

Cognitive bias in rats evoked by ultrasonic vocalizations suggests emotional contagion



Yumi Saito^a, Shoko Yuki^a, Yoshimasa Seki^{a,b}, Hiroko Kagawa^{a,b}, Kazuo Okanoya^{a,b,*}

^a Graduate School of Arts and Sciences, The University of Tokyo, Meguro, Tokyo, Japan

^b Okanoya Emotional Information Project, ERATO, Japan Science and Technology Agency, Kawaguchi, Saitama, Japan

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ABSTRACT

Emotional contagion occurs when an individual acquires the emotional state of another via social cues, and is an important component of empathy. Empathic responses seen in rodents are often explained by emotional contagion. Rats emit 50 kHz ultrasonic vocalizations (USVs) in positive contexts, and emit 22 kHz USVs in negative contexts. We tested whether rats show positive or negative emotional contagion after hearing conspecific USVs via a cognitive bias task. We hypothesized that animals in positive emotional states would perceive an ambiguous cue as being good (optimistic bias) whereas animals in negative states would perceive the same cue as being bad (pessimistic bias). Rats were trained to respond differently to two sounds with distinct pitches, each of which signaled either a positive or a negative outcome. An ambiguous cue with a frequency falling between the two stimuli tested whether rats interpreted it as positive or negative. Results showed that rats responded to ambiguous cues as positive when they heard the 50 kHz USV (positive vocalizations) and negative when they heard the 22 kHz USV (negative vocalizations). This suggests that conspecific USVs can evoke emotional contagion, both for positive and negative emotions, to change the affective states in receivers.

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1. Introduction

1.1. Empathy in rodents

Empathy is defined as the capacity to share, react to, and understand the experiences and associated mental states of others (Davis, 1994). One of the primitive phenomena underlying the ability to feel empathy is “emotional contagion,” in which one acquires the emotional or arousal state of other individuals. Emotional contagion is important for survival because vicarious experiences motivate empathic reactions, which benefit both the individual and those nearby, for example, by enabling individuals frightened by the alarm of others to flee rapidly, or through the actions of a mother distressed by her baby’s crying. (de Waal, 2008).

Studies suggest that rodents show empathy (Langford et al., 2006; Panksepp and Lahvis, 2011), and that it is generated through emotional contagion, particularly contagion of negative or high-arousal states (e.g., pain; Langford et al., 2006). In order to measure this, researchers must observe behaviors that provide an indication

of the animal’s internal state. For example, licking and biting of the paw after formalin injection are considered indicators of pain or discomfort (Wheeler-Aceto et al., 1990). Negative emotional contagion is exhibited after a painful or stressful stimulus such as acetic acid (Langford et al., 2006), or electric shock (Bredy and Barad, 2009), is given to an individual (the demonstrator) in the presence of another individual (the observer). When the demonstrator performs a behavior in response to the induced pain or stress, the observer may in turn change or facilitate its own behavior as if experiencing the pain or stress caused to the demonstrator.

In addition, Bartal et al. (2011) showed that empathy might motivate pro-social behaviors in rodents, similar to what is seen in humans. In their research, rats rapidly learned to help free their trapped cagemates from a narrow cage. Even when rats were faced with either helping a cagemate or receiving a food reward, they performed both tasks with similar latencies. This means that receiving a reward was not chosen over helping a cagemate. In over half of the trials, rats also shared the reward with the previously trapped cagemate. The authors’ interpretation was that even in rodents, motivation for alleviating stress or pain and providing joy to others was not less than the motivation for receiving a direct reward.

In considering the methodology of the above stated studies, there exist two limitations. First, researchers are only able to infer internal affective states if they are expressed as innate behav-

* Corresponding author at: Graduate School of Arts and Sciences, The University of Tokyo, Meguro, Tokyo, Japan.

E-mail address: cokanoya@mail.ecc.u-tokyo.ac.jp (K. Okanoya).

iors such as writhing (Langford et al., 2006). If these behaviors are used as indicators of affective states, we cannot separate true empathic responses from innate releasing mechanisms, which occur physiologically and are hard-wired to respond to specific stimuli to produce stereotyped behaviors or fixed action patterns. Second, the field of empathy is dominated by research into negative-oriented contagion (Panksepp and Lahvis, 2011). Studies of positive-oriented contagion are much more limited because the phenomenon is more difficult to demonstrate. Thus, we utilized a “cognitive bias task”. Although the cognitive bias task still relies on measuring behavior, these behaviors are learned, and thus the task can compensate for these limitations by not relying on existing stimulus–action links that are inherent in innate behaviors.

1.2. Cognitive bias task

It is known that emotional states influence cognitive functioning in humans by inducing biases on attention (Mogg et al., 1992), memory (Christianson, 1992), and judgement (Nygren et al., 1996). Judgment biases in particular are quite prevalent. For example, anxious individuals tend to interpret ambiguous stimuli such as homophones more negatively (e.g. die/dye, guilt/gilt, pain/pane; Mathews et al., 1989), than individuals who are not anxious. They may also overestimate the likelihood of negative outcomes, or underestimate the likelihood of positive outcomes (Wright and Bower, 1992), while people with positive affect tend to interpret situations conversely (Nygren et al., 1996). That is, when faced with ambiguous situations, emotional state can influence one’s judgement—positive emotions can cause a tendency for “optimistic” judgments (optimistic biases) whereas negative emotions can cause a tendency for “pessimistic” judgments (pessimistic biases).

Some studies suggest that animals showed optimistic or pessimistic behaviors based on emotions and from this, we can infer their affective states (Mendl et al., 2009; Paul et al., 2005). Under the influence of positive emotions, animals tend to respond positively to an ambiguous stimulus as if they judge the stimulus as rewarding (optimistic biases; Brydges et al., 2011; Rygula et al., 2012), whereas, they tend to perceive ambiguous stimulus as avoidance stimuli when under the influence of negative emotions (pessimistic biases; Harding et al., 2004; Papciak et al., 2013). Therefore, these biases can be used as an indicator of positive or negative affective state in animals (Harding et al., 2004). Even though cognitive bias is not an empathic response but rather an index of affective state, we can use it to infer whether positive or negative contagion is occurring during the task. If the emotional state of an animal (measured via cognitive bias) changes after exposure to the specific signals of another individual, we can infer that emotional contagion has occurred.

We trained animals to perform either optimistic or pessimistic behaviors via a lever press in an operant conditioning task. We used a two choice task with two discriminative stimuli and two response levers. An overview of the paradigm used in our study is as follows: First, either a reward stimulus or an avoidance stimulus is presented as an anchor stimulus. The two stimuli have the same modality (i.e., sound, light, etc.) and constant physical values. They only differ in pitch. When presented with one stimulus (the reward-associated stimulus), animals are conditioned to respond to receive a reward by pressing one lever. Conversely, the animals are conditioned to avoid punishment when presented with the other stimulus (the avoidance-associated stimulus) by pressing the other lever. After animals acquired the discrimination, they are presented with an ambiguous stimulus, which has an intermediate value between the two anchor stimuli. Thus, we can infer the emotional states of the animals by measuring the frequency of the conditioned behaviors. If animals respond to the ambiguous

stimulus as they would respond to the reward associated stimulus, we can assume they are in a positive affective state. On the other hand, if they respond in the same way as they would to a negative, avoidance-associated stimulus, we can assume they are in a negative affective state (For detail of procedure, see Methods).

By using this procedure, we aimed to measure rats’ emotional states after presenting specific ultrasonic vocalizations (USVs), which are considered the emotional signals used by rats in communication. When an animal achieved the discriminative criterion, of 85% correct lever presses for reward and avoidance tones, the animal was run in a test session with the ambiguous stimuli. The USVs were presented immediately before the test session to manipulate the emotional state of the subject rats.

1.3. Ultrasonic vocalizations as emotional signals

Rats emit 50 kHz USVs, which have short durations (3–65 ms; Sales, 1972), show rapid frequency changes (bandwidth 2–50 kHz; Sales and Pye, 1974) and have a frequency range of 35–70 kHz (Wintink and Brudzynski, 2001). They also emit 22 kHz USVs, which are longer (300–3400 ms; Sales, 1972), have little frequency change (bandwidth 1–5 kHz; Sales and Pye, 1974), and have a frequency range of 18–32 kHz (Sales and Pye, 1974).

We focused on these USVs in rats as an index of positive/negative affective states (Knutson et al., 2002). It is known that rats emit short, 50 kHz USVs in positive/approach contexts such as being tickled (Panksepp and Burgdorf, 2003), when playing (Knutson et al., 1998), during mating (McIntosh and Barfield, 1980), and in a social approach to a conspecific (Blanchard et al., 1993; Brudzynski and Pniak, 2002). Thus, 50 kHz USVs have the contextual similarity to a primitive laughter (Panksepp, 2007). In contrast, rats emit long, 22 kHz USVs in negative/aversive contexts such as being exposed to a predator (Blanchard et al., 1991), pain (Tonoue et al., 1986), or when showing social defeat (Thomas et al., 1983). These vocalizations also function as a distress call (Litvin et al., 2007).

In addition, some researchers claim that 50 kHz and 22 kHz USVs function as social signals. For example, playback of 50 kHz USVs enhanced female receptive behaviors during mating (Thomas et al., 1982), while playback of 22 kHz USVs induced freezing or avoidance behaviors (Brudzynski and Chiu, 1995). Therefore, USVs can transmit information about the affective and motivational state of the sender, or can predict rewards or punishments for the receiver (Knutson et al., 2002).

In our study, once a rat acquired the operant contingency in the cognitive bias task, we attempted to manipulate the emotional states of the rat via playback of USVs. We tested whether 50 kHz or 22 kHz USVs can evoke positive or negative emotions (emotional contagion) and cause optimistic or pessimistic biased-like behavior in response to ambiguous stimuli. Our object was to examine emotional contagion in rats evoked by USVs through cognitive bias tests.

2. Materials and methods

2.1. Subjects and housing

The experiments were conducted in accordance with experimental implementation regulations of the University of Tokyo and were approved by the animal experimental committee at the University of Tokyo, Graduate School of Arts and Sciences (Permission Number: 22-5).

Sixteen male Sprague–Dawley (SD) rats were used in the study. Four rats were used to record stimulus vocalizations and to establish the experimental protocols (pilot study). The other 12 rats participated in the discrimination task and the probe test. Rats

were housed in individual cages on the same shelf at a temperature of 20–22 °C, in an air conditioned colony room under a 12/12-h light/dark cycle (lights on at 8:00 am). Rats were 6-months old at the start of the study. Rats were fed ad libitum for 7 days to obtain their free-feeding weights (460.3 ± 33.1 g). During the training and test periods, access to food was restricted (15 g–18 g of food per day; Lab Diet, PMI Nutrition International, St. Louis, MO) to maintain $85 \pm 2.5\%$ of the free feeding weight for each rat. Operant conditioning and behavioral tests were carried out during the hours of noon to 6:00 pm, which is the light phase of the light/dark cycle. Experimenters handled the rats for 10 min before conducting the behavioral tests. At the beginning of training, all animals were fully matured adults. All experimental procedures were done within 90 days of the start of training and the weight gains were very slight during this period, so we assumed that animals maintained their healthy body weights.

2.2. Apparatus

Two computers controlled two sound-attenuated Skinner boxes (Interior: 12.0" L \times 9.5" W \times 11.5" H, ENV-007-CT, Med Associates, St. Albans, Vermont, the USA). Each box was equipped with a light, a speaker, a liquid dispenser, a grid floor and two retractable levers. The liquid dispenser, which dispensed a sucrose solution, was located between the two levers.

2.3. Recording and editing of USV stimuli

Before training and test sessions, we recorded 50 kHz or 22 kHz USVs from the four individual rats, which were not used for the training or the probe tests. Two individuals were placed in a sound-attenuated box containing a recording system (UltraSoundGate and Recorder-USG, Avisoft, Glienicke, Germany) and a monitoring camera (Debut Video Capture Software, NCH Software, Canberra, Australia). Soon after the rats entered the box, they emitted the 50 kHz and 22 kHz USVs.

After recording, we classified USVs as either 50 kHz or 22 kHz by visual inspection of the sound spectrogram (FFT length: 512 points; Resolution: 488 Hz) and selected syllables for 50 kHz and 22 kHz USVs playback. Sound analysis software (SasLab pro, Avisoft) was used to make a single wav file 7 s in duration that contained multiple syllables in the order in which they were emitted. We chose USV syllables from one pair of rats as stimuli for each file. We made 3 files for 50 kHz playback stimuli (24.5 syllables emitted in 6.80 s on average) and 3 files for 22 kHz stimuli (5.8 syllables emitted in 7.26 s on average) from the vocalizations of two pairs of rats. Eventually, we prepared 6 files for the 22 kHz USVs playback because the 22 kHz USVs had fewer variations in sound intensity so we had to duplicate the original 3 files and vary the sound amplitude. This procedure equated variance in sound pressure levels in two sets of files.

The frequencies outside 30–70 kHz for 50 kHz stimuli were rejected using a band-pass filter (a function of SASLab). Likewise, stimuli falling outside 18–22 kHz were rejected for 22 kHz stimuli. One example each for the 50 kHz and 22 kHz stimuli is shown in Fig. 1a and 1b, respectively. 50 kHz vocalizations showed slight variations in frequency modulation (e.g., Fig. 1c,d,e). USV stimuli were amplified (Integrated-amplifier A-10, Pioneer, Kawasaki, Japan) after converting from digital to analog signals (Quad-Capture, Roland, Hamamatsu, Japan), and played through a speaker (PT-R4, Pioneer) that was fixed above the Skinner box. USV stimuli were adjusted so that each stimulus was played at about 60 dB sound pressure level (SPL) on average. We monitored the output of the sound stimuli using an ultrasonic-recording microphone (Ultra-sound gate, Avisoft).

2.4. Stimuli and behavioral training

Rats were divided into two groups at random. One group consisted of individuals who were trained to press the positive lever in response to a 2 kHz tone and to press the negative lever upon hearing a 9 kHz tone (Group A: 6 individuals). The other group was trained to press the positive lever upon hearing a 9 kHz tone and to press the negative lever upon hearing a 2 kHz tone (Group B: 6 individuals). Prior to conducting the training phase, rats were placed inside the Skinner box two or three times so that they became habituated to the chamber. During this time, they also learned to press the left and right levers to gain access to the sucrose solution.

During both the initial and second training phases, rats were trained to press one lever to receive a reward (20% sucrose solution dispensed for 2 s) when one tone (30 s, 2 kHz at 75 dB SPL or 9 kHz at 75 dB SPL, counterbalanced) was presented. The tone became the signal of the positive value and was referred to as the reward tone, and the lever was also referred to as the reward lever. In the same session, rats were also trained to press the other lever to avoid a punishment (95 dB SPL white noise presented for 30 s) when a different tone (60 s, 9 kHz or 2 kHz at 75 dB SPL, counterbalanced) was presented. In the negative trials, the stimulus duration was 60 s, whereas in the positive trials the stimulus duration was only 30 s. This was done to compensate for the fact that rats tend to learn positive trials more rapidly than negative trials. Presentation of white noise has been shown to be effective in punishment and avoidance training (Bolles and Seelbach, 1964). The tone and the lever were referred to as the avoidance tone and avoidance lever, respectively.

We selected the frequencies of ambiguous tones based on the responses of four rats used in the pilot study, which used a similar behavioral training paradigm to the current study. We decided to use 3 intermediate tones, each at a different frequency to ensure that rats did not respond to a given tone in a specific way due to innate sensitivities for certain frequencies. Tones were 4 kHz, 6 kHz and 8 kHz (30 s, 75 dB SPL) for Group A, and 4 kHz, 3 kHz and 2.5 kHz (30 s, 75 dB SPL) for Group B. These tones were chosen because these could evoke similar responses between Group A and Group B as in the pilot study. Specifically, 6 kHz and 3 kHz for Groups A and B in the pilot study, evoked about 50% positive responses (pressing reward levers) and 50% negative responses (pressing avoidance levers) in the absence of emotional manipulation.

In Groups A and B, the 4 kHz tone was termed the "RP tone" (Reward Proximate tone). The 6 kHz tone in group A or 3 kHz tone in Group B was termed the "N tone" (Neutral tone). Lastly, the 8 kHz tone in Group A or 2.5 kHz tone in Group B was termed the "AP tone" (Avoidance Proximate tone).

The initial training was carried out as follows. First, a reward or avoidance tone was played at random (termed a reward or avoidance trial) and both left and right levers were presented. If the rats pressed the reward lever when reward tone was played, they received a reward. If they pressed the avoidance lever when avoidance tone was played, they avoided a punishment but no reward was presented. If they pressed the incorrect lever in either reward or avoidance trials, they received the punishment and the same trial was continued in order to reform the false response (correction trial). In addition, if they did not respond to reward tones within 30 s or avoidance tones within 60 s, the punishment was not presented, but a correction trial was inserted. Rats could not advance to the next trial unless they pressed the correct lever. The correction trials continued until this occurred. Within a 60-min period, we performed 150 trials (75 reward trials and 75 avoidance trials, not counting the correction trials). Trials were separated by a 10 s inter-trial interval (ITI). Training sessions occurred once per day. During the early days of training, nearly all rats were unable to finish the 150 trials within the 60-min time period. In these cases, we

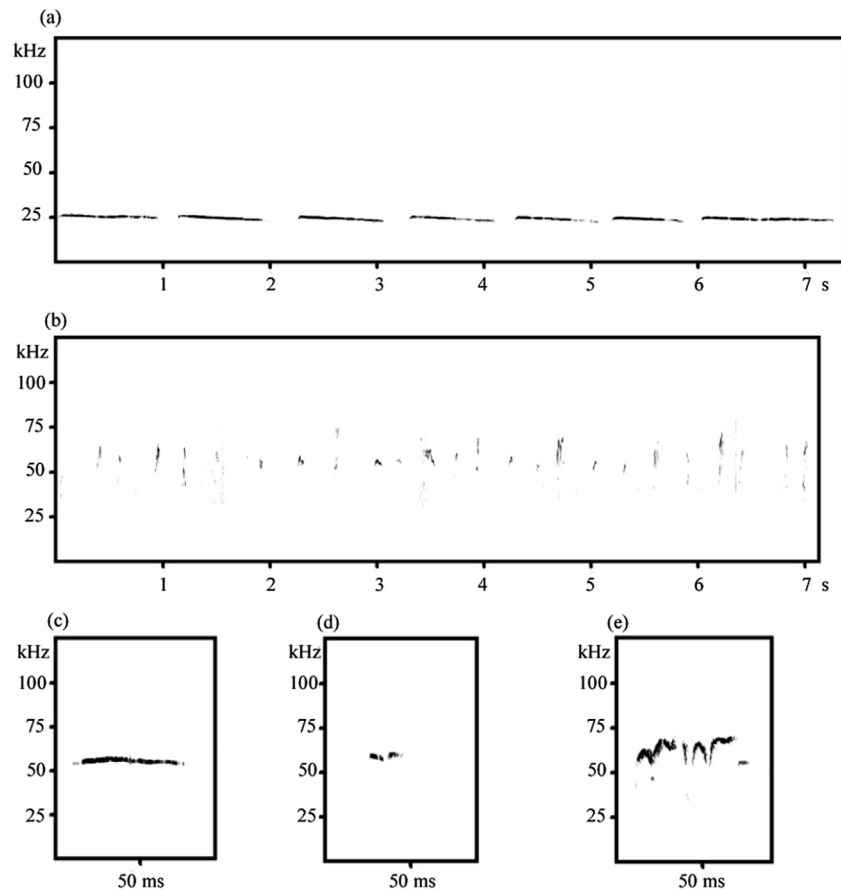


Fig. 1. Sample of recorded ultrasonic vocalizations.

Examples of stimuli used in our experiments. For each spectrogram, the x-axis represents time, and the y-axis represents frequency. (a) One sample of the 22 kHz stimuli, which was composed of 7 syllables which had long durations (300–3400 ms; Sales, 1972). (b) One sample of the 50 kHz stimuli, which was composed of 24 syllables which had short durations (3–65 ms; Sales, 1972). Both (a) and (b) are about 7 s in total duration. (c–e) Variations of 50 kHz USVs. (c, e) have long durations, whereas (d) is very short. (c, d) have little frequency modulation and appear flat, whereas (e) exhibits frequency modulation.

stopped the training at the 60-min time limit. However, within one month, rats were able shape their discriminative responses to the two tones. We continued with a second training if a rat showed a correct response rate of more than 85% two days in a row for both reward and avoidance trials.

In the second training, the following trials were added to the original training. In 10% of trials, there was no reward or punishment given if rats responded at any time during the presentation of the reward or the avoidance tone (no reinforcement trial). These trials were added in order to prepare rats for the ambiguous-cue trials on the probe tests since ambiguous-cue trials do not have any rewards or punishments associated with them. This procedure was modified from a previous study (Parker et al., 2014). Within a 60-min period, we performed 160 trials (72 reward trials, 72 avoidance trials, 8 no reinforcement reward trials and 8 no reinforcement avoidance trials). Trials were separated by a 10 s ITI and this second training also occurred once per day. If a rat showed a correct response rate of more than 85% two days in a row for both reward and avoidance trials, we considered the rat to have learned the “correct” stimulus-response contingency for the training stimuli and the rat was run in a test session on the next day.

2.5. Testing

2.5.1. Presenting USVs

If rats satisfied all the conditions mentioned above, they continued on to the testing phase. The 50 kHz or 22 kHz USV stimuli

were presented in the Skinner box (termed 50 kHz condition or 22 kHz condition) 20 min prior to the probe test. Alternatively, in the control condition, no stimulus was presented and rats could move freely in the box for 20 min. In the 50 kHz condition, we presented 3 wav files of 50 kHz USV stimuli in a random order, each wav file presented 20 times (60 times total, 13.0 s Inter-Stimulus Interval (ISI), 59.9 ± 4.63 dB SPL, 6.80 s average duration). In 22 kHz conditions, 6 wav files of 22 kHz USV stimuli were presented in a random order, each presented 10 times (60 times total, 13.0 s ISI, 59.7 ± 4.45 dB SPL, 7.26 s average duration). All individuals were assigned to these 3 conditions in a random order.

2.5.2. Probe test

We conducted the probe test session immediately after the USV presentation phase. The 3 different ambiguous-cue trials (RP, N or AP tone trials) were added to reward and avoidance trials and presented at random. In the ambiguous-cue trials, neither reward nor punishment was given to the rats. The probe test session consisted of 150 trials (65 reward, 70 avoidance, and the 3 ambiguous cues presented for 5 trials each). Trials were separated by a 10 s ITI. During the session, we recorded the proportion of pressing the reward or avoidance levers on ambiguous-cue trials. After test sessions, rats continued to perform the second training for one week and were given a second probe test a week later in order to avoid habituation to USVs and ambiguous stimuli. All individuals were tested once for each condition in random order, so they experienced three test

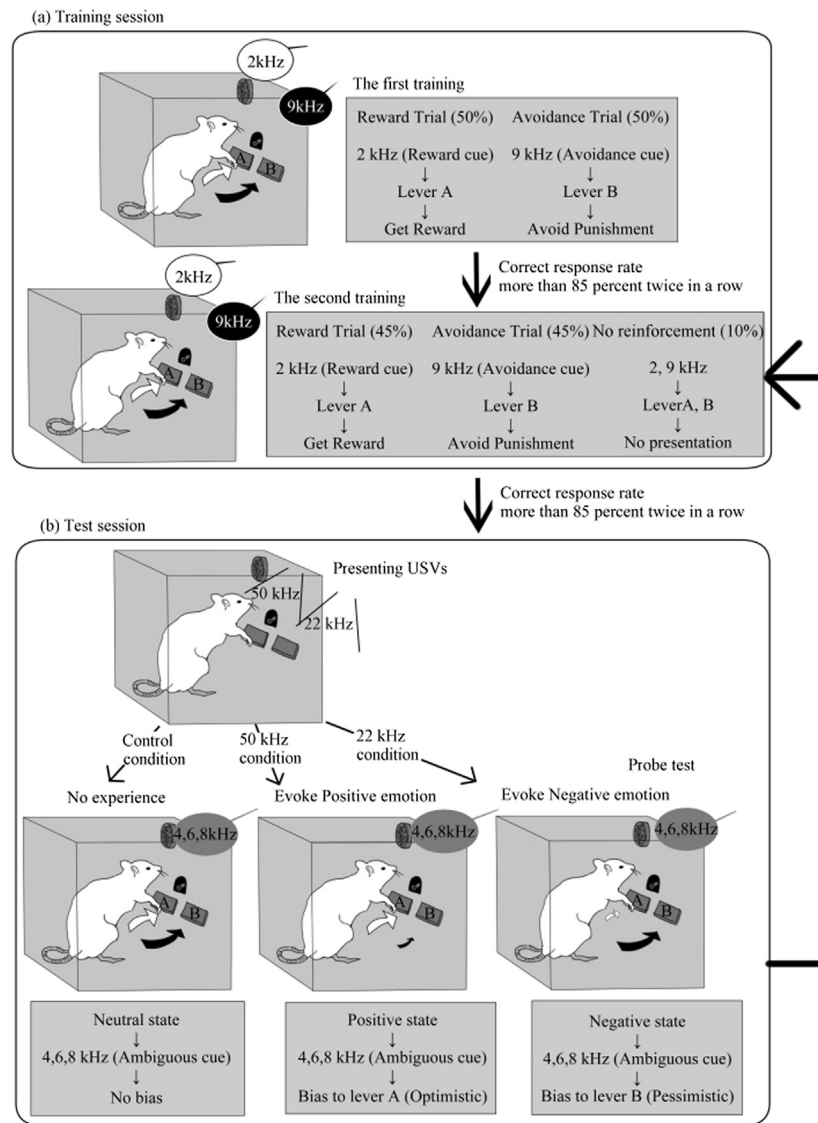


Fig. 2. The procedures for training and testing in the cognitive bias task.

The figures depict the procedures for the training and test sessions in Group A. (a) The training sessions. In the first training, rats are conditioned to press lever A to receive a reward after hearing a 2 kHz tone (reward trial), and to press lever B to avoid a punishment after hearing a 9 kHz tone (avoidance trial). If a rat showed a correct response rate of more than 85% two days in row for reward and avoidance trials, the rat moved onto the second training. In the second training, there were a proportion of trials in which there was no reward or punishment if rats responded at any time during the presentation of 2 kHz or 9 kHz tone (no reinforcement trial). No reinforcement trials accounted for 10% of trials; the remaining trials were reward or avoidance trials. If a rat showed a correct response rate of more than 85% two days in a row, the rat moved onto the test session. (b) The test session. First, we presented 50 kHz stimuli (50 kHz condition), 22 kHz stimuli (22 kHz condition) or no presentation (control condition) for 20 min. Once the presentation phase finished, rats moved onto the probe test, which included ambiguous tone trials. When an intermediate tone of 4, 6 or 8 kHz was presented (ambiguous trials), rats with a positive emotional state are expected to press the lever A in response to the stimulus while rats with a negative emotional state are expected to press lever B. Rats were tested in each USV condition. Each test session was separated by one week of the second training, so that rats did not habituate to the 50/22 kHz USV stimuli. Conversely, in Group B, we used the 9 kHz tone as the reward cue and the 2 kHz tone as the avoidance cue. In this case, ambiguous cues used were 4, 3 and 2.5 kHz tones.

sessions in total. Fig. 2 illustrates the procedure for the training and test sessions, including the cognitive bias task.

2.6. Analysis and statistics

We analyzed the data of 12 individuals to determine the rate at which each lever (reward or punishment) was pressed. For the data analysis, a 2-way (condition by probe tone) repeated (animals) within subject analysis of variance (ANOVA) was used to assess whether the 50 kHz or 22 kHz USV presentation had an effect on the rate of pressing reward levers or avoidance levers. Following that, the Bonferroni multiple comparison tests were performed to compare among the three conditions (control, 50 kHz, and 22 kHz condition) with each of the three probe tones. All data

were analyzed using BellCurve software (Social Survey Research Information, Tokyo, Japan).

3. Results

It should be noted that only 3 of the 540 ambiguous trials (0.56%) were trials in which no response was given due to time out of stimulus presentation. The statistical results were practically the same (p values were the same until the third decimal place) between the rate of pressing the reward and avoidance levers, because animals had to choose between the two levers. A high rate for the reward lever would automatically result in a low rate for the avoidance lever, and vice versa. Thus, we only report statistics for the reward lever.

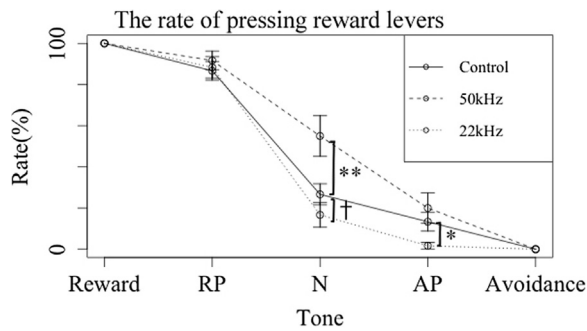


Fig. 3. The average rate of pressing reward levers for the three ambiguous stimuli and the reward and avoidance stimuli.

Error bars indicate the standard error. The symbol of ** means $p < 0.01$, * means $p < 0.05$ and † means $p < 0.10$. Under the 50 kHz condition, N tone was perceived as more similar to the reward stimulus (optimistic bias), while under the 22 kHz condition, AP tone was perceived more similar to the avoidance stimulus (pessimistic bias). The graph for the avoidance lever would look like the upside-down version of this graph, because animals responded either of the levers.

Fig. 3 shows the average rate of pressing reward levers; a significant difference was observed among the 3 conditions (ANOVA: $F(2,96) = 9.24$, $p < 0.001$) and the 3 stimuli ($F(2,96) = 167.46$, $p < 0.001$). Our analysis treated the differences among individuals as a repeated measure. There was a significant interaction between USV conditions (50 kHz and 22 kHz) and ambiguous stimuli ($F(4,96) = 3.51$, $p < 0.05$). When N tone was presented as ambiguous probe, multiple comparisons detected a significant difference between the control and 50 kHz condition ($p < 0.01$), a marginal difference between the control and 22 kHz condition ($p < 0.10$), and a significant difference between the 50 kHz and 22 kHz condition ($p < 0.01$). When AP tone was presented as ambiguous probe, multiple comparisons detected a significant difference between the control and 22 kHz condition ($p < 0.05$), and a significant difference between the 50 kHz and 22 kHz condition ($p < 0.01$).

In sum, under the 50 kHz condition, N tone was perceived as more similar to the reward stimulus (optimistic bias), while under the 22 kHz condition, AP tone was perceived more similar to the avoidance stimulus (pessimistic bias).

4. Discussion

Our results show that rats tend to respond more positively to ambiguous stimuli (by pressing the reward lever) when 50 kHz USVs are presented and more negatively when 22 kHz USVs are presented. These results suggest emotional contagion occurs via conspecific USVs in rats for both 50 kHz and 22 kHz USVs, supporting our hypothesis.

As stated in the introduction, our procedure was designed to compensate for the limitations of previous studies. First, we were able to quantitatively measure emotional states of the animals, for both positive and negative contexts, using the same behavior (lever pressing). We did this by calculating the bias of the response rate (pressing the reward lever vs. the avoidance lever) because the association of these conditioned behaviors with positive or negative events was mutually exclusive. The previous studies were limited to the case of specific, negative-oriented contagion, such as pain (e.g. Langford et al., 2006) because rodents more readily express negative affective behaviors than positive ones. Using this cognitive bias task, we can more comprehensively study emotional valence in animals because we can examine both negative and positive-oriented contagion.

Second, this cognitive bias task is able to measure the internal affective states of animals because the conditioned behaviors

(pressing reward or avoidance levers) in this task change in accordance with animal emotion (i.e., positive or negative states). In contrast, previous research was not able to examine internal states unless they were expressed as innate behaviors. This fact limited the study to innate responses evoked by specific stimuli (i.e., pain), and researchers cannot disentangle empathetic responses from the innate releasing mechanisms resulting in these behaviors. On the other hand, because pressing reward or avoidance levers is a behavior learned specifically through operant conditioning, it is only influenced by emotional states and not by any other innate behavioral patterns. It should be noted, however, that measuring internal states via innate responses has more ecological validity than our cognitive bias task, and that we can discuss only affective changes that result from emotional contagion using this task. Therefore we consider our method for measurement to be complementary to that of previous studies. Taken together, both methods tell us a great deal about the emotional state of animals.

In conclusion, our cognitive bias task complements past research into animal emotion, and suggests that there is empathic communication, both for positive- and negative-oriented empathies in rats. This communication is thought to share emotional states via specific acoustical signals, and may have a relationship to non-linguistic primitive communication in humans, such as laughter or crying. It is important to clarify the common underlying neural basis of this phenomenon. For example, neural controls of USVs originate in the amygdala and periaqueductal gray matter, and these areas of the brain are also related to emotional expression and perception (Panksepp, 2007). It is possible to clarify the relationships between emotional expression, perception, and behavior by examining and comparing neural systems among mammalian species; rodents provide a good model for this purpose.

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