrawal or avoidance behaviors during aggression (Knutson et al. 2002; Burgdorf et al. 2008) and to be emitted when rats are being submissive (Sales 1991), and we found that rats emitting these 22-kHz ultrasonic calls received more aggressive behaviors than they dealt out, which supports the use of these 22-kHz calls as a losing criterion. Activity of rats consistently decreased after presentation of 22-kHz calls (Sales 1991; Brudzynski & Chiu 1995) and remained low for 5 min afterward, which was suggested to allow a loser to escape and a winner to prevent wasting time in pursuit (Sales 1991). Thus, 22-kHz calls possibly inhibit or reduce the aggression of social partners for some time. As this had not been our initial aim, we did not record data in a way allowing for a direct test of the function of the 22-kHz calls.

As predicted, we found that in the test encounter losers received less aggressive behavior (lateral approach, chase, and fight) than in the experience encounter, and they emitted fewer ultrasonic calls. Lateral approaches imply costs; in 75% of cases, fighting followed a lateral approach. Therefore, it may be advantageous to be submissive quickly and as a result receive fewer lateral approaches, as this might reduce the risk of injury. In addition, the effort of producing ultrasonic calls can thus be reduced. A potential reduction in injury risk by behavioral adjustment of former losers was also suggested in Syrian Golden hamsters, where repeated defeat of resident males leads to early flight from intruders before being attacked, whereas without losing experience resident males would attack novel intruders introduced into their home cages (Potegal et al. 1993). Similarly, in male copperheads, prior losers lost their subsequent encounter by passive defeat, that is to say they avoided fights by showing submissive behavior (Schuett 1997).

Being able to decide a contest faster saves time and energy, as fighting is metabolically costly (e.g., Copeland et al. 2011), and might decrease the risk of injuries. Even superior individuals are at risk of being injured in an escalated encounter. In our experiments, no injuries occurred apart from minor scratches and plucked hairs. Prolonged fights might also reduce the time available for vigilance, thereby increasing exposure of combatants to attacks by predators in a natural environment (Wittenberger 1981).

On an ultimate level, we would expect winner and loser effects to evolve under circumstances where the saving of costs is relatively high compared to the value of the ownership of a resource (Rankin & Taborsky subm.). In this case, a winner might show a strong display of strength and aggression early in a contest, preventing an escalated fight that can involve injury. A loser, on the other hand, might lose less in missing a minor resource such as one food item than when it loses an escalated fight. Winner and loser effects may also result from the assessment of the average opponent's fighting ability or of one's own relative fighting ability within the population (Rutte et al. 2006). Previous social experience may modify an individual's self-assessment of its fighting ability: Winner effects may enhance an individual's self-assessment of its RHP (Hsu et al. 2006, 2008; Arnott & Elwood 2009), while loser effects may reduce individual perception of RHP, which in turn may alter subsequent contest behavior. Saving costs during sequential encounters as suggested by our results and assessment of fighting ability might be two non-exclusive adaptive explanations for the existence of winner and loser effects.

Winner and loser effects are functionally related to generalized reciprocity, where anonymous social experience also influences behavior in subsequent interactions by affecting the tendency to cooperate (Rutte & Taborsky 2007), suggesting that in both cases a rather simple mechanism leads to contingent behavior based on prior experience. So far, little is known about proximate mechanisms underlying the winner and loser effects (for review see Hsu et al. 2006). A simple physiological mechanism underlying the loser effect might be exhaustion. Shortly after losing, i.e., 20 min in the present study, an animal may still be exhausted when the next contest occurs and thus lose again. Although exhaustion could underlie the loser effect, it cannot explain the winner effect. Based solely on exhaustion, an individual with recent fighting experience would not be expected to win the subsequent encounter against a 'fresh' opponent, even if it won the previous fight (see also Chase et al. 1994). Many studies found endocrine changes and adjustments in neural pathways after agonistic contests (Cooper et al. 2009; Gleason et al. 2009; Fuxjager et al. 2010). Testosterone levels decrease after defeat in male mice and rats, whereas adrenocorticotrophic hormone (ACTH) and corticosterone increased (Brain 1980; Leshner 1980; Schuurmann 1980). In California mice, testosterone increased significantly in males having won more than two previous encounters compared to controls, but not compared to males with no or one prior winning experience, whereas corticosterone levels remained the same regardless of winning experience (Oyegbile & Marler 2005). Winning fights increased also the expression of androgen receptors, a key brain area that controls social aggression, which might motivate winners to fight again (Fuxjager et al. 2010). In male Japanese quail (*Coturnix coturnix*), the aggressive and submissive behaviors did not relate to their circulating levels of corticosterone and other hormones (Ramenofsky 1984). Recently, Hirschenhauser et al. (2008) found that fighting behavior itself and conflict outcome alone did not explain the observed variation of post-conflict androgen responses, leading the authors to suggest that gaining some information on relative fighting ability may influence the androgen response. In male cichlid fish Mozambique tilapia (*Oreochomis mossambicus*), treatment with anti-androgen was found to block the winner effect, whereas androgen administration failed to reverse the loser effect, leading the authors to suggest an involvement of androgens on the winner but not on the loser effect (Oliveira et al. 2009). Thus, it is unclear whether in general changes in hormone levels or neural pathways are the proximate causes of winner and/or loser effects. It is unlikely, though, that winner and loser effects are mere by-products of physiological processes underlying the regulation of agonistic behavior during a fight [for a review of literature on winner and loser effects and discussion of ultimate and proximate causes, see Rutte et al. (2006)]. Our results show that rats reduce costs in a subsequent encounter when behaving according to their social experience, which clearly hints at an adaptive advantage of winner and loser effects.

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