

## BRIEF COMMUNICATION

# Cooperative Behavior of Laboratory Rats (*Rattus norvegicus*) in an Instrumental Task

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Cooperation is a cognitively demanding, complex social behavior, found primarily in primates. Here we investigated mutualism in rats (*Rattus Norvegicus*), a simple form of cooperation in which two subjects work on operant task, receiving immediate and simultaneous sucrose reward for a joint action. To receive the sucrose reward, familiar pairs of rats were required to nose poke simultaneously. Following 44 training days, we examined the relation of social contact and ultrasonic vocalizations to the rat's cooperative behavior by testing the effects of inserting opaque, wire-mesh, or no partition - between subjects. Cooperative behavior (simultaneous nose-poking): (a) increased gradually during initial training; (b) decreased with the opaque partition (restricting visual, acoustic, and physical communication); (c) increased with a wire mesh partition restricting only physical contact; and (d) increased with the number of 50 kHz USV "happy" calls and the intensity of social interaction. The possibility of studying the development of cooperative behavior in laboratory rats using a simple procedure based on commercially available equipment may prove useful in modeling determinants of social behavior.

**Keywords:** cooperative behavior, communication, rats

In natural conditions cooperative behavior has been demonstrated in several contexts, such as foraging, mate attraction, predator avoidance, territory defense, and parental care (Kappeler & van Schaik, 2006; Krebs & Davis, 1993). Cooperation can be defined as the voluntary joint action of two or more individuals that benefits the recipient(s) (Brosnan & de Waal, 2002; Hamilton, 1964). An act of altruism benefits the recipient at the cost to the actor (Hamilton, 1964), while mutual benefits follow a joint action resulting in the simultaneous benefit for all individuals involved (Dugatkin, 2002; Krebs & Davis, 1993). Cooperation involves mostly related subjects, suggesting that kin selection plays an essential role. Since cooperation requires advanced cognitive abilities, such as individual recognition, social learning and memory, temporal discounting, delayed gratification, and so forth, (Stevens & Hauser, 2004), it is uncommon among nonrelatives except in humans and other primate species. Field observations (Calhoun, 1962) as well as laboratory data with feeding/footshock avoidance (Daniel, 1942), body pulling behavior (Aquino, Wong, & Rodriguez, 1985), shuttling in a runway (Schuster, 2002), and reciprocal feeding (Rutte & Taborsky, 2007) strongly suggest that rats also demonstrate a native propensity to cooperate. However, little is known about the mechanisms allowing cooperative behavior in

this species. Here we investigated the role of social interactions and ultrasonic vocalizations in the simple instrumental mutualistic cooperative task in rats.

## Materials and Methods

### Animals

Twenty male Sprague–Dawley rats (*Rattus Norvegicus*; Charles River, Germany), weighing approximately 225–250 g at the start of experiment, were housed in groups of four in standard plastic cages in the temperature ( $22 \pm 1^\circ\text{C}$ ) and humidity (45–50%) A/C controlled animal colony with an automatic 12-hr light/dark cycle (lights on: 07:00). Food and water were provided ad libitum. The experiment was conducted in agreement with the NIH Guide for the Care and Use of Laboratory Animals and was approved by the Ethics Committee for Animal Experiments, Institute of Pharmacology.

### Apparatus

The experiment was conducted in slightly modified four identical standard operant chambers (Coulbourn Instruments) measuring  $29 \times 29 \times 20.5$  cm (H  $\times$  W  $\times$  D). One of the rear panels was replaced with the small glass window allowing video-recording; the ceiling contained a hole, to which ultrasound microphone was attached. During the *training phase*, 2.5 cm above the floor a single nose-poke operandum was placed centrally on the front panel, which was also equipped with the liquid feeder that upon activation delivered 10  $\mu\text{l}$  of sucrose solution (20%) and turned on feeder light bulb for 4 s. Above the feeder there was a 5-W house

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light, which served as a cue-light signaling the availability of the reinforcer. During the *experimental phase*, only two operant boxes were used, each equipped with pairs of nose-poke operandi, pairs of liquid feeders, the close-circuit video camera (Sony) and the ultrasound microphone (Ultrasound Advice, U.K.) connected to the A/D converter DAQ device (USB-6251 1.25 MS/s M Series). High-frequency (HF;  $\sim 50$  kHz) “happy” USV calls were recorded on a PC computer running Raven Pro 1.4 (Cornell Lab of Ornithology, Bioacoustics Research Program) interactive sound analysis software. At the no-partition phases of experiment—allowing free interactions between rats—the social behaviors were video-recorded on the second PC computer. A third PC computer running Coulbourn Instruments software controlled operant chambers and recorded and analyzed the instrumental behavior data.

### Preexperimental Training

The rats were individually trained for 4 weeks during single daily sessions lasting for 30 minutes each, under a fixed ratio 1 (FR1) schedule of reinforcement.

### Experimental Procedures

During the “no-partition” phase #1 lasting for 14 sessions, pairs of rats were trained to nose poke almost simultaneously (within 2 s), and only then both rats received simultaneously the reinforcer. When the nose poke responses had happened outside the 2-s time window (i.e., were not nearly simultaneous), the subjects were not reinforced. The number of individual (noncooperative) and mutual (cooperative) nose poke responses were recorded automatically. To investigate the impact of social interaction and communication reduction, during the “opaque partition” phase #2 lasting for the 10 following sessions (Days 15–24), the black opaque plastic partition was inserted in the middle of the operant chambers. During the second “no-partition” phase #3 lasting for 10 sessions (Days 25–34), the partitions were removed to check

whether the cooperative behavior was altered by opaque partition insertion. During the last “wire-mesh partition” phase #4 lasting for the following 10 sessions (Days 35–44), the operant chambers were divided by a metal wire mesh ( $\sim 1 \times 1$  cm; wire diameter  $\sim 0.5$  mm) partitions, limiting physical contact, but allowing unlimited visual, olfactory, and auditory communication.

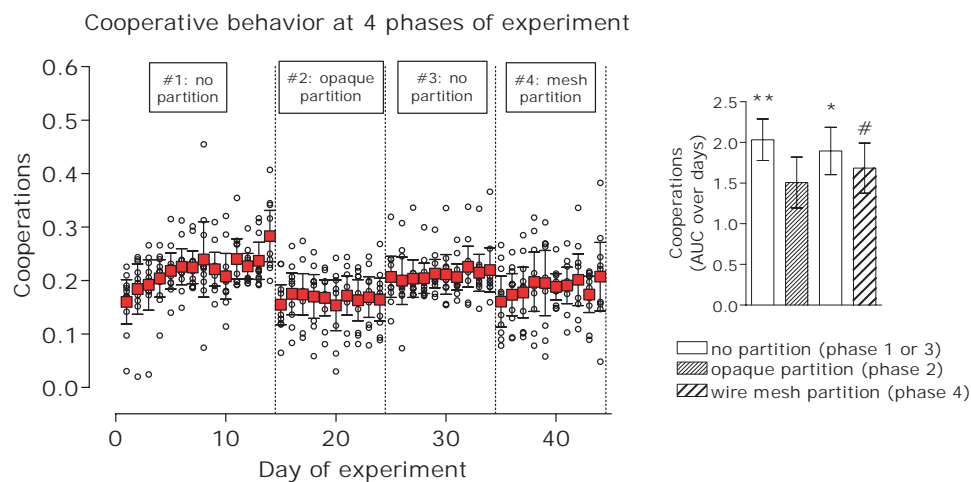
### Statistics and Data Presentation

Due to the high variability of responding in individual pairs, the data are expressed and analyzed as a ratio of cooperative (reinforced) responses divided by all nose-poke responses. In addition, we analyzed off-line a variety of social interactions (Barnett, 1967), in particular, the approach behavior occurring around the time of cooperative action (moving toward or watching the partner). The number of HF USV was also analyzed off-line. All assessments were done by the observer unaware of the given phase assignment.

For statistical analyses (Statistica 7), data were expressed as the area under the curves (AUCs) over days of a given phase of experiment (for the comparison, phase #1 data lasting for 14 days were normalized to 10 days). Paired *t* test, within-subjects ANOVA with Duncan’s post hoc test or Friedman’s ANOVA for nonparametric data (HF USV) were used throughout. Regression analyses were used to detect associations between cooperation behavior and the days of the given phase, the social behavior, and the USV calls.

### Results

Figure 1 shows that the intensity of cooperative behavior was the highest at no-partition phases and the lowest at the opaque partition phase:  $F(3, 27) = 3.59, p < .05$  (Figure 1, inset). Separate regression analyses demonstrated positive correlation between the day of experiment and the number of cooperative responses for phases #1, #3, and #4 ( $r^2: 0.71, p < .05$ ;  $r^2: .59, p < .05$  and  $r^2:$



**Figure 1.** Cooperative behavior in rats depends on the conditions restricting social interactions and visual, acoustic and physical communication. Subjects were simultaneously reinforced in the instrumental task with sucrose, only by simultaneous nose-poke response. Mean ( $\pm 95\%$  CI) raw data with cooperative responses; Inset: same data as area under the curve (AUC); \*  $p < .05$ . \*\*  $p < .01$  versus opaque partition phase. #  $p = .066$  versus no-partition phase #1.

.40,  $p < .05$ , respectively); but not for opaque partition phase #2 ( $r^2 = .002$ , NS). In addition, the mean number of cooperative responses at all 44 days of experiment (but not of particular phases) correlated positively with the number of mean HF USV calls:  $r^2 = .174$ ,  $p < .01$ . Due to the high variability, the number of HF calls did not differ among four phases ( $F = 3$ , NS).

The intensity of social interactions at two phases allowing free interaction (see Figure 2) was higher at the second no-partition phase #3 than at the first no-partition phase #1 ( $t = 6.16$ ,  $df = 7$ ,  $p < .001$ ; Figure 2, inset). Regression analyses demonstrated that cooperative responses positively correlated with the number of social interactions at phase #1 ( $r^2 = .46$ ;  $N = 14$ ,  $p < .01$ ) and at phase #3 ( $r^2 = .60$ ,  $N = 10$ ,  $p < .01$ ).

### Discussion

Present results suggest that laboratory rats can cooperate to receive sucrose reinforcement in an instrumental task. This cooperative behavior developed gradually as the number of cooperative responses increased during the training; in addition it was reduced by a partition restricting visual, acoustic and physical communication but not by a partition restricting only physical contact. Detailed analyses have demonstrated that the cooperative behavior was related to the number of  $\sim 50$  kHz USV “happy” calls and to the intensity of social interactions (approaches). A positive correlation between cooperative behavior and the day of a given phase suggests that this behavior is learned, supporting the results of Schuster’s (2002) experiment using a shuttling task.

To our knowledge, this is the first demonstration of HF calls associated with cooperative behavior. Rats emit low-frequency ( $\sim 22$  kHz) “alarm” calls and high-frequency ( $\sim 50$  kHz) “happy” calls observed during play and mating (Burgdorf et al., 2008). Since the “happy” calls accompany “positively valenced appetitive behavior,” our data suggest that cooperative behavior was reinforcing for the subjects. A limitation of present findings lies in the fact that this observation—perhaps due to the high variability—

was based on regression analysis and thus does not implicate causality. Since we found that the calls both preceded (i.e., purportedly facilitating the response of the partner) and followed the simultaneous nose-poke response (i.e., indicating a positive emotional state associated with reinforcement), we could not firmly establish their role in facilitating cooperative action. However, pilot recordings done during the preexperimental training may favor the former hypothesis. This is because when the rats were trained individually, on average they emitted  $9.3 \pm 1.3$  of HF calls, while at the first and second no-partition phases there were  $63.8 \pm 6.7$  ( $N = 112$  observations) and  $65.4 \pm 7.9$  ( $N = 80$  observations) of HF calls, respectively. Overall, present data suggest that the HF calls were emitted before the cooperative behavior and facilitated its occurrence.

However, the HF calls may not completely account for the increase in cooperative behavior, since in the mesh partition phase—restricting only physical contact—the level of cooperation was not as high as at the no-partition phases. This would imply that in the no-partition phases, the subjects used also other—than HF calls—aims for coordinating cooperative behavior. In our experiment, analyses of various social interactions indicated that cooperation behavior positively correlated with social approach behavior. Consistent with this observation, Gardner et al. (1984) reported that rats cooperated better if they could see each other, suggesting that social interactions further facilitated cooperation (see also Noe, 2006). Approach behavior (moving toward or watching the partner) could be considered a form of an encouragement or an invitation by one partner toward another for the cooperative action to occur (Barnett, 1967). In the footshock avoidance/feeding cooperative paradigm, Daniel (1942) reported that “as the experiment progressed, the rats directed more and more toward each other.” Social behaviors inserted by partners into their coordination strategies were described as touching, crawling on the other’s back, tail biting and pulling (Daniel, 1942). Apparently, similar behavioral encouragement played a role in our experimental conditions. How-

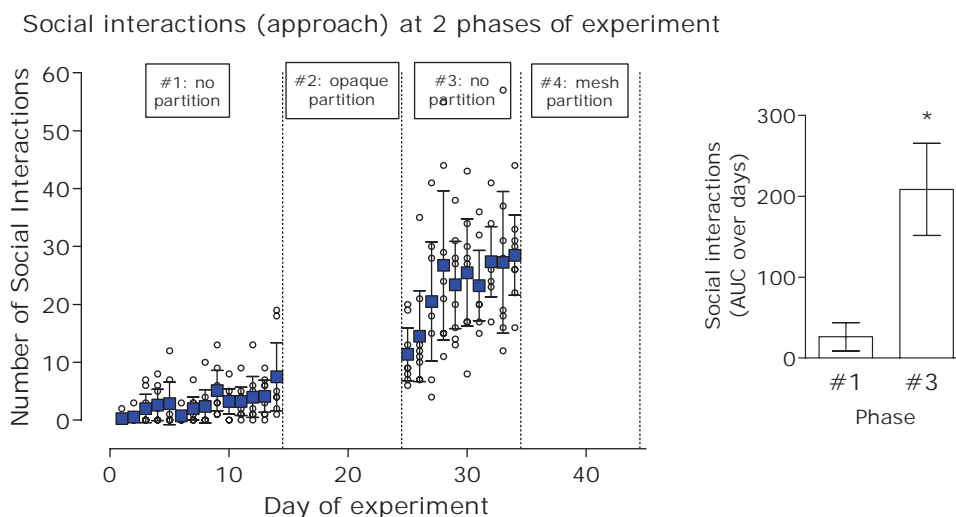


Figure 2. Social interactions during the cooperative task are higher in rats that cooperated; raw data (mean  $\pm$  95% CI); Inset: the same data expressed as AUC \*  $p < .05$  versus phase #1. Note that social interactions were assessed in only 8 (10) pairs due to technical reasons.

ever, the much higher number of approaches in the second no-partition phase is difficult to explain in light of the similar amount of cooperative behavior in these two phases. It could be that the partners encouraged each other more actively, but the intensity of cooperation had already achieved a "ceiling" level. Alternatively, the maximal level of cooperation resulted in satiation and thus the rats started behaving more socially as a result of satiation.

Overall, the present findings suggest that under different conditions rats use different means of communication, and that severe restriction in social communication reduces the intensity of cooperation to the level observed at the beginning of training. Apparently, the physical presence of the partner allowed the most intense cooperative behavior to occur, confirming and extending the results of Berger and Schuster (1982) and Schuster (2002). In addition, the high incidence of physical contacts and HF USV could suggest that rats might experience positive affective states during cooperation task because they might "enjoyed" working together. In the natural conditions cooperating male lions interact socially (e.g., displaying mutual grooming) prior to start their joint action and at the end of successful cooperation (Packer & Pusey, 1982) that additionally enhances relationships between individuals in a group. These acts per se appear to be reinforcing. Thus, cooperation might increase the incentive value of the achieved outcome and cooperators may even prefer "a company" rather than working alone.

The present procedure is based on the instrumental conditioning (Skinner, 1966) using commercially available and relatively inexpensive equipment. It enabled us to assess behavior qualitatively and quantitatively and to vary numerous conditions influencing the output of operant behavior. Since the procedure seems likely to be relatively simple to replicate under similar experimental conditions, it appears to be well-suited for studying the neurobiology of cooperative behavior. In contrast, previous, pioneering experiments of Schuster (2002) on shuttling behavior in a runway, and of Rutte and Taborsky (2007) on reciprocal feeding in instrumental setting required the use of custom-made apparatuses. Because poor social skills have been implicated in the etiology of mental disorders such as major depression (Spirito, Hart, Overholser, & Halverson, 1990), the present technique might be useful for an assessment of social communication in animal models of depression.

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