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„Body temperature responses to USV stimuli in adult male rats"

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Table of contents

Introduction

Reliable assessments of emotional states are a core aspect to animal welfare research. Tools used for these assessments should be both cost efficient and non-invasive to be applicable on a larger scale. Infrared thermography technology provides a noninvasive method of measuring surface temperature (Speakman & Ward, 1998). Studies in homoiothermic species such as rats, chickens, dogs, cows, and humans have shown that changes in an animal's body temperature reflect emotional states (Briese & Cabanac, 1991; Moe et al., 2017; Proctor & Carder, 2015; Salazar-López et al., 2015; Travain et al., 2016). Here we set out to further our understanding on infrared thermography as a non-invasive method of measuring body temperature in relation to emotional states.

Emotions can be described in two dimensions: arousal and valence (Mendl et al., 2010). Valence describes if an emotion is perceived as positive or negative ranging from pleasant to unpleasant while arousal describes the intensity ranging from low (e.g. calm) to high (e.g. excited) (Mendl et al., 2010). Previous studies show a strong correlation between emotions of negative valence and changes in the peripheral temperature (Briese & Cabanac, 1991; Edgar et al., 2013; Gjendal et al., 2018; Moe et al., 2017; Vianna & Carrive, 2005). When rats were confronted with a stressful situation (e.g. removal from home cage and being subjected to invasive handling) the mean surface temperature of the tail base decreased initially. This initial decrease was followed by a sharp increase due to vasodilation until it reached a plateau (Briese & Cabanac, 1991). In contrast to that, brain temperature rose from the beginning until it reached a plateau (Briese & Cabanac, 1991). This thermogenesis during a fear response is a result of the activation of the sympathetic nervous system causing vasoconstriction in the extremities which leads to a drop in the temperature of the extremities and a rise in the core temperature (Marks et al., 2009). Stress related drops of peripheral temperature have been recorded across different vertebrate species (Briese & Cabanac, 1991; Edgar et al., 2013; Gjendal et al., 2018; Moe et al., 2017). It remains unclear though if the temperature change is correlated with emotional valence, arousal level, or both.

A study on hens (*Gallus gallus domesticus*) proposed that a stress related drop in peripheral temperature reflects the intensity of a stressor: stimuli that cause a greater stress response led to a bigger drop in peripheral temperature compared to stimuli that cause a smaller stress response (Herborn et al., 2015). Chicken anticipating treats, and therefore most likely in an emotional state of positive valence, show a similar decrease in peripheral temperature (Moe et al., 2012). These results led to the proposal of the peripheral temperature being an indicator of arousal rather than emotional valence (Moe et al., 2012). Contrasting Moe and colleagues (2012), Proctor and Carder (2015) suggested that a drop in peripheral temperature indicated a change in valence regardless of the direction (Proctor & Carder, 2015).

To better understand the relationship between emotional states and temperature, our study examines the effect of stimuli of different emotional valence (positive, negative and neutral) on the surface temperature of male rats. The same valanced stimuli can induce different surface temperature changes in different body parts (Dezecache et al., 2017). To investigate the effects of differently valanced stimuli on the changes in surface temperature of different body parts, the surface temperature of three different body parts (eyes, body, tail) was recorded in this study.

Rats are highly social animals with a complex acoustic communication system (Simola & Brudzynski, 2018). Adult rats produce two different types of ultrasonic vocalization (USV), 22kHz and 50kHz calls, to communicate their emotional state (Brudzynski, 2013).

50 kHz calls are produced when rats are in a positively valanced emotional state (Brudzynski, 2013; Burgdorf et al., 2008) like positive social context, rough-and-tumble play, and mating (Burgdorf et al., 2008). Rats were shown to prefer frequency modulated 50 kHz calls in the Self Administration Box (Burgdorf et al., 2008) and respond to the playback of 50 kHz USVs with approach behavior and dopamine release in the nucleus accumbens (Willuhn et al., 2014). 22 kHz calls are produced when rats are in negative affective states (Brudzynski, 2013). They are emitted during aversive or withdrawal behaviors during play, mating, as aggressive response and in fear conditioned contexts (Kim et al., 2010, Burgdorf et al., 2008). 22 kHz vocalizations of fear conditioned rats lead to increased freezing behavior in their conspecific partner rat if this individual had previously experienced an aversive event (Kim et al., 2010). This indicates that the 22 kHz vocalizations are one key factor for social transmission of fear in rats that have experienced fear before (Kim et al., 2010).

USV playbacks are not only perceived but they also have the capacity to change the rats' emotional state (Brudzynski, 2013).

To sum up, emotional states have the potential to influence the peripheral temperature of animals (Dezecache et al., 2017; Moe et al., 2017; Proctor & Carder, 2015; Travain et al., 2016; Watanabe, 2015) and USVs have the capacity to change the emotional state of rats

(Brudzynski, 2013). Therefore, we hypothesize that the body temperature will respond to the playback of 50 and 22kHz ultrasonic vocalizations. The 22-kHz stimulus in our study is intended to elicit an emotional state of negative valence while the 50-kHz stimulus was intended to elicit an emotional state of positive valence. For playback of 22kHz USVs we predict that the rats' tail surface will show the same temperature pattern as that of the stressed rats in study of Briese and Cabanac (1991). If emotional valence is the decisive factor for the temperature changes, then we predict the temperature response after playing 50 kHz calls to differ from both the negative (22 kHz) and the neutral stimulus.

Methods

All housing and testing procedures were approved by the University of Vienna, Faculty of Life Sciences Animal Ethics and Experimentation Board ethics (ethical approval number 2020-18).

Animals and housing

We used a population of 8 adult male rats (Rattus norvegicus domestica) in this study. One rat died of old age before completing the experiment and another rat chose to not participate in this study, which led to 6 rats completing this study. Willingness to participate in this study voluntarily was assessed by the rats entering the transport carrier. At the start of data collection, the rats' age ranged from ten months (two individuals) to eleven months (four individuals). All rats were housed as a group, sharing a two-floor enclosure with the dimensions 80 x 75 x 161 cm in the animal care facility at the Department of Cognitive and Behavioral Biology. Enrichment was distributed across the entire cage. Food and water were provided ad libitum. This study was conducted between the 8th of September 2020 and the 10th of December 2020. Since body temperature of rats fluctuates with the circadian rhythm (Kittrell & Satinoff, 1986) all measurements were taken at the same predefined time slot (16:00 - 18:30).

Thermal imaging

Pictures of the surface temperature of eye, body and tail were taken with a handheld infrared camera (FLIR E8). For the habituation and experiment phase of the study the ambient temperature and air humidity was measured. To measure the reflected temperature a patch of black tape was placed on the rats' enclosure during baseline measurement and stuck to the bottom of the plastic test box in the experimental set up. In a previous study the emissivity of the rat fur and skin was established to be 0.98 (Vianna & Carrive, 2005); we adopted this emissivity value during all our rat body temperature measurements. The infrared pictures were analyzed with the software FLIR Tools (version 5.13). Where visible, the three sites of interest (eye, body, tail) were marked, and the temperatures noted. In FLIR Tools, the maximum temperature of the eyes was measured by placing a box around the eyes, the maximum temperature of the body by placing a line down the center of the body between the shoulders and pelvis, the maximum temperature of the base of the tail by placing a box at the point where the hairless part of the tail begins (Fig. 1.).

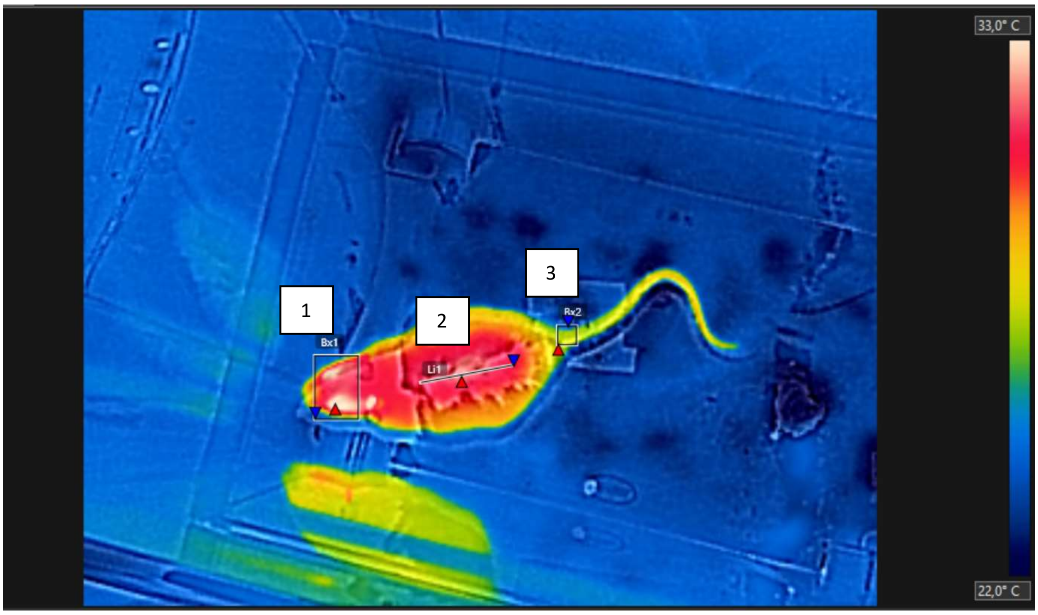

Figure 1. Infrared image of a rat. The value of the surface temperature is indicated by the color scale. The lowest temperatures of the color scale (22 °C) are shown with dark blue and the highest temperatures of the color scale (33 °C) are shown with white. The three sites of interest (eye, body, tail) that were measured are indicted with numbers: 1. The maximum temperature of the eyes. 2. The average temperature of the body (midline of the body between shoulders and pelvis). 3. The maximum temperature of the tail base.

For each individual picture the corresponding room temperature, humidity and the reflected temperature were input into the software to calculate surface temperatures of the measured body parts. For the baseline period the thermohydrometer was discovered missing leading to missing data points for environmental temperature and humidity. Since the animal care facility is air conditioned and ventilated, temperature and humidity remain stable. We therefore compensated the missing data by using the average temperature and humidity measured after one week of baseline measurements. This temperature and humidity data was collected on 5 days (September 21st, 22nd, 23rd and October 6th and 8th) during the same time slot (16h -18h) in which the baseline measurement had taken place. For those 5 days an average temperature of 22.86 °C (sd = 0.69 °C) and an average humidity of 45,2% (sd = 7.5%) were recorded.

Experimental setup

There were two parts of the experiment: first, the baseline measurements, which was conducted in the home range cage in the rats' normal social groups (i.e. a more naturalistic setting) and second, the experimental measurements, which consisted of the habituation and the experiment and was conducted in the experimental room (i.e. controlled environment). The baseline measurements were conducted for two reasons: First, to test how the rats' behavior in their home environment affected their temperature, and second, to control for the effects of the experimental setup. After the baseline the habituation was conducted (at least three sessions of letting the animal habituate to test room with no stimuli being presented). During habituation, the rats were acclimated to the experimental arrangement to make sure the rats were habituated to our setup and the effects measured were indeed in response to the stimulus and not the environment. Infrared images were taken during habituation to obtain temperature data for comparison to baseline. After successful habituation the experiment was started. In the experiment we wanted to test if the type of stimulus had an effect on body temperature. For this the rat was presented with one of the three stimuli (22 kHz calls-negative stimulus, 50 kHz calls-positive stimulus, background noise-neutral stimulus) before the infrared images were taken. For the measurements during the habituation and experiment the rat had to be transported from the home enclosure to the experimental room. The rats' participation in the study was voluntary to avoid stress responses that could have been triggered by handling. If a rat entered the transport carrier it received a food reward and was transported to the test room where it received another food reward after leaving the carrier and entering the test arena. Room temperature puréed Spaghetti Bolognese baby food was chosen as a reward.

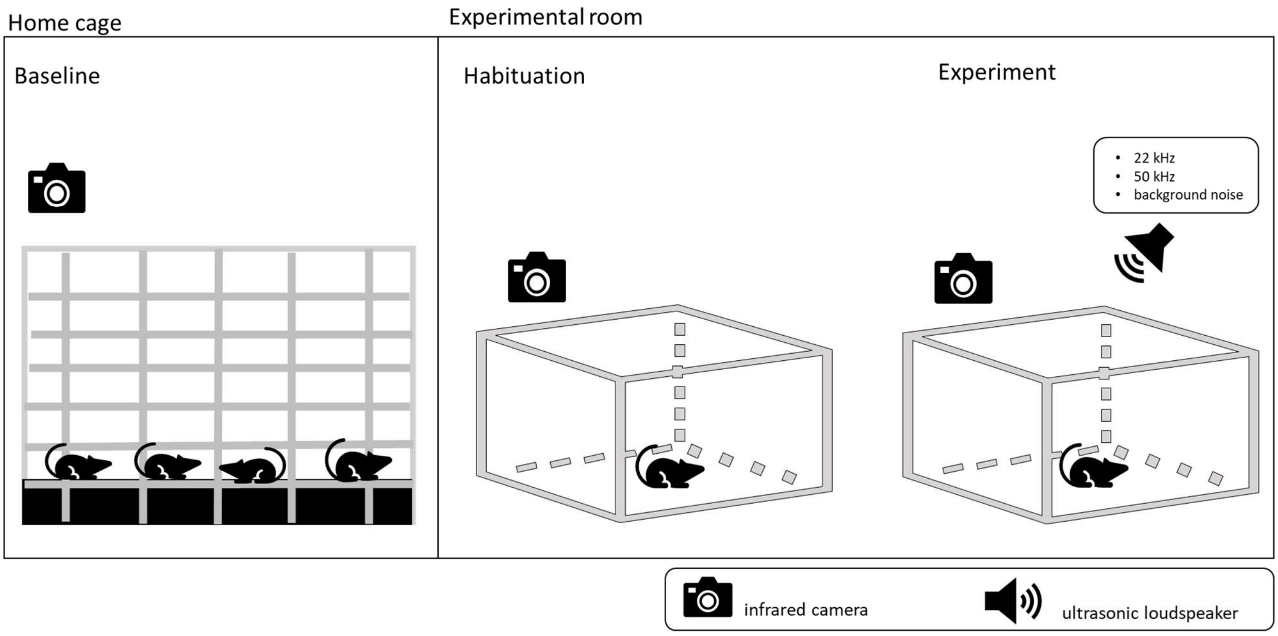

Figure 2. Summary of the three phases of the study. For the Baseline measurements infrared images of each rat were taken in their home cage environment. Habituation and experiment took place in the experimental room with a single rat being brought into the experimental set up. During the habituation phase, the rat remained in the test area for 30 minutes without being presented with any stimuli, while in the experiment phase, the rat remained in the test area for 30 minutes after being presented with one of the three stimuli. Infrared images of each rat were taken every minute while the rat was in the test area.

Baseline measurements

The baseline measurements were taken in the animals' normal living environment, their home cage. Infrared images were taken of each rat at 30-minute intervals for two hours.

The behavior of the rats was recorded during each infrared measurement. The baseline measurements were conducted on five weekdays between the 8th of September 2020 and 14th of September 2020.

Table 1. Overview of the recorded behaviors

Behavior	Explanation
Sleeping alone	Sleeping without body contact to another rat
Sleeping together	Sleeping in body contact with at least one rat
After sleeping alone	Less than 1 minute before the picture was taken the rat was sleeping alone
After sleeping together	Less than 1 minute before the picture was taken the rat was sleeping together
Active	Not sleeping
Eating	Consumption of food

Experimental measurements

The experiment was run between the 22nd of September 2020 and the 10th of December 2020. During the experiment the rats were placed in a plastic box (45 x 57,5 37.5 cm) that was surrounded by a metal frame with a height of 80 cm. This frame provided attachment points for all equipment and a stable point of equal distance above ground on which the infrared camera rested while taking pictures. Before each session the room temperature, humidity and the reflected temperature were measured. Two video cameras a Nikon Coolpix B500 (HD 1,920 × 1,080, mp4 format) and a GoPro Hero6 (HD 1,920 × 1,080, mp4 format) were used to film the rat's behavior during the session. A bat USV detector (Echo Meter EM3+ from Wildlife Acoustics; https://www.wildlifeacoustics.com/) was used to record the ultra-sonic vocalizations and an H4N recorder was used to record the comments of the experimenter. The recordings were saved in wav format.

For the habituation phase the rat was put in test area with no stimuli being presented. An adult rat's body needs approximately 31 minutes after stimuli presentation to revert to baseline body temperature (Briese & Cabanac, 1991). Therefore, every individual habituation session lasted 30 minutes. Before each session, the name of the rat, date, time, humidity, and room temperature were recorded. At the beginning of every minute an infrared picture was taken which led to a total of 30 pictures per session. After 30 minutes the rat was transported back to the home cage. A rat was habituated a maximum of once per day. The habituation process was seen as successfully if the rat did not show any stress related behaviors three sessions in a row. These behaviors include freezing, 22 kHz vocalization or trying to escape the test arena multiple (≥ 10) times.

After successful habituation three different playback stimuli were presented during the experiment: 22kHz (negative), 50kHz (positive) and background noise. These stimuli were derived by using the software Praat (www.fon.hum.uva.nl/praat/) from a 2-minute-long audio recording of one male rat from a different rat population that never came into contact with the tested rats. The 22 kHz and 55 kHz stimuli were created from the audio file by always inserting a pause after each call and then another call until 1.4 seconds total playback time were reached. The control stimulus was derived by extracting 1.4 seconds of background noise from the original recording. All stimuli were saved and played back in wav format.

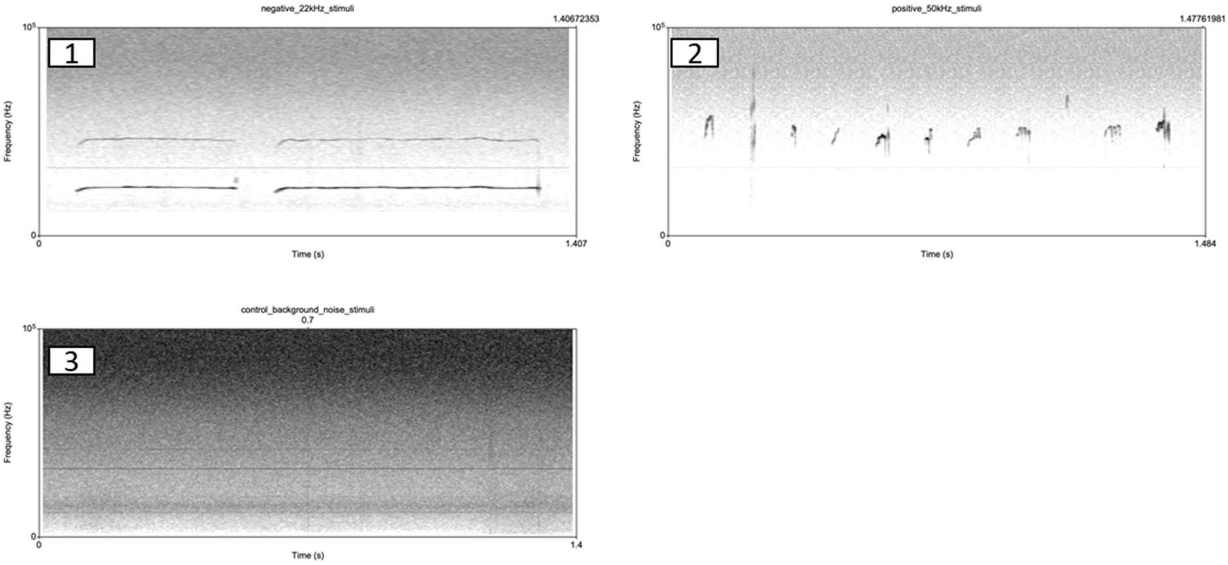

Figure 3. Spectrograms of the three stimuli used in the study: 1. picture: 22 kHz calls (negative stimulus), 2. picture: 50 kHz calls (positive stimulus), 3. picture: background noise (neutral stimulus). All stimuli lasted ~1.4 seconds

For the playbacks, an ultrasonic loudspeaker (Ultrasonic Dynamic Speaker ScanSpeak : http://www.avisoft.com/discontinued/scanspeak.htm) was placed on a table next to the experimental box. Before the experiment was started, we checked that the setup produced playbacks as intended.

During each experimental session the rat was placed in the setup rewarded with food for completing the transfer and was given 5 minutes to acclimate before one of the three playback stimuli was presented. The stimulus was played back once. The test lasted for 30 minutes after presentation of the playback stimuli, leading to 30 temperature recordings for each session. Each rat was tested twice in each condition leading to a total of six test sessions (2x22kHz, 2x50kHz, 2x white noise) per rat. The stimuli were presented counterbalanced and pseudorandomised with no direct repetitions of a stimulus. Each rat was tested a maximum of once per day.

Statistical analysis

Statistical analyses were performed in R (Version 4.0.5; www.r-proje ct.org/) and RStudio (Version 1.4.1106; www.rstudio.com/) with the packages plyr, car, ggplot2, readxl, dplyr, ggpubr, reshape2, tidyverse, psych, pastecs, tidyverse, lme4, doBy, performance and lmerTest. All models were created by using with the function “lmer” from the package “lme4”. All models were full- null model tested and examined for residual distribution, visually examined through histograms, qqplots and residual-fitted value plots. I did not find indications regarding deviations from the model assumptions.

Statistical analysis: baseline measurements

To see how the rat's behavior in the home cage is correlated with temperature, the behavioral data was compared with the temperature data from the infrared pictures. I created a separate linear mixed-effects model for each measured body part (tail, eye and body). For the three models the response variable was the measured temperature so either tail-, body- or eye temperature. The behavior and subject identity were included as fixed effects and to control for effects of date this variable was included as a random intercept.

The significance of each full model (model with all variables) was compared with the null model (identical model to the full model but without the fixed effect behavior). Full model and null model were compared by using the R function ANOVA (test = "Chisq"). The models included 123 data points for the eye temperature, 105 data points for the tail base temperature and 99 data points for the body temperature, collected from 6 individuals on 5 days.

Statistical analysis: baseline vs habituation

Since stress causes a drop in average tail temperature in rats (Briese & Cabanac, 1991), we compared the minima of the tail temperatures between baseline (home cage) and habituation (experimental room) to determine whether the experimental setup had an effect on the tail temperature. The minimum of the tail temperature was calculated for each rat per session and compared between baseline (home cage) and habituation (experimental room). To provide an accurate comparison, only baseline data collected when the rat was active and not asleep were selected for comparison with the habituation. In total these two datasets consisted of 42 observations, 23 for the baseline and 19 for the habituation phase.

A linear mixed-effects model was created with the minimum tail temperature as the response variable. The type of environment (home cage or experimental room) and the subject identity were included as a fixed factor. To control for effects of date this variable was included as a random intercept. The significance the full model (model with all variables) was compared with the null model (identical model to the full model, but without the fixed effect type of environment). Full model and null model were compared by using the R function ANOVA (test = "Chisq").

Statistical analysis: experiment

To compare the effect of the three experimental conditions (22 kHz playback, 50 kHz and background noise) on the temperature three linear mixed-effects models were created with delta temperature values as the response variable. We used temperature delta values to control for individual and daily variation of temperature. The delta values were calculated by subtracting the value of temperature of minute 30 (last recording of the temperature for each session) from all other values from this session. If the last temperature recording (minute 30) was missing the session was excluded from the dataset. The dataset for delta values of the eye temperature included 23 individual sessions with a total of 356 temperature recordings. The dataset for delta values of the tail base temperature included 34 individual sessions with a total of 980 temperature recordings, and the dataset for delta values of the body temperature included 32 individual sessions with a total of 945 temperature recordings.

For each measured body part (tail, eye and body) a separate linear mixed-effects model was created. The type of playback stimulus (22 kHz playback, 50 kHz or background noise), ratID, order of stimuli (ranging from 1 to 6, since each rat had 6 sessions) were included as a fixed effect. To control for effects of date and session these variables were included as random intercepts. The significance of each full model (model with all variables) was compared with the null model (identical model to the full model, but without the fixed effect playback stimulus). Full model and null model were compared by using the R function ANOVA (test = "Chisq").

Results

Baseline measurements

Rats´ behavior had a significant effect on the measured maximum tail base temperature: (full-null comparison likelihood ratio test: df = 5, χ^2= 35.124, P= 1.421e-06; null model: AIC= 478.84, full model AIC= 453.71). Overall, the maximum tail base temperature was higher for the behaviors “after sleeping together” ,“sleeping together” and “sleeping alone” compared to the behavior “active” (Table 2, Fig. 4).

The behavior had no significant effect on either eye temperature or body temperature. (eye: df = 5, χ^2= 4.1397, P= 0.5295 , body: df = 5, χ^2= 9.98, P= 0.0758).

Table 2. Result of tail base temperature model

		Estimate	**Std. Error**	**df**	**t value**	**Pr(>|t|)**	
	(Intercept)*	28.64644	0.75959	34.06641	37.713	< 2e-16	
Behavior							
	After sleeping alone	1.09308	1.43807	91.98164	0.76	0.449138	
	After sleeping together	1.06083	0.51264	92.14897	2.069	0.041315	*
	Eating	-1.13744	0.65811	93.43615	-1.728	0.087229	
	Sleeping alone	2.28741	0.63757	91.72258	3.588	0.000538	***
	Sleeping together	3.05901	0.72437	92.84279	4.223	5.62E-05	***
Rat individual							
	b	0.06793	0.80636	92.72104	0.084	0.93305	
	c	0.76534	0.84322	92.0881	0.908	0.366439	
	d	0.69609	0.7443	92.07037	0.935	0.352119	
	e	-0.35799	0.79497	92.40683	-0.45	0.653539	
	f	0.45668	0.75926	91.43435	0.601	0.549013	

*Intercept includes the behavior “active” and the rat individual “a”

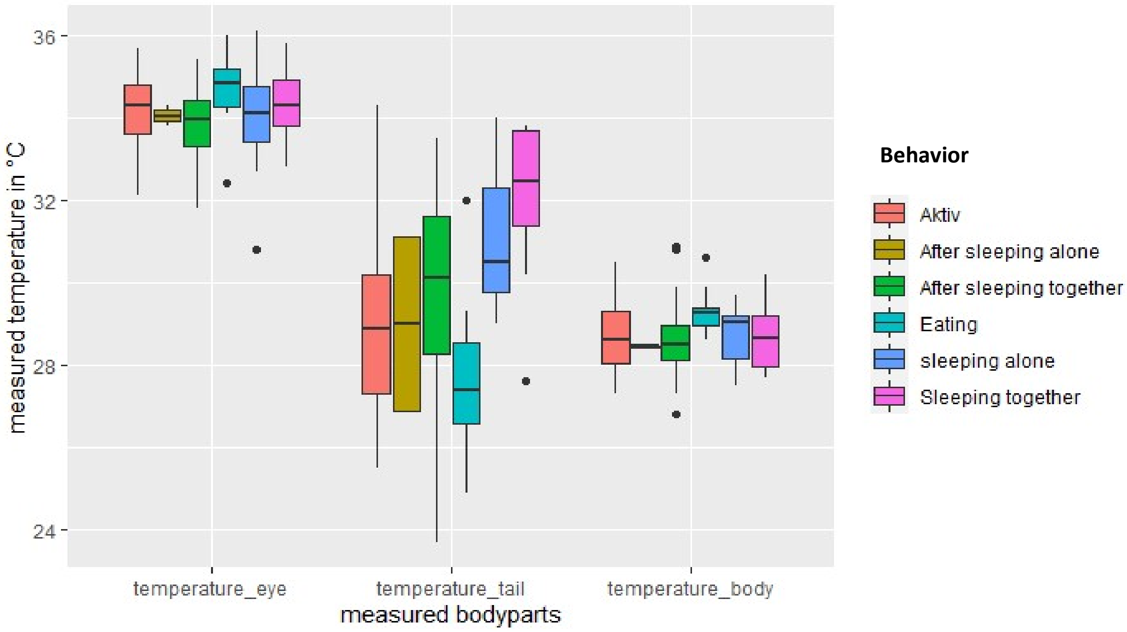

Figure 4. Boxplots for the eye, tail and body temperatures of the 6 rats measured in the home cage. The different behaviors are indicated by the different colors. The x-axis shows the measured body part by behavior. The y-axis shows the measured absolute temperature in Celsius.

Comparing Baseline and Habituation period

The type of environment (home cage or experimental room) had a significant effect on the minimum temperature of the tail base: (full-null comparison likelihood ratio test: df = 1, χ 2= 26.776, P= 2.285e-07 ***, null model: AIC = 200.01, full model AIC= 175.23). Overall, a significantly higher minimum temperature was found in the home cage compared to the experimental room (Table 3, Fig. 5).

Table 3. Result of minimum tail base temperature model

		Estimate	Std. Error	df	t value	Pr(>|t|)	
	(Intercept)*	23.3925	0.7528	35	31.074	< 2e-16	
Environment							
	Homecage	4.0816	0.5365	35	7.607	6.40E-09	***
Individual							
	b	0.1333	0.9947	35	0.134	0.894	
	c	0.5833	0.9947	35	0.586	0.561	
	d	0.894	0.9329	35	0.958	0.345	
	e	0.9177	0.9085	35	1.01	0.319	
	f	0.2465	0.9593	35	0.257	0.799	

*Intercept includes the environment “habituation” and the rat individual “a”

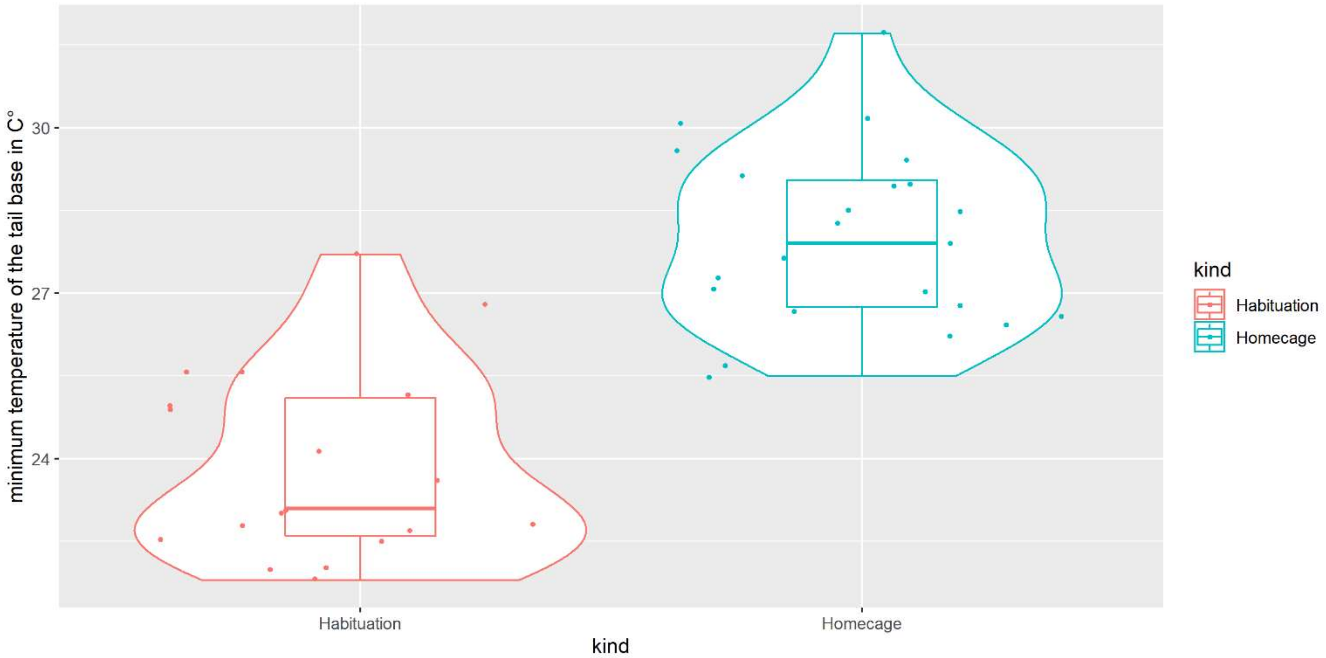

Figure 5. The graph is showing the for the habituation and the home cage period**.** Each point represents the minimum temperature of each rat per day. The x-axis shows the measured kind of environment (habituation or homecage). The y-axis shows the calculated minimum per day per rat of the measured tail base temperature.

Experiment

The type of the stimulus (22 kHz, 50 kHz, background noise) had a significant effect on the delta values of the measured body temperature (full-null comparison likelihood ratio test: df = 2, χ 2= 8.0724, P= 0.01766 *, null model: AIC = 1870.2, full model AIC= 1866.1). Overall, the delta values for measured body temperature differed significantly between the 22kHZ stimulus and the background noise (Table 4, Fig. 6). There was only a significant difference in body temperature response between the neutral and negative stimuli. No significant difference was found either between the negative and the positive stimulus or between the positive and the neutral stimulus.

The type of the stimulus had no significant effect on the delta values of either the measured tail base temperature (Fig. 6) or eye temperature (tail base: df = 2, χ 2= 0.6659, P= 0.7168, eye: df = 2, χ 2= 1.1568 , P= 0.5608).

Table 4. Result of minimum body temperature model

		Estimate	Std. Error	df	t value	Pr(>|t|)	
	(Intercept)*	0.89003	0.42388	21.6819	2.1	0.0476	*
Stimuli							
	neutral	-0.54947	0.25648	25.24859	-2.142	0.042	*
	positive (50kHz)	-0.4863	0.27403	21.14718	-1.775	0.0904	.
Individual							
	b	-0.4255	0.35124	17.71315	-1.211	0.2417	
	c	-0.44808	0.37452	18.36336	-1.196	0.2468	
	d	0.19416	0.31491	10.49621	0.617	0.5507	
	e	-0.45779	0.39297	21.26162	-1.165	0.2569	
	f	-0.42701	0.37339	21.15724	-1.144	0.2656	
Order							
	2	-0.22948	0.39041	20.18513	-0.588	0.5632	
	3	-0.21488	0.41148	21.53125	-0.522	0.6069	
	4	-0.39504	0.39566	18.59518	-0.998	0.3309	
	5	-0.57655	0.38778	20.43016	-1.487	0.1523	
	6	0.02172	0.36233	19.04862	0.06	0.9528	

*Intercept includes the stimuli “negative (22kHz)”, the rat individual “a” and the order “1”

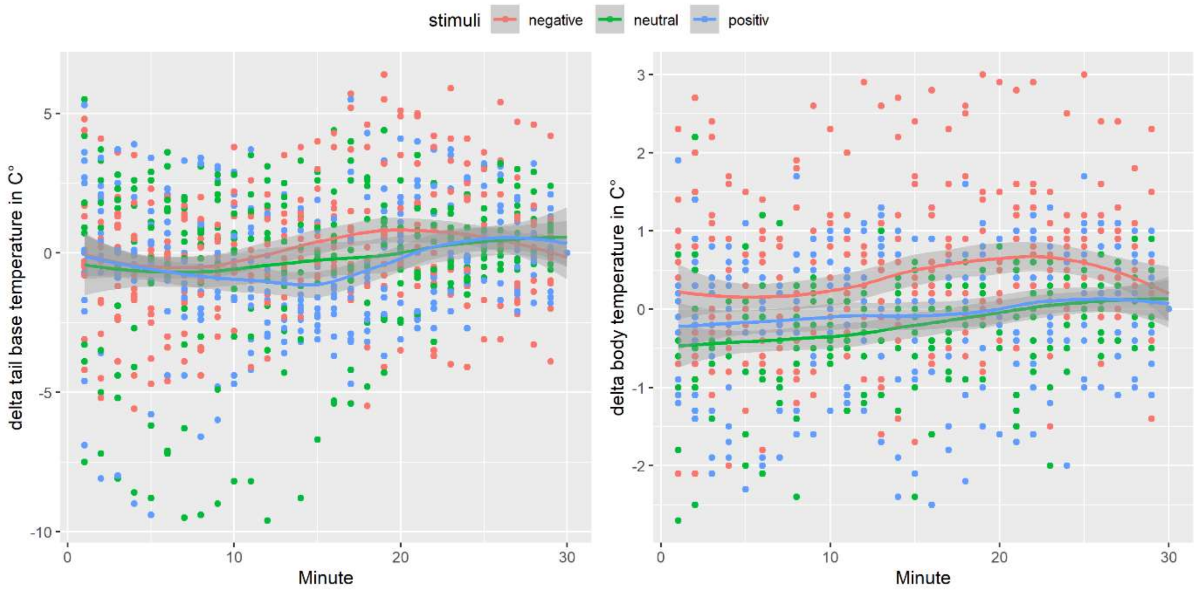

Figure 6. Plots of the delta temperatures of the 6 rats combined throughout the test and all three stimuli. The left plot is showing delta temperatures measured at tail base and the right plot is showing delta temperatures measured at the body. Temperature in Celsius is plotted along the y-axis over the progression of time on the x-axis. The measurements start at minute 1 of the experiment and end after 30 minutes. Each dot represents a measurement of the delta temperature of one rat in one session. The different colors represent the type of playback stimulus. Red lines represent 22kHz (negative) playback stimuli responses, green lines 50kHz (positive) playback stimuli responses, and blue lines background noise (neutral) playback stimuli responses. The type of stimulus had a significant effect on the delta values of the measured body temperature (right diagram), but no significant effect on the tail base temperature (left diagram).

There was no general pattern in the way the delta body temperature of each rat responded to the different stimuli: two individuals showed a clear difference between the negative stimulus compared to the other two stimuli, while the temperature curves of the three stimuli overlapped in the other rats (Fig. 7).

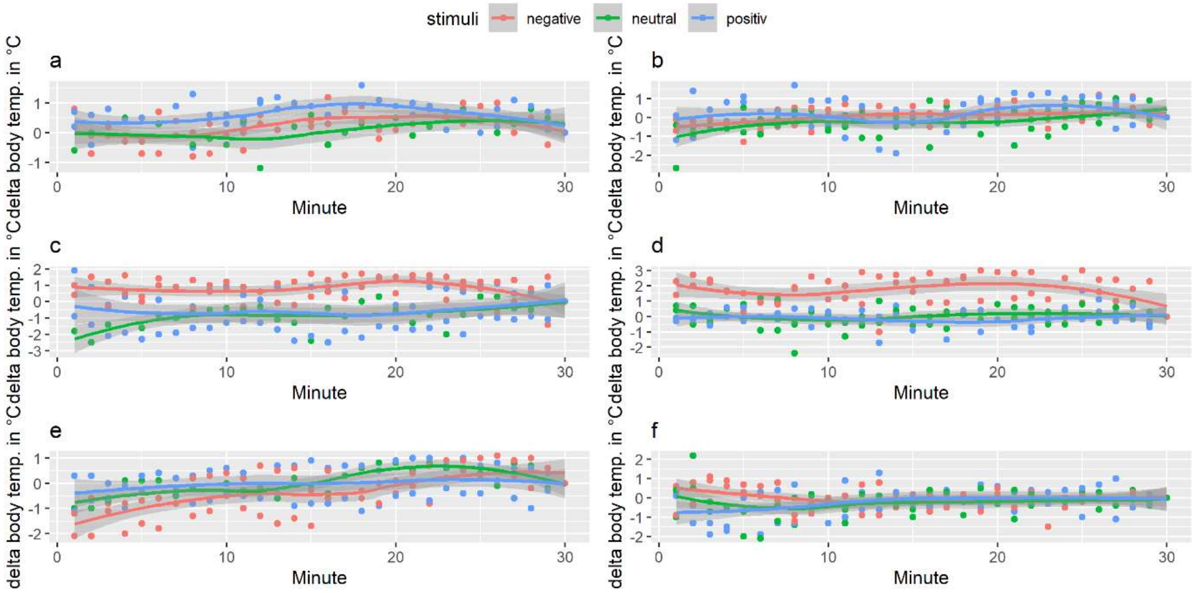

Figure 7. Plot of the delta body temperature for each of the 6 rats throughout the test for the 3 stimuli. For each rat a separate plot is shown. The letters a-f stand for the rat ID. The delta temperature in Celsius is plotted along the y-axis over the progression of time on the x-axis. The measurements start at minute 1 of the experiment and end after 30 minutes. Red lines represent 22kHz (negative) playback stimuli responses, green lines 50kHz (positive) playback stimuli responses, and blue lines background noise (neutral) playback stimuli responses

Discussion

The aim of the present study was to investigate the use of infrared thermography as a tool to assess the well-being of rats by exposing them to three different playback stimuli (22 kHz, 50 kHz, background noise), each of which was expected to evoke different emotional states in rats. The 22-kHz stimulus was intended to elicit an emotional state of negative valence, while the 50-kHz stimulus was intended to elicit an emotional state of positive valence, and the background noise was intended to serve as a neutral control stimulus. To our knowledge, is this the first study investigating how the body temperature in rats responds to different playbacks of ultrasonic vocalization (USV).

Briese & Cabanac, 1991 showed that the surface temperature of the tail base in rats exposed to a stressor first decreased and then increased sharply after about 12 minutes (Briese & Cabanac, 1991). We therefore expected the 22 kHz stimulus to have the same effect on tail base temperature. In contrast to our predication, the tail base temperature did not show the expected temperature response pattern after the 22 kHz stimulus (Fig. 6). Interestingly, the type of stimulus (22 kHz, 50 kHz, background noise) had no significant effect on tail base temperature or eye temperature but it had a significant effect on the delta values of the body temperature, with the temperature response after 22 kHz playback being significantly different from the temperature response after background noise playbacks. Mean delta body temperature values were higher after the 22-kHz playback than after the control stimulus, background noise. Surface body temperature in mice has been shown to correlate with core temperature (Mei et al., 2018) therefore these results presented here may indicate that we measured a stress related increase of the core temperature. This is consistent with a study by Watanabe (2015), showing that body surface temperature of mice increases in response to the stress caused by restrain (Watanabe, 2015).

There was only a significant difference in body temperature response between the neutral and negative stimuli. No significant difference was found either between the negative and the positive stimulus or between the positive and the neutral stimulus. However, the negative stimulus did not have the same effect on all rats: When looking at the individual responses of each rat, there was no general pattern in the way the body temperature of the rats responded to the different stimuli (Fig. 7).

There are several potential explanations for why the rats did not appear to show a clear difference in their temperature response between the three stimuli (except perhaps for body temperature between the negative and neutral stimuli):

First, the ecological relevance of the stimuli. Ultrasonic vocalization (USV) playbacks used in the experiments are social stimuli that are normally produced in a social context (Brudzynski, 2013; Burgdorf et al., 2008; Simola & Brudzynski, 2018). Since the USV playbacks were presented without social context the rats might have perceived them as irrelevant. In this context, it is reasonable to assume that the negative stimulus (22 kHz) might be more ecologically relevant to rats than the positive stimulus (50 kHz). Rats may experience more situations in which it is important to respond to the call of a non-visible stressed rat. In contrast, the 50 kHz calls used to initiate social play/mating might be more relevant when rats are in direct contact. Second, the age of the rats may also have influenced their receptivity to the USV stimuli. Studies suggest that social motivation decreases with age in rats (Perkins et al., 2016; Shoji & Mizoguchi, 2011). Since the age of the subjects ranged from ten to eleven months, these rats might have been less receptive to social stimuli due to their advanced age. It would be interesting to investigate if age has an influence on how rats respond to this experimental setup.

Further it could also be the case that the experimental setting had a greater influence on the emotional state of the rats than the stimuli. To control for the influence the experimental setup had on the measured temperature we compared the minima of maximum tail temperatures between baseline (home cage) and habituation (experimental room). The measured minimum of the tail base temperatures was significantly lower in the experimental setting than in the rats' home cage. This suggests that the experimental setup itself had an effect on the rat´s peripheral temperature in the habituation phase. The difference in the rats' tail base temperature might also have been influenced by slight differences in the ambient temperature between the experimental room and home cage though. However, differences in ambient temperature do not affect the stress-induced decrease in the tail base temperature in rats (Briese & Cabanac, 1991). One factor that may have triggered a stress-induced temperature drop during the habituation phase could have been the novelty of the test environment. However, during the actual experimental phase, the novelty of the test environment should not have played a role, since each rat had to undergo a habituation phase of at least three 30-min sessions in the experimental environment before starting with the actual experiment.

The treats the rats received upon entering the test area could have also influenced the emotional state of the rats. In other vertebrates, positive stimulation with food resulted in changes in surface temperature: after receiving treats, the comb temperature of chickens decreased, while the eye temperature of dogs increased (Moe et al., 2012; Travain et al., 2016). Therefore, there is the possibility that the treats had a bigger effect on the emotional state of the rats than the stimuli themselves. We chose to give the rats treats for entering the transport container and the experimental room so that the rats' participation in the experiment would have been voluntary and thus would not have elicited a stress-related response due to unwanted handling. Since the procedure was the same for all stimuli, the potential effect of the treats should have been stable under all three conditions.

Further it could be that the surface temperature may not be influenced by emotional valence or arousal alone. More complex interactions between emotional valence and arousal could be the underlying cause: e.g in human participants the nose temperature decreased when they had an empathic response, either by watching funny videos or watching others suffer, but when they experienced love, their face showed an overall increase in temperature (Salazar-López et al., 2015).

Conclusion:

While a significant difference in body temperature was found between playback of 22 kHz and playback of background noise, the study failed to show that there is a general pattern to how the surface temperature of rats' changes in response to different USV playbacks. Since this was a pilot study with only 6 animals, it may be that a larger sample would indicate clearer patterns. Considering that even these 6 animals had very different individual temperature responses with a lot of overlap of these three stimuli, it is debatable if more generalizable patterns would emerge with a higher N. Further it could be that the stimuli were not salient enough to elicit different temperature responses, or surface temperature may not be influenced by emotional valence or arousal, but by a more complex interaction between the two. This questions infrared thermography as a simple/single tool to assess animal welfare. To better understand how emotional states effect an animal it would be vital to not only record temperature data but other physiological correlates such as heart rate and the corresponding behavior of different individuals over a long period of time. Such extensive data recordings would provide large data bases demanding analysis in (semi-)automated systems.

Abstract

The aim of the study was to find out whether different emotional states in rats have an effect on their surface temperature. For this purpose, male adult rats (n=6) were presented with three different stimuli (22 kHz, 50 kHz, background noise), each of which was intended to evoke different emotional states in the rats. The background noise was intended to serve as a neutral control stimulus, while the 22 kHz stimulus was intended to evoke an emotional state of negative valence and the 50 kHz stimulus was intended to evoke an emotional state of positive valence. In a test arena the respective stimuli were played back to the rat followed by infrared recordings every minute for 30 minutes, measuring the peripheral temperatures of the eye, body, and tail base. A linear mixed-effects model was created for each of the three measured areas to determine the effect of the stimulus on the temperature values. Although a significant difference in body temperature was found between playback of the 22 kHz stimulus and playback of background noise, there was no obvious universal pattern to how the surface temperature of rats responded to different stimuli. Our results suggest that either the stimuli were insufficient to elicit different emotional states in the rats, or that surface temperature is not influenced by emotional valence or arousal alone, but by more complex individual interactions between the two.

Zusammenfassung

Ziel der Studie war es, herauszufinden, ob verschiedene emotionale Zustände bei Ratten einen Einfluss auf ihre Oberflächentemperatur haben. Zu diesem Zweck wurden männlichen erwachsenen Ratten (n=6) drei verschiedene Reize (22 kHz, 50 kHz, Hintergrundgeräusch) vorgespielt, die jeweils unterschiedliche emotionale Zustände bei den Ratten hervorrufen sollten. Das Hintergrundrauschen sollte als neutraler Kontrollreiz dienen, während der 22-kHz-Reiz einen emotionalen Zustand negativer Valenz und der 50-kHz-Reiz einen emotionalen Zustand positiver Valenz hervorrufen sollte. In einer Testarena wurden der Ratte

die jeweiligen Reize vorgespielt, und anschließend wurden 30 Minuten lang jede Minute Infrarotaufnahmen gemacht, bei denen die peripheren Temperaturen von Auge, Körper und Schwanzansatz gemessen wurden. Für jeden der drei gemessenen Bereiche wurde ein lineares Modell mit gemischten Effekten erstellt, um den Einfluss des Reizes auf die Temperaturwerte zu bestimmen. Obwohl ein signifikanter Unterschied in der Körpertemperatur zwischen der Wiedergabe des 22-kHz-Stimulus und der Wiedergabe von Hintergrundgeräuschen festgestellt wurde, gab es kein offensichtliches universelles Muster, wie die Oberflächentemperatur der Ratten auf verschiedene Stimuli reagierte. Unsere Ergebnisse deuten darauf hin, dass entweder die Reize unzureichend waren, um unterschiedliche emotionale Zustände bei den Ratten hervorzurufen, oder dass die Oberflächentemperatur nicht allein durch die emotionale Valenz oder Erregung beeinflusst wird, sondern durch komplexere individuelle Wechselwirkungen zwischen diesen beiden.

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