Analyzing Ultrasonic Vocalization-Evoked Changes in Rat Facial Expressions using the Pose-Estimation Software, DeepLabCut

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This summer I had the pleasure of conducting a pilot-study in Professor Jennifer Honeycutt's behavioral neuroscience lab that focused on utilizing deep-neural networks to build upon current behavioral testing methods used with laboratory rats. We looked at new ways to quantify and understand the emotional states of rodents in areas that commonly used behavioral tests, such as the Open Field Test and Elevated Zero Maze, fall short. These methods rely on exploratory-behavior being a direct indicator of anxiety and depression in rodents have only been validated in male rats. Previous research in the Honeycutt Lab identified conflicting displays of anxious behavior in male and female rats. These limitations of current testing methods present significant challenges in attempting to understand the complexities and individual differences in the emotional states of laboratory rats.

Inspired by The Rat Grimace Scale, a pain assessment tool that compares the presence of certain facial expressions, we induced positive and negative emotional responses in our subjects by using two different frequencies of ultrasonic-vocalization (USV) playback (Sotocinal et al., 2011). USVs are a type of communication used by rats that humans are unable to hear and are effective in eliciting emotional contagion in rats (Demaestri et al., 2019 & Saitom et al., 2016). We recorded videos focusing on the rats' faces which was then used to train a DeepLabCut network to detect 15 points on the rats faces for analysis. After habituating our 10 Sprague-Dawley rats to a small, movement-restricting holding chamber we conducted two days of experimental testing. On the first day, a rat was placed into the chamber and heard 2 minutes of silence followed by 3 minutes of USV playback. On the first day they heard either a positive or negative USV call (randomly assigned) and the opposite on the second day. We then used our video recordings to begin the process of training a DeepLabCut network and analyzing our data, which we are still in the process of.

Video recordings were first used to train a neural-network to track points on the rats' faces. The software selected numerous diverse frames from each video. The 15 desired facial points were then manually identified and used to train the network. Once the network was trained, we analyzed videos and assessed the network's accuracy. Initially, the network struggled to accurately place all of the points so re-training was required. More diverse frames were selected and their points were adjusted from where the network placed them, to their correct positions. The re-training process occurs multiple times, to ensure that the points we are tracking are accurately placed. Once we have an accurate network, we plan to use R-Studio to analyze our data, and track changes in the geometry of these points in response to the different behavioral states. We are excited to share that we were able to see distinct qualitative changes in the rats behavior in response to the USV calls, which confirms that the emotional states we were attempting to elicit with the USV calls are actually being elicited. Continuing to explore new ways to assess behavior in laboratory animals is incredibly important in ensuring that the results from our research are being applied to their fullest extent.

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