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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 ᴶ ɢ such as th Ŀ C Œ R

The field of affective behavioral neuroscience research is an important one in understanding emotions and behaviors. Specifically, in emoton research involving rats, there are several current behavioral testng methods that are used to assess affective states based on behavioral paterns. For example, there is the open field test (OFT) which observes locomotor activity such as frequency and duraton of entering the center of the open field (Gould et al., 2009). Additonally, 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 limitatons. First, each of these behavioral testng 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 limitaton to these models is whether exploratory behavior is a valid indicaton of emotonal state. For example, specific amounts of tme 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

playbacks are often used to model emotonal contagion because different USVs elicit different affective states. Emotonal contagion is the idea that an individual acquires emotonal states via social cues (Saito et al., 2016). There are two main types of USVs emited by adults: 22kHz and 55kHz. The 22kHz USVs indicate aversive or negative states. They are emited during fear- or anxiety- provoking situatons and are used as an alarm call.

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.

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.

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 fe

Laviola, G., Macrì, S., Morley-Fletcher, S., & Adriani, W. (2003). Risk-taking behavior in adolescent mice: Psychobiological determinants and early epigenetic influence. , 27(1–2), 19–31. https://doi.org/10.1016/s0149-7634(03)00006-x.

Mathis, A., Mamidanna, P., Cury, K. M., Abe, T., Murthy, V. N., Mathis, M. W., & Bethge, M. (2018). Deeplabcut: Markerless pose estimation of user-defined body parts with deep learning. , 21(9), 1281–1289. https://doi.org/10.1038/s41593-018-0209-y.

Saito, Y., Yuki, S., Seki, Y., Kagawa, H., & Okanoya, K. (2016). Cognitive bias in rats evoked by ultrasonic vocalizations suggests emotional contagion. , 132, 5–11. https://doi.org/10.1016/j.beproc.2016.08.005.

Shepherd, J. K., Grewal, S. S., Fletcher, A., Bill, D. J., & Dourish, C. T. (1994). Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. , 116(1), 56–64. https://doi.org/10.1007/bf02244871.

Sotocina, S. G., Sorge, R. E., Zaloum, A., Tuttle, A. H., Martin, L. J., Wieskopf, J. S., Mapplebeck, J. C., Wei, P., Zhan, S., Zhang, S., McDougall, J. J., King, O. D., & Mogil, J. S. (2011). The rat grimace scale: A partially automated method for quantifying pain in the laboratory rat via facial expressions. , 7. https://doi.org/10.1186/1744-8069-7-55.

Toledo-Rodriguez, M., & Sandi, C. (2011a). Stress during adolescence increases novelty seeking and risk-taking behavior in male and female rats. , 5. https://doi.org/10.3389/fnbeh.2011.00017.