Designs for Understanding Empathy in the Rodent Brain

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Abstract

It has previously been shown that rats behave in a pro-social manner in response to a conspecific's distress, and that there are biological roots of empathy. However, whether rodents in fact display truly empathic behaviors and the neural mechanisms underlying these behaviors are still unclear. We have developed a novel task using aversive airflow which is administered in alternating trials to one of the two rats that are placed in a triple chambered apparatus, separated by plexiglass dividers. By triggering a beam with a nose poke, only one rat (the active rat) can stop the aversive airflow delivered to himself or the other rat. Pilot studies have shown that the active rats are able to identify the distress of the passive rat, and are able to nose poke to alleviate that distress. To further understand the neural mechanisms behind this behavior, the rats will be implanted with electrodes in the insular cortex (where interoceptive information is encoded) and the amygdala, as well as with flexible electrodes on the chest muscle to obtain heart and respiration rates during the task. We will then be comparing various neuronal activities from these regions, as well as the heart and respiration rates of the rats when the air is flowing on the active rat versus the passive rat. By doing so, we hope to obtain a clearer picture of the neural mechanisms of empathy and pro-social behavior in rats.

Introduction

Empathy is defined as the ability to understand and perhaps even 'feel' the emotions of others. Prosocial behavior refers to an act that is done to benefit another. In our environment, where social interaction and collaboration are keys to success in society, empathy and prosocial behavior are essential features that a human being must possess. However, not much is known about the neural mechanisms and biological roots of empathy. Although it is known that social interactions involve the insular cortex, amygdala and autonomous nervous system, it is not quite known how these system collaboratively interact with each other to enable empathetic and prosocial behavior. Therefore, this study aims to investigate the neurobiological mechanisms of empathy and prosocial behavior by developing a tightly controlled paradigm that is capable of isolating the elements that give rise to empathetic and prosocial actions. In particular, this study utilizes the combination of hardware and software tools to create a robust and well-controlled paradigm for studying empathy and prosociality.

Background

Up until recently, it was thought that only humans and some non-human primates exhibited empathy and prosocial behavior. However, recent studies by Bartal et Al suggest that rats may also exhibit these behaviors. Bartal et Al's research used the restrainer test to elicit empathic and prosocial behaviors from the rats. Two rats were placed in an arena: one rat (dubbed as the 'trapped rat') was trapped inside a restrainer and the other rat (dubbed as the 'free rat') was outside in the arena. The free rat had the ability to open the restrainer for the trapped rat. With various trapped and control conditions, the researchers concluded that the free rat exhibited some form of empathy for its trapped cagemate and thus, acted in a prosocial manner and opened the restrainer door for their cagemate. They also concluded that this provided some biological roots for empathy, (Bartal et Al, “Empathy and Pro-social Behavior in Rats”, 2011).

A follow up study was conducted where the researchers were interested in probing the motivation of rodent helping behavior. They hoped that this would lead to a deeper understanding of biological influences on human pro-sociality. (Bartal et Al, “Anxiolytic Treatment Impairs Helping Behavior in Rats”, 2016). In particular, they were interested in seeing whether an affective response motivated door-opening. Rats received a benzodiazepine anxiolytic called midazolam and were tested in the restrainer task. Results indicated that midazolam-treated rats showed less helping behavior than rats that received no injection or saline treated rats. Further results indicated that rats with greater cortisol responses showed the least helping behavior than others with lower cortisol levels. The study emphasized the importance of affect in motivating prosocial behavior in rodents. The study stated that the rats resonated with the negative arousal of the trapped cagemate and opened the restrainer because of the affective response. The researchers finally concluded that this response that the rats’ had to their trapped cagemate, shares many commonalities with elements found in empathetically motivated humans. Lastly, they predicted that this could serve as a model for studying the biological mechanisms of human pro-sociality.

Other studies have delved into understanding whether rats reciprocate help differentially in response to the quality of their partner’s help. (Dolivo & Taborksy, “Norway rats reciprocate help according to the quality of help they received”, 2015) In particular, they were interested in understanding whether rats used the value of help received as a decision criterion to help back. This study tested whether rats could differentiate between different conspecifics depending on the quality of the help received. The results indicated that rats adjusted their reciprocity depending upon the perceived quality of the partner's previous help. The study also showcased that rats differentiated between helping a cagemate (direct reciprocity) and helping another, unknown rat (generalized reciprocity.) (Dolivo & Taborksy, "Norway rats reciprocate help according to the quality of help they received", 2015). This study concluded that the helper rat is rewarded by the recipient of the cooperative act according to the benefits it had previously received.

All of the studies discussed above focus on the behavioral aspects of empathy, prosocial behavior and reciprocity. However, these studies have not shown whether rats truly display empathic behavior or not and have not shown the neurobiological processes that underlie empathy and prosocial behavior in rats.

Methods

Interoceptive Box (iBox)

Interoception is defined as the sense of the internal state of the body. In this experiment, we placed two rats (cage mates) into two different chambers separated by a plexiglass. Aversive air flow is administered into the chambers. This aversive air flow helps to change the internal state of the rats, thus earning the name 'interoceptive box'. The interoceptive box experiment is a novel experiment to test for empathy in rats. It controls for confounds found in previous experiments, such as rats opening the restrainer doors in order to interact with the other rat, rats being over trained to open restrainer doors or because the rats simply enjoyed opening the restrainer doors. Furthermore, the restrainer experiments did not provide isolated time-intervals under which the trapped rat underwent stress. This paradigm enables the isolation of time-controlled intervals during which the rats will experience aversive air flow. This is especially useful for studying the neurobiological roots of empathy and for comparing the neuronal and autonomic activity of the two rats during the time period when the aversive air flow is administered.

Subjects

In total, 32 male Sprague-Dawley rats will be tested in the interoceptive box.   In the first experimental design with the double chambered apparatus, 4 male rats (2 dominant-submissive pairs) were tested.

Software Tools

The programming language Python was used to create a graphical user interface that researchers can easily manipulate during the experiment and adjust the parameters according to experiment needs. The researchers are able to manipulate parameters for stage number, number of trials and delay time. The graphical user interface showcases the chamber in which the aversive air flow is currently being administered in. It also shows real-time footage of the two rats in the interoceptive chamber.

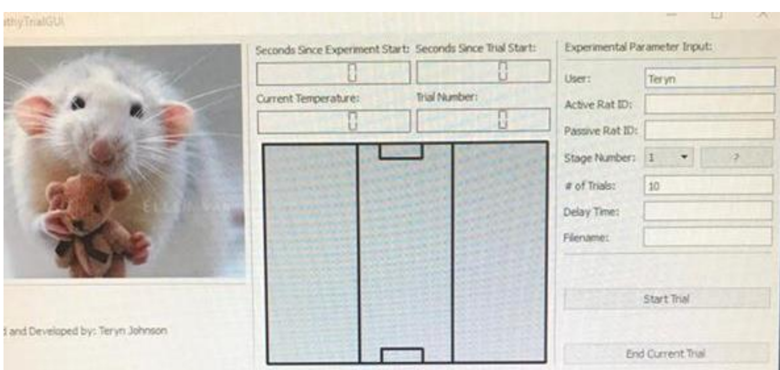


Image 1: Depicts a screenshot of the graphical user interface during experimentation

Hardware Tools

The Arduino platform, which is an open-source computer hardware was used to control the motors for the air tubes and for the sensors in the nose ports.

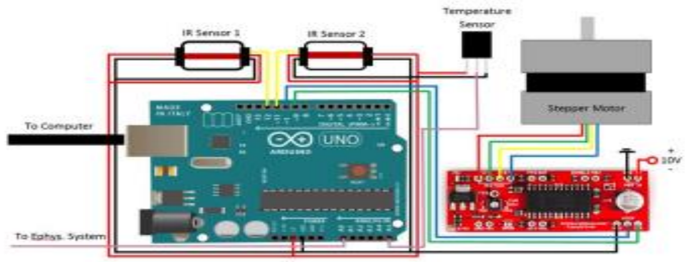


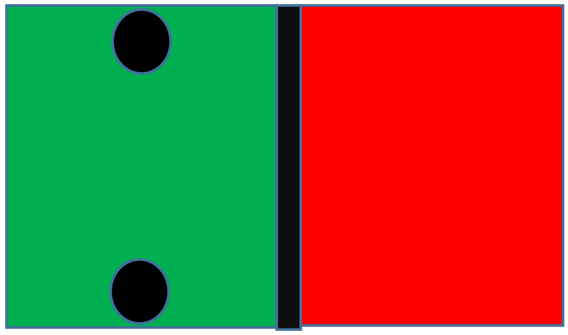
Image 2: This depicts an animated version of the hardware interfaces for the interoceptive box

Experimental Design 1: Double Chambered Apparatus

The first experimental design contains two chambers: the active chamber and the passive chamber. These two chambers are separated by a clear plexiglass. Air flow is administered into these chambers via an air tube controlled by the Arduino code.

Active Chamber: The rat in this chamber is dubbed as the ‘active rat’. The active rat has control over stopping the aversive air flow. He has access to two nose ports, one that stops the airflow for himself and the other nose port stops the airflow for his friend in the other chamber. When the active rat presses the nose port, a sensor is activated which informs the Arduino code to stop the air from flowing into the respective chamber.

Passive Chamber: The rat in this chamber is dubbed as the ‘passive rat’. The passive rat has no control over stopping the aversive air flow.



Active Chamber with two nose ports

Passive Chamber with no nose ports

Image 3: Cartoon representation of the Double Chambered Apparatus

Important Parameters to Manipulate

The graphical user interface was designed in a way so that the researchers can easily manipulate the different parameters for the experiment. The rats underwent thirty trials with a length of sixty seconds each and an interim period of twenty seconds. The interim period is between two trials and is important especially when the motors are moving to different chambers. During the interim period, the sensors are deactivated so that if the active rat pokes the nose port, it does not register the signal to the motor.

Parameter	**Purpose**
Stages	We used different stages to train the rats how to turn off the airflow. The different stages depended on the order of the air flow to the active and passive chamber. The different stages also assure that the air flow is randomized and that there is no pattern.
Trials	The number of times the air flow will be administered.
Delay Period	The length of each trial (in seconds).
Interim Period	Period between different trials where the sensors are not activated.

Training Stages for Double Chambered Apparatus

Different training stages were used to train the active rat on how to turn off the airflow for himself and for the passive. The training stages are crucial especially when one is trying to communicate with a non-verbal creature. The different training stages help the rats learn the rules of the task.

Stage 1: The air flow was administered only to the active chamber. Only the nose port for the active chamber was enabled. The rat was trained to poke this nose port when the air flowed onto his chamber. This stage lasted until the rat learned to turn off the air flow for himself with an 80% accuracy rate.

Stage 2: The air flow was successively alternated between the active chamber and the passive chamber. Both of the nose ports were enabled. The rat was trained to press the nose port for his friend when the air flow was administered in the passive chamber. This stage lasted until the rat learned to turn off the air flow for himself and for his friend with an accuracy rate of around 60%.

Stage 3: The air flow was randomly alternated between the active chamber and passive chamber. This was done so that the active rat did not develop any bias or learn any pattern towards poking the nose ports. In this stage, the air flow could not be administered to the same chamber more than two consecutive turns.

Experimental Design 2: Triple Chambered Apparatus

This design used three chambers instead of two. The active and passive chambers remained the same. A third chamber labelled the 'empty chamber' was added in this design to address the potential confounds that resulted from the first experimental design. This design used four nose ports. One for the active chamber, one for the passive chamber, one for the empty chamber and one unresponsive port to maintain the symmetry of the design.

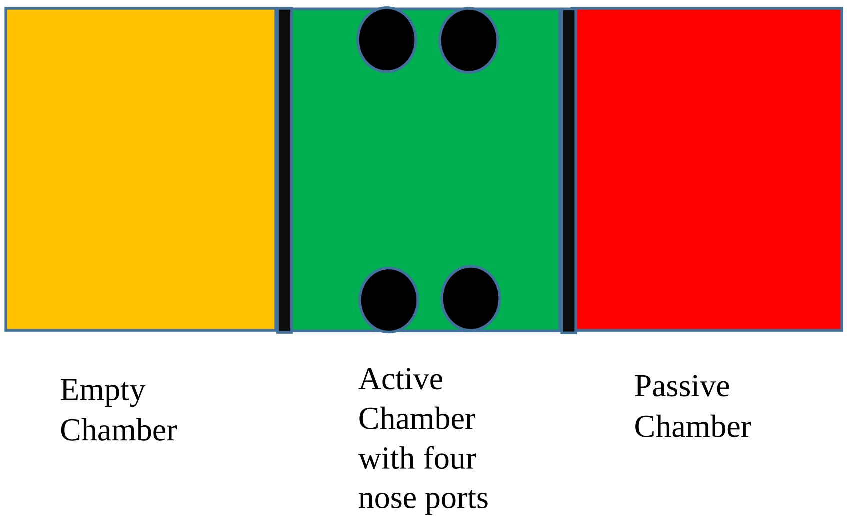


Image 4: Cartoon representation of the Triple Chambered Apparatus

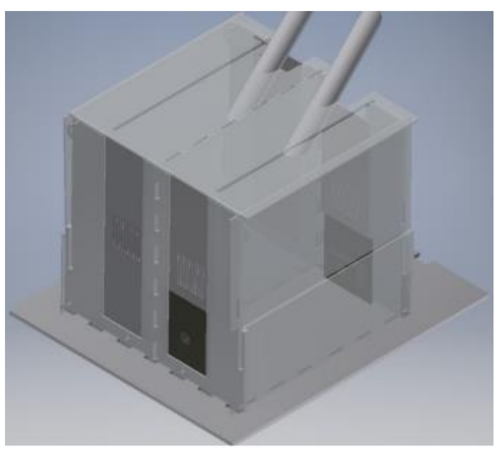


Image 5: This depicts a computer aided three-dimensional representation of the triple chambered apparatus

Training Stages for Triple Chambered Apparatus

Stages 1-3 that were used from the double chambered apparatus remained the same for the triple chambered apparatus. Three new stages were added to teach the rat on how to respond to the air flow that is administered into the empty chamber.

Stage 4: The air flow is randomly alternated between the active and empty chamber. The rat is taught to poke the nose port for the empty chamber.

Stage 5: The air flow is randomly alternated between the empty chamber and the passive chamber.

Stage 6: The air flow is randomly alternated between all three chambers.

Results

Results for Double Chambered Apparatus

For the double chambered apparatus, our results indicate that the active rat was able to discern the passive rat's distress. The active rat was able to turn off the air flow for himself with 80% accuracy and was able to turn off the air flow for his friend with about 77% accuracy. The active rat was quicker to turn off the air flow for himself than for his cagemate.

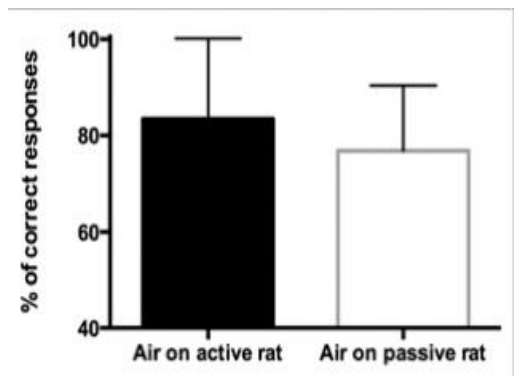


Image 6: This depicts the percentage of correct responses made by the active rat

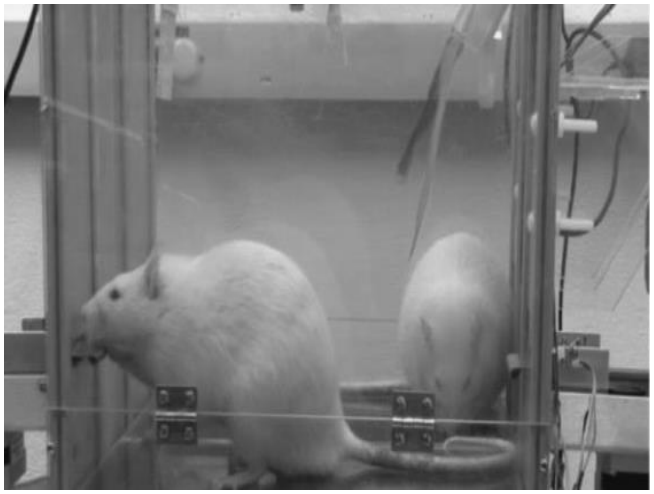


Image 7: In this picture, the active rat is seen poking the nose port to stop the air flow from flowing to the passive rat.

Confound for Double Chambered Apparatus

After testing the double chambered apparatus, we ran into a potential confound. Was this experiment truly testing for empathy and prosocial behavior? As there were only two chambers and two nose ports, it could have been plausible that the active rat had been trained to always press a nose port. This could indicate that the active rat was not actually emphasizing with the distress of his cagemate and could imply that he simply liked to press the nose ports. For that reason, we introduced the triple chambered apparatus with an empty chamber. The empty chamber served as a control condition.

Anticipated Results for Triple Chambered Apparatus

For the triple chambered apparatus, we hope to see that the active rat pokes significantly more for his chamber and his friend's chamber than for the empty chamber. This would indicate that the active rat is certainly emphasizing with the passive rat's distress due to the airflow and is acting in a prosocial manner.

Discussion

The experimental design of the interoceptive box experiment is indicative of empathy and prosocial behaviors of one rat towards their cagemate in order to relieve their distress. From the double chambered apparatus set up, we can infer that the rats are able to accurately turn off the airflow for themselves and for their cagemate. However, that design alone is not sufficient for claiming that the rats truly do experience empathy towards their cagemate and therefore act in a prosocial manner to alleviate the distress that is being caused upon their cagemate. The triple chambered apparatus design aims to solidify this claim and hopes to address the confounds presented by the double chambered apparatus.

Future Directions

In the future, we aim to implant the rats with flexible sensors and a custom-made manipulator that will overly the diaphragm and the basolateral amygdala complex and insular cortex, respectively. We will be comparing the neuronal encodings from the active and passive rats to see if there are any similar patterns amongst the two. The neuronal recordings will include single unit and local field potentials.

We will also be implanting flexible electrodes into the chest muscles of the rat to obtain heart and respiration rates. The autonomic activity of the active rat will be recorded, before and during interoceptive stimulation using cold air and during the time point preceding both the action to turn the air off on himself and the prosocial action. This will be compared with the autonomic activity of the passive rat before and during the stimulation of the cold air.

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