Detailed Material and Methods

Subjects

We used 20 female wild-type Norway rats at an age of approximately 22 months (source: Animal Physiology Department, University of Groningen, Netherlands) from 10 different cages. The rats were housed in groups of five sisters per cage (80 cm x 50 cm x 40 cm; in four cages which contained 5 of the tested individuals, one non-tested littermate was not a sister). Each cage interior was enriched with wooden and paper toys, a tunnel and a wooden shelter. Water and rat pellets were provided ad libitum. In addition, the rats received special treats (seeds, fruits, vegetables, pasta or rice) in the afternoon. As rats are predominantly nocturnal, an inversed 12:12 light:dark cycle with lights on at 20:00 hours allowed us to work during the animals'natural activity phase.

Four unrelated female wild-type Norway rats from the same breeding source were used as cooperators. These animals lived in four different group cages located in a separate room. For the experiment, they were split into two pairs, each of which was assigned to 10 focal test individuals.

Experimental food items

Observations of our test animals revealed that bananas were obviously much more appreciated than carrots (see Results). Bananas contain over 2 times more energy than carrots on average (89 kcal versus 41 kcal per 100g; USDA Database), and animals usually attempt to maximize the rate of energetic returns when foraging [1].

Pre-experimental training

Rats were trained in a cage split in two compartments by wire mesh (Fig. 1a). During 11 sessions, focal individuals initially learned to pull a stick fixed to a baited platform towards the cage to obtain food for themselves; the reward, a cereal flake, could be reached by the rat when the platform had moved inside the cage. During subsequent 18 sessions, the rats learned to pull the stick to provide food only to a partner positioned in the other compartment; the reward could only be reached by the partner when the platform had moved inside the cage. During the course of these 18 training sessions, the interval between the exchange of roles of the two partners was gradually increased towards a condition in which the same rat provided food for her experimental partner during the whole session (7 minutes), without getting any returns. This criterion was reached at session 15, after which the roles of the partners were exchanged after an interval of 30 minutes. At the two last training sessions (17 and 18), this interval was increased to one day. Cooperators had the same training as focal individuals, but in contrast to the latter they were regularly rewarded directly after pulling for a partner to enhance their pulling motivation.

Statistical analysis

The preference of rats for bananas or carrots was tested with a Fisher's exact probability test. As a clear preference was thereby established, one-tailed Wilcoxon matched-pairs signed-ranks tests were used for the comparisons between banana and carrot treatments.

Wilcoxon tests were used to analyze the number of pulls and the latencies before the first pull in dependence of treatment. Tests were performed on global results (sum of pulls for both test days in dependence of treatment, mean of latencies before the first pull between both days in dependence of treatment), and for each test day separately.

In a number of encounters mainly with previous carrot providers, test rats did not pull at all. In these cases, we assigned the maximum time period of 420 seconds to the latency before the first pull, which is the duration of the experiment. This is a conservative estimate, because the latency may have been much longer if the experimental period had been longer.

References

1. Stevens JR, Hauser MD (2004) Why be nice? Psychological constraints on the evolution of cooperation. TRENDS in Cognitive Sciences. Vol.8, No.2: 60-65