Analyzing Ultrasonic Vocalization-Evoked Changes in Rat Facial Expressions

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ABSTRACT:

It is important to be as accurate and precise as possible when it comes to analyzing the affective state of an organism. Despite past reliance on exploratory behavior for determining emotional states, behavioral assays such as the open field test, elevated zero, and elevated plus maze may not be the most effective methods. We present herein the use of DeepLabCut using adaptations from the Rat Grimace Scale as a method of accuracy, efficiency, and reproducibility to quantify the facial expressions elicited in response to ultrasonic vocalization playbacks.

INTRODUCTION:

The field of affective behavioral neuroscience research is an important one in understanding emotions and behaviors. Specifically, in emotion research involving rats, there are several current behavioral testing methods that are used to assess affective states based on behavioral patterns. For example, there is the open field test (OFT) which observes locomotor activity such as frequency and duration of entering the center of the open field (Gould et al., 2009). Additionally, there are the elevated plus and elevated zero mazes (Shepard et al., 1994). Each of which look at exploratory and/or risk-taking behavior which is seen to be correlated with different levels of stress or anxiety in rats (Laviola et al., 2003). While these are commonly used methods, they have some limitations. First, each of these behavioral testing models were originally validated in male rats, and as shown by Toledo-Rodriguez, there are sex-specific differences in psychopathologies and stress-related behaviors, so the conclusions based on past studies cannot be generalized to all rats (Toledo-Rodriguez & Sandi, 2011). Another limitation to these models is whether exploratory behavior is a valid indication of emotional state. For example, specific amounts of time in the open arms of the elevated zero maze or in the center of the open field are seen as risk-taking behaviors. Which, according to previous studies is associated with stress and anxiety, but it is possible that the rats are engaging in risk taking behaviors for more than just one reason.

When attempting to assess the emotional state of a human, potential solutions may include verbally asking them or analyzing their facial expressions. The current study hopes to understand how the same can be done with rats precisely and accurately. One model for assessing behavior that does not rely on exploratory behaviors is the Rat Grimace Scale. The Rat Grimace scale is a facial coding scale for the quantification of pain in rats. It consists of five facial “action units” including orbital tightening, nose/cheek flattening, ear changes, and whisker changes. Each behavior is scored on a 0-2 scale for their prominence in still photographs (Sotocina et al., 2011). There are a few limitations correlated with Sotocina et al.’s rat grimace scale from 2011. For example, the use of the current model provides an inconsistent quantification of results due to its limited 0-2 scale. Additionally, the current scale was designed for use in pain assays and therefore focuses on negative emotions. In this study, we hope to investigate the expansion of its use on both positive and negative emotions.

In rat behavioral research, different types of ultrasonic vocalization (USV) playbacks are often used to model emotional contagion because different USVs elicit different affective states. Emotional contagion is the idea that an individual acquires emotional states via social cues (Saito et al., 2016). There are two main types of USVs emitted by adults: 22kHz and 55kHz. The 22kHz USVs indicate aversive or negative states. They are emitted during fear- or anxiety- provoking situations and are used as an alarm call.

Differently, the 55kHz USVs indicate appetitive states. These are emitted during non-aggressive social interactions such as during play or in anticipation of a reward (Burke et al., 2017). The current study used pre-recorded 22 and 55kHz recordings to elicit both appetitive and aversive states within the rats in order to analyze their corresponding behaviors and more specifically facial expressions. DeepLabCut (DLC) was used to analyze their facial expressions. DLC is a type of deep learning pose estimation software (Mathis, 2018). After an initial training process to identify specific established salient points on a few clips, DLC creates an automated process allowing it to label these points among large quantities of photo and video data. By plotting these salient points, DeepLabCut can recognize various patterns that appear within the skeleton which can be correlated to common behavioral patterns exhibited by rats in a variety of affective states. While this data has been manually collected in the past, such work is labor intensive and time consuming. DeepLabCut is a more efficient way to collect the same and additional data that in turn is more accurate and reproducible.

We formulated a behavioral study to be analyzed using DLC to understand if we can monitor the emotional state of rats by tracking patterns in facial expressions as well as how different USV frequencies induce changes in rat facial expressions. We hope to assess the rats' emotions in response to the 22 and 55kHz playbacks, supporting the hypothesis that 22kHz USVs elicit aversive behaviors and 55kHz USVs elicit appetitive behaviors.

METHODS:

Subject Assignment:

Ten Sprague Dawley rats were randomly assigned to one of two experimental groups. Five female rats and five male rats in each group were exposed to two different types of USV playback stimuli (22kHz and 55kHz). On Day 1 of testing, Group 1 was exposed to the 22kHz playback whereas Group 2 was exposed to the 55kHz playback. On Day 2 of testing, both experimental groups were exposed to the opposite playback from Day 1.

Conditioning Procedure:

Rats were habituated to the holding containers for 10 mins each day for 3 days, during which they heard silence. The apparatus was set up as it would be during testing in order to minimize any potential confounds.

Apparatus:

A 10x9x17.5cm plastic holding container was used to enclose the rat for habituation and video recording. The holding container included a divider adjustable for rat size along with a cover on top.

Data Collection Procedure:

On testing day, the rats were placed in the holding chamber and recorded for facial expression data. The first 2 minutes of testing was done in complete silence to be used as baseline control data. The 2 minutes of silence was immediately followed by 3 minutes of one of the two USV playbacks (22kHz or 55kHz, determined by random assignment). On testing Day 2, the procedure was followed precisely, though the opposite USV playback from testing Day 1 was played.

DeepLabCut Data Analysis

The steps for using the DLC interface were laid out in various GitHub sites created by Alexander Mathis and his team, who created the DLC program, as well as Lucy O'Sullivan, who did previous DLC work in the Honeycutt lab. These resources were followed for means of extracting frames from the video data, manually labeling various salient points on these frames, training, evaluating, analyzing, and retraining the network. The specific salient points used in this study included: upper, lower, inner, and outer left and right eyes, tip and base of left and right ears, left and right cheeks, and the nose. Through various steps of network trainings, the DLC network was able to identify these salient points on the rats' faces from inputted video data.

RESULTS:

Based on the initial facial expression recordings in the pilot study, there appears to be behavioral differences among rats in each of the different exposure scenarios. In this within subject's design, we were able to compare behavior during exposure to silence, 22kHz, and 55kHz playbacks within one rat. Across all subjects, behaviors were similar with respect to each of the experimental groups. The silence recordings showed the most exploratory behavior. Rats rarely froze during silence. When exposed to the 55kHz playback, rats still underwent exploratory behaviors. There was an immediate pause in behavior upon the beginning of the 55kHz playback, which was then followed by more exploratory behavior. When exposed to the 22kHz playback, the rats seemed to freeze in response to the audio. There appeared to be periods of grooming during each of the experimental groups and during silence. There are no apparent behavioral differences between rats exposed to the 22kHz playback on testing Day 1 compared to those who were exposed to the 55kHz playback on testing Day 1.

The use of DLC proved effective in tracking the given salient points on the rats' face, following training. Further analysis is necessary in order to correlate the identified salient points to particular facial expressions and corresponding affective states.

CONCLUSION:

Some of the behaviors that were observed in the video data included freezing and grooming. The pause in behavior seems to occur in synchrony with the start of the playback during both the 22 and 55 kHz playbacks. The freezing during the 22kHz seems more fearful as when they freeze during this exposure it tends to be immediate freezing with little to no movement following. During the 55kHz exposure, there seemed to be a pause in activity immediately upon hearing the playback. This behavior seems more curious where the rat freezes with the audio and then moves around in exploration as if to find where the sound is coming from. These subtle differences are indicative of the different behavioral responses elicited by the different USV playbacks. In congruence with additional DLC data analysis of facial expressions we will be able to further understand the specificities to their emotional state in response to a specific playback. Facial grooming in rats is a compulsive behavior and is typically increased during stressful situations. Grooming may also be indicative of anxiety, boredom, and even de-arousal (Estanislau et al., 2019). In this study, the facial grooming on its own does not support any specific claims about the playbacks.

The initial findings of this study are promising for recording rat facial expressions. After just a few rounds of training, the system was able to identify the various salient points on its own. Next steps for this pilot would include further analysis of the DLC output data. This data processing will allow for the reading and calculating of the labeled skeleton including analyzing behavioral counts to quantify the facial expression data. In order to refine the current study for future iterations we plan to adjust the data collection method as well as continue to improve the DLC model. Specifically, future iterations would include a modified holding chamber. In order to allow for more consistent capturing of the rat's face on video, the holding chamber may need to be smaller or more restrictive in order to limit their ability to face away from the camera. This will be done while limiting external stressors as a confound. Another idea to simulate this adjustment would be to direct the rat's attention towards the camera during habituation so that they are more likely to face the camera when it is recording. Additionally, as with any automated computer model, the more training it has, the more accurately it will be able to identify points on its own. In future iterations, the network will undergo more training with additional video data. In a similar vein, we will include additional salient facial points to train the network. These may include inner and outer left eyes and whiskers. Additional points will allow for more precise behavioral counts and analysis.

The use of deep neural networks is becoming of greater importance in the field of behavioral neuroscience. Most importantly, deep learning networks such as DLC increase efficiency, reliability, and accuracy in data collection. For these reasons, they can be used as a supplement to behavioral testing. Tracking the rats' emotional states through facial behaviors using a neural network allows for potential use in the real-time monitoring of animal welfare. DLC can also be used in combination with other programs such as Deepsqueak (a deep neural network designed to detect and identify various rat calls) to compare facial expression behavior to calls they may be emitting simultaneously.

FIGURES:

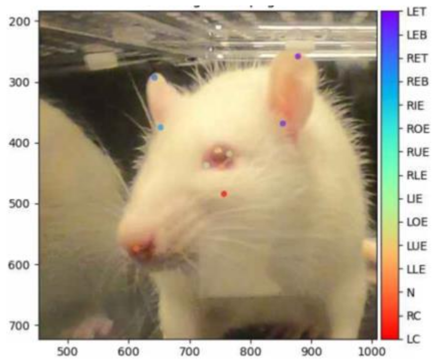

Fig 1. Screenshot from DLC program of a still rat face labeled with the salient points that are visible within the frame.

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