**Rat ultrasonic vocalizations as social reinforcers – implications for a multilevel model of the cognitive representation of action and rats' social world**

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Rats are social animals that live in groups and tend to display actions that benefit conspecifics. In a previous experiment, we have shown that rats exhibit mutual-reward preferences, meaning that they prefer choices that yield a reward to themselves and to a conspecific, over choices that yield a reward to themselves only, but not to the conspecific. Such mutual-reward preferences might be caused by reinforcing properties of ultrasonic vocalizations (USVs) that are emitted by the conspecific. In rats USVs are socio-affective signals with important communicative functions that can provide information about the current situation and/or state of the calling rat to other rats in its vicinity. Roughly, USVs of adult rats can be subdivided into two classes: 50-kHz USVs that are emitted during appetitive situations and 22-kHz USVs that are emitted during stressful and aversive situations. To test the effect of USVs on choice behavior, we trained rats to enter one of two compartments in a T-maze setting (Fig. 1).

**Figure 1:** T-maze setting to test the influence of USVs of the choice behavior in rats. Rats are placed in a start box and can choose freely to enter either a left or a right compartment. In both compartments, rats receive a food reward, paired with a different type of USV. Please note that in the current experiment only one rat was tested in the respective maze.

Entering either compartment yielded identical food rewards as well as playback of either 50-kHz USVs, which are expected to be appetitive and therefore potential positive reinforcers, or 22-kHz USVs predicted to be aversive and therefore potential negative reinforcers. In three experimental conditions, rats could choose between compartments yielding 50-kHz USVs versus a non-ultrasonic control stimulus (condition 1), 22-kHz USVs versus a non-ultrasonic control stimulus (condition 2) or 50-kHz versus 22-kHz USVs (condition 3). Results show that rats exhibit a transient preference for the 50-kHz USV playback over non-ultrasonic control stimuli, as well as an initial avoidance of 22-kHz USV relative to non-ultrasonic control stimuli (Fig. 2).
Each testing day consisted of 16 trials, during which rats could choose between the USV-associated compartment and the control compartment. The above-mentioned preference for the USV-associated side was most pronounced during the early block of trials (trials 1-5), compared to the later blocks of trials (trials 6-10 and trials 11-16), in line with previous findings (Fig. 3). Our results support the hypothesis that USVs have (transient) motivating and potentially reinforcing properties that can influence the choice behavior of rats.

These results might have implications for understanding the structure of social cognition and learning in animals and potentially humans. The reaction of rats towards USVs can reflect a previously learned association of a certain type of USV with either a positive or a negative event. This type of learning can be explained by the cascade approach, which assumes that the cognitive representations of stimuli and actions are multilevel. The basic physical level of rat USVs is merely acoustic but USVs also possess emotional, motivational, and social significance to the rats. Behavior exhibited in the present experiment might consist of such multilevel representations of stimuli, which then, as a whole, guide action.

**Figure 2:** Rats chose more often for the compartment associated with 50-kHz playback (blue bar) compared to the compartment associated with 22-kHz playback (red bar) when the USVs were paired with a control stimulus in the other compartment.

**Figure 3:** The preference for the USV associated compartment was most pronounced during the early trials of a testing day (block 1).