DISTRESS AND THE MICROBEHAVIORS OF FREEZING

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Introduction

When considering inter-person interactions, empathy is commonly accepted as a key component of positive social interactions between people. However, empathy is not a trait commonly associated with other animals, including rats. Stress is another commonly accepted emotion in humans, and some animals, including mice and rats, are also generally accepted to have feelings of discomfort in a novel or stressful situation (Morton et al., 1985). Rats also demonstrate co-distress and can pick up the affect of another conspecific. This distress can come out in a range of behaviors including freezing. Freezing has traditionally been looked at as a unilateral behavior in response to a stressor or pain. This is too simplistic and this paper will explore a way to analyze the microbehaviors of freezing.

Aims of the Honors Thesis

The first aim was to observe behavioral dynamics of distress. The second was to develop stable markers for behavioral distress and the third was to develop a pipeline for automated coding in the future.

Background

While often casually used interchangeably, stress and distress are not the same thing. Stress is a response to an outside stimulus where one feels threatened. Enough stress can lead to distress. Distress occurs when one cannot successfully adapt, cope, or habituate to the stressors in their life (Clark et al., 1997). Long term distress has numerous negative impacts on wellbeing, with physical, psychological and behavioral health possibly impacted.

The amount of stress that one is facing exists on a sliding scale. Following the Yerkes Dodson Curve, stress acts in an upside down U curve with arousal (the body's response to mobilize and react) on the X axis and performance on the Y axis. On one extreme there is a lack of stress where one is unable to perform a task and the other extreme, one undergoes extreme levels of stress. Again, one is unable to perform a task. In the middle, there exists a healthy balance where there is optimal performance (Yerkes et al., 1908). Further research shows the Yerkes Dodson Curve also extends to rats. Just like in humans, there's some variability between rats in terms of levels of neuroticism and emotionality (Broadhurst et al., 1957).

The Yerkes Dodson curve applies to all forms of stress and demonstrates one's ability to cope. Stress comes in various forms, as either "concrete", such as a or an act of violence but it also can be "implied", such as a threat being given or a response to an unpredictable or uncontrollable situation such as the beginning stages of a romantic relationship (Lupien et. al, 2006). While most humans are instinctively aware of concrete stressors, not all are as cognizant to implied stressors (Lupien et al., 2007). This is also demonstrated in rats where some rats are able to demonstrate "anxiety-like behavior" in response to an implied stressor.

Empathy can be defined as an understanding of another's affective state and a reactive emotional response that comprehends and responds to the other being's state without confusing self with another (Decety et al., 2015 & de Waal et al., 2017). For example, if Person A sees Person B in a painful car crash, if Person A has an empathetic response, they may also feel pain seeing the crash and try to help Person B.

Since one often engages in empathy when another is in pain or under a negative stimulus, previous research shows that there is "*emotion transmission*" which suggests that the emotional distress of the pain can transmit from one being to another. Another facet of empathy is "*emotional resonance*" where the observer feels contagiously distressed by the other's distress which may lead to emotional mimicry which can be defined as one animal mimicking the behavioral side effects of the other animal's affective state (de Waal et al., 2017).

These negative emotions in response to another's distress can lead to prosocial, helping behavior. "*Prosocial behavior*" occurs when an animal engages in behavior assisting another animal where the animal doing the action gains no direct personal benefit or comes at a personal cost (Church et al., 1959 & Bartal et al., 2014). These behaviors are another component of empathy where one must not only have "the natural ability to perceive and be sensitive to the emotional states of others" but also the additional pairing of "a motivation to care for their well-being" (Decety et al., 2016).

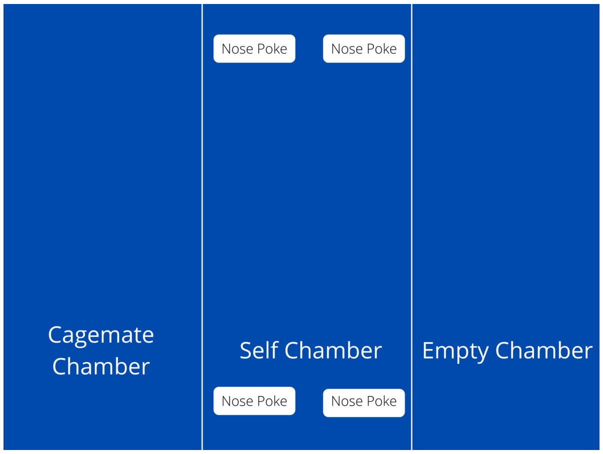
Previous research has demonstrated that rats recognize another animal's distress and can also engage in behavior to help alleviate another's distress. For example, when a mouse is injected with 0.9% acetic acid, the mouse demonstrates a hypersensitivity to pain if they see another mouse undergo the same injection, but not if they are alone or if they see another mouse that did not receive an injection (Langford et al., 2006). In terms of prosocial behavior, when a rat had a choice of either chocolate chips (a high value treat) or helping another rat with no direct benefit to themselves, the rat would choose to help the other cagemate (Bartal et al. 2011).

In response to a stressor, rats often demonstrate "*freezing*" behavior. Freezing is defined as at least one second of immobility not caused by some other physical disability (Boguszewski et al., 2007). This is a universal "form of behavioural inhibition" and is demonstrated with both unconditioned and conditioned fear stimuli. In times of stress and when used as a defensive mechanism, freezing behavior occurs when both the sympathetic and parasympathetic nervous system are aroused but there is parasympathetic dominance. Rats also freeze in response to pain, as seen in numerous animal care post-surgery manuels (National Research Council, 1992). When a rat is frozen, there is "increased arousal and physical symptoms that support the freezing response [such as] increased heart rate and cardiac output, increased arterial pressure, inhibition of digestive function and increased respiration, in its turn increasing perfusion of active tissue. There is also increased muscle tone and pain suppression" (Roelofs, 2017).

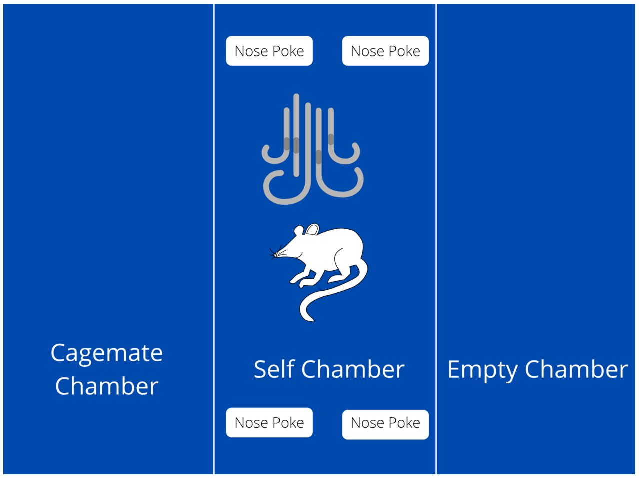
iBox and Video Coding

The rats observed in this thesis were Sprague Dawley adolescent females. Sprague Dawley rats were used due to their increased levels of prosocial behavior. A cagemate pair (EW_3 and EW_4) of rats were observed with EW_4 being the main rat carefully observed at a micro level.

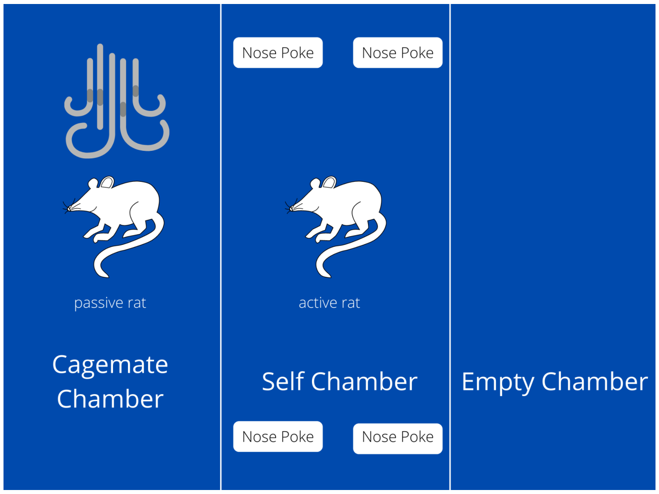
The distress marker of freezing was viewed in the context of a three chambered device named iBox which tested prosocial behavior levels in rats.The walls of each chamber are made of plexiglass, so that rats can visually observe each other when in the iBox and each chamber holds a vent where compressed air is intermittently (via a mechanized timer) blown through to stress the rat. The "self" chamber has four nose poke portals with a laser beam inside each one that can be turned on and off. The laser beam serves like a button, and when the rat pokes its nose through the nose poke portal, it breaks the laser beam's stream of light. This turns off the air in the iBox. The "cagemate" chamber has no buttons in it and holds the cagemate during the cagemate condition. The "empty" chamber has never held a rat. Below is an image of the iBox.



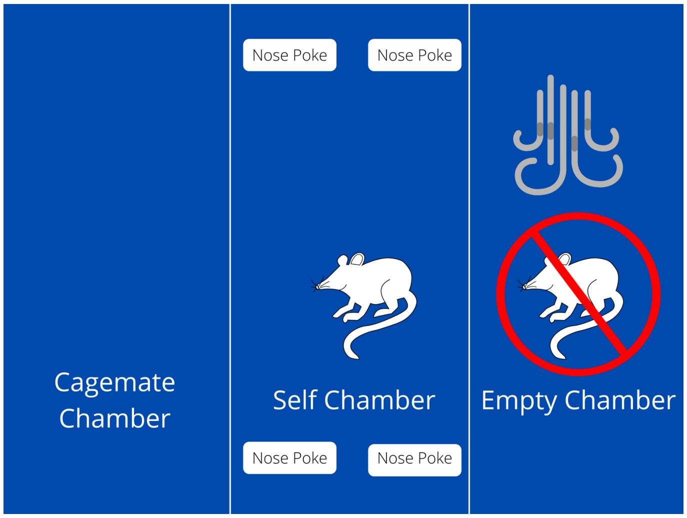
In the “self” condition, a rat is placed in the “self” chamber to acclimate itself to the iBox and is trained to associate completing a “nose poke” with the cessation of the negative air stimulus blowing on itself. By doing so, a baseline for its learning speed and level of its own stress can be established for this rat. This stage serves as the control setting. The self condition occurs for twelve days. Below is a diagram of the iBox during the self condition.



After acclimating the rat, the rat undergoes the “cagemate” condition. In this condition, there are two rats, the active rat and the passive rat. The active rat is in the “self” chamber and has access to the controls, with no air being blown on itself. The passive rat is the cagemate of the active rat and is in the “cagemate” chamber with air being blown on it intermittently, but it has no ability to turn the air off for itself. The cagemate condition occurs for 7 days. Below is a diagram of the iBox during the cagemate condition.



After the cagemate condition, the rat undergoes the “empty” condition, where air is blown onto the empty chamber that was never occupied by any rat. Again, the rat is placed in the “self” chamber, with the nose poke portals and the ability to turn off the air being blown into the “empty” chamber. This is done to test whether the rat was turning off the air primarily when there was another animal, or if the rat turned off the air indiscriminately. If it demonstrated greater amounts of turning off the air when another animal was present than not, the rat would be demonstrating prosocial behavior. If the rat just turned off the air indiscriminately, it would suggest that there was no prosocial behavior going on and that the air turning off was more a novelty behavior than anything significant. Below is a diagram of the iBox during the empty condition.



The data analyzed was from one video camera placed next to the empty chamber. The analysis of the videos was conducted using ELAN 5.9, an open access video-coding software. When coding in ELAN, I would play the video at 175% speed to efficiently find the behaviors I was looking for. Once spotting a behavior, I would back up the video prior to when the behavior began, and then went video frame-by-frame to accurately mark the beginning and end of the behavior, aiming for accuracy within 0.01 of a second.

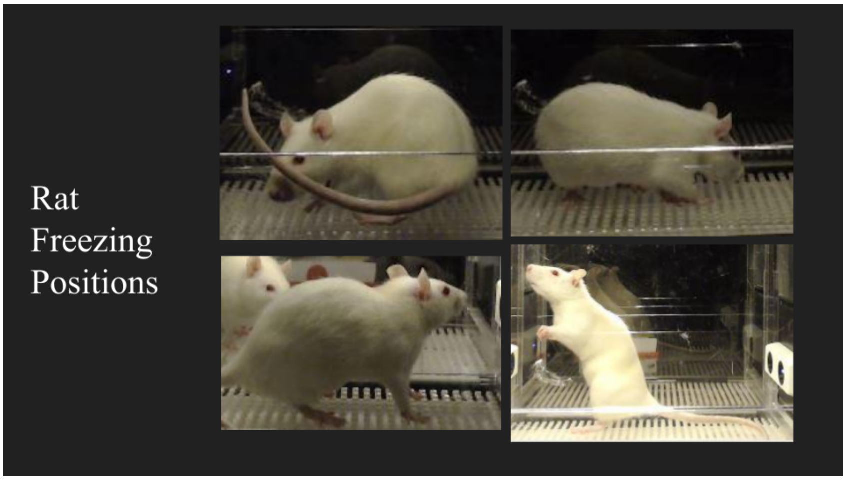
While I initially looked for nose poke, grooming, and freezing behaviors in both the active and passive rat, I ultimately ended up solely using the freezing data for the active rat. Freezing was demonstrated when a rat held its position for at least one second without moving its nose, tail, limbs, etc. (Boguszewski et. al, 2007). It was measured by the number of seconds frozen.

Preliminary Freezing Data

Based on preliminary freezing data seen in both EW_3 and EW_4, demonstrated that freezing did occur. Notably, it appeared that freezing occurred in greater amounts when a cagemate was present, suggesting greater distress when seeing another's distress.

Freezing, Microbehaviors, And Circularity

Currently in the literature, freezing is being treated as a unitary behavior, with all freezes being solely determined by a cessation of motion. However, in the below image, are four still images of freezing in the same rat.



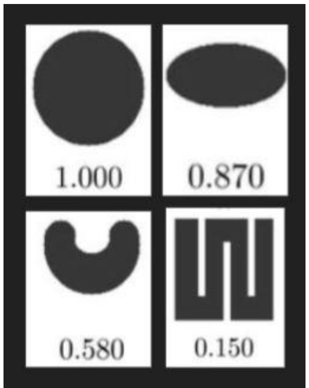
It is evident that these four body positions are drastically different, despite all being classified as freezing. Therefore, in this project, I wanted to find a more fine-tuned and objective behavioral marker of distressed freezing.

Microbehaviors, which is the concept of taking a behavior and breaking it down, is not uncommon from both a casual perspective or an academic perspective. We accept that different body positions mean different things. For example, in dogs, many people understand that a tail held in a neutral or medium position, ears forward, and movement reflect a relaxed or neutral state but a tucked tail, trembling, cowering, and hiding behind objects all demonstrate fear (Dowling-Guyer, 2011 & Gutiérrez, 2019). Similarly in humans, when we are sick, in pain, or distressed we may likely demonstrate hunching posture and in response to a traumatic event, fetal positions are not uncommon (Hennessy 2001 & Eekhoff, 2021).

Thus, taking the concept of relating behavioral position and one's affect and expanding it to rats is not unreasonable. Previous literature shows that rats' bodily position indicates information about distress. For example, abnormal posturing of head and neck can indicate moderate to severe pain levels and body posture can also indicate distress. The rats' spinal curvature also can be indicative. For example, a hunched, C-shape back can signal distress (Ebbesen, 2021). Thus, looking at the microbehaviors of freezing is a replicable, objective method of looking at distress in rats.

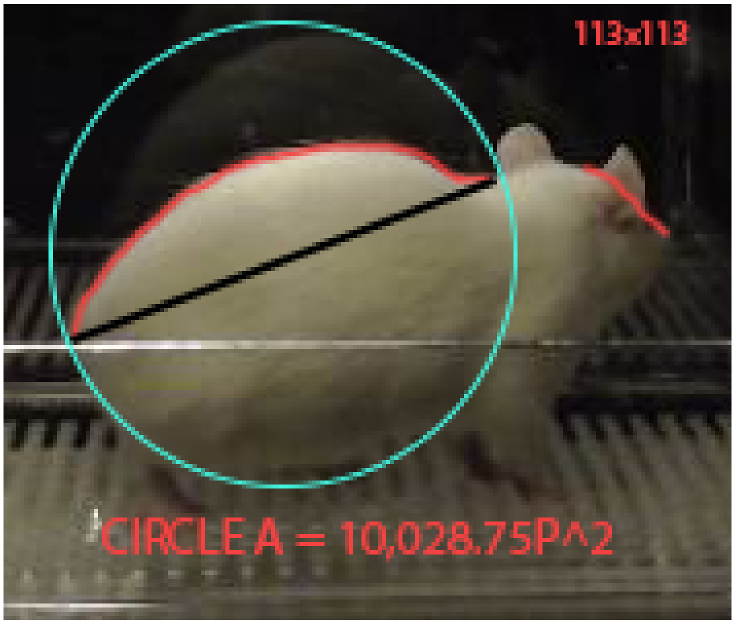
In order to analyze the hunched C-shape position, a variety of methods were attempted such as printing and hand-calculating the angles of the spine and making a visual estimation by committee. However, none were sufficiently objective or reliable.

A new method was developed, using the concept of circularity. In essentials, circularity measures how closely an object resembles a perfect circle. Thus, a perfect circle is equal to 1.00 with a straight line approximating 0.00 (Giraud, 2017). This is a previously used technique in other fields. For example, it has been used to evaluate the health of penguin populations in Antarctica, analyze rocks on Mars and classify tuberculosis cells (Hall, 2021 & Fox, 2002 & Rulaningtyas, 2011). Below is an image demonstrating different shapes and their according measures of circularity. Most notable is the one on the bottom left with a circularity of 0.580. This one has a C-shape and is curved, similarly to the rats's spines, and an average score of 0.58 was not uncommon either.



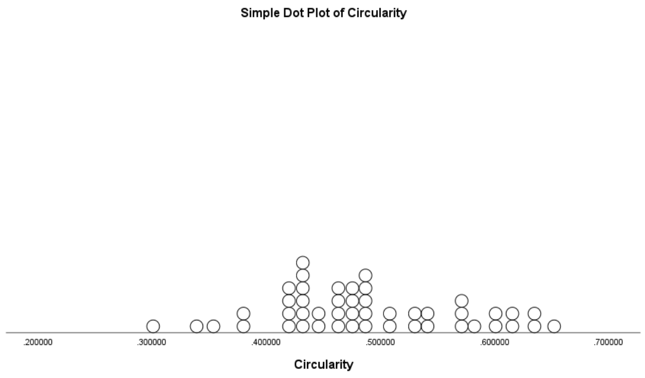
Giraud, R., Ta, V. T., & Papadakis, N. (2017).

Since occasionally, rats will curve their body into a shape resembling a ball, it was reasonable to try and use this circularity and see how tightly the rat's spine would resemble a circle. In order to measure the rat's spine, Photoshop was used to outline the curvature using the Brush Tool. The first step was pulling all the still images of freezing rats and sorting them based on whether the rat was sideways with its body parallel to the camera and separating all instances of when the body was parallel to the camera but the rat was rearing, since rearing is often associated with exploratory behaviors instead of a fear response and this could easily be a confounding factor. After tracing the rat's spine from mid-neck to just above the tail, a line was drawn to connect them. After selecting the outline, I ran the Analysis function in Photoshop, looking at the cingularity measure. Below is an image of how the software was utilized.



The red line traced out the spinal curvature from mid-neck, in order to get a sense of which direction the head was going (up/down), through the spine to right above where the tail met the spine. The black line shows the angle between the neck and the tail, which makes it easier to further trace when calculating the circularity. The blue circle shows the circle against which the circle against the selected area between the red and black lines will be calculated. The label for Circle A helped me see the area of pixels squared in the circle and the 113x113 showed me the diameter of the circle, which helps in the calculation process.

Ultimately, of the 179 freezes that noted, 43 of the freeze images could be used to calculate the circularity with an additional 37 being side-ways but with at least one paw up making those images a rear-freeze and disqualifying them from calculation. After creating a dot plot of the qualifying freezes, there was an obvious distribution, with a significant difference in circularity on a scale from 0.00 - 1.00. This indicates that the freezes were objectively very different from each other and warrant further research.



Limitations & Further Directions of Research

There were several key limitations to the process of developing the circularity method. The first was that there were not enough freeze events to repeatedly test the method due to the fact that the camera would need to be a 360 degree camera so that it could constantly catch the rats in a way so that they are parallel to the camera. The second limitation was that the method was hand-coded on a computer. This meant that there was a moderate margin of human error, since using a mousepad to trace an image on a screen is not the most accurate method. The third limitation was not enough time to further do this experiment with more rats to further test the methods. The final limitation was that I was the only person coding and calculating the circularity.

Some of these issues could be resolved using neural networks. This is not a new concept, and machine learning has already been utilizing neural networks to automatically classify tuberculosis bacteria (Rulaningtyas, 2011). In the case of mechanising neural nets to calculate the circularity of rats, software like Deep Lab Cut and SLEEP could be trained and utilized to develop a skeleton framework on top of the videos of the rats and track their movements. After training this, if nodes were placed on the mid-neck, throughout the back, and where the tail meets the spine, it would be a simple task to train the neural network to calculate the circularity of the rat. This could be extremely beneficial because not only would it eventually save time when calculating the circularity but it would also be more accurate since it would be more reliable than coding by hand.

Another thing noted previously was that freezing is a socially complex emotion for rats and looking at gaze direction when freezing may also be interesting. When one rat (Rat A) watches another (Rat B) undergo a shock, Rat A may also freeze vicariously. This demonstrates emotional contagion and also teaches Rat A that the stimuli is painful, without Rat A ever directly undergoing the stimuli (Atsak et. al, 2011). In relationship to empathy and prosocial behavior, having another animal nearby may help with “social buffering”. Social buffering is defined as “a phenomenon in which the presence of an affiliative conspecific (associate) mitigates stress responses in a subject” (Kiyokawa et al., 2018).

Generally speaking, when freezing a rat could face one of three ways (as seen below). The first is that they could be looking and facing the empty chamber. The second would be that they were looking sideways facing neither the cagemate nor empty chamber. The third was looking at the cagemate chamber. When coding the video of EW_4, it was notable but also unsurprising that the active rat was looking at the passive rat. Interestingly, even after the cagemate condition, during the empty condition, the active rat continued to primarily freeze facing the cagemate chamber at a significantly higher rate when compared to the self-chamber.

Conclusion

Clearly, the current unilateral understanding and approach to looking at empathy and freezing requires further fine tuning. With the technological advances, neural networks can be utilized to further refine and calculate a rat's spinal circularity. With this development, there is now a new way to objectively measure distress and co-distress, and eventually better understand prosocial behavior and empathy in rats.

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