NON-HUMAN PRIMATE INTEROCPETION AFFECTS DECISION-MAKING DURING APPROACH-AVOIDANCE CONFLICT TASK

By

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Abstract

Interoception involves the signals within the body used to interpret its physiological condition. These signals may include heartrate, breathing rate, and even the sensation of how full the stomach is with food. We investigate how interoception influences decision-making and what would occur if we could alter interoception from its normal state. More specifically, we induced a heartrate increase (a sympathetic-dominated visceral state) in our monkey subjects using a drug, glycopyrrolate, and had our subjects perform in an approach-avoidance conflict task. This type of decision-making task involved subjects choosing between 1) receiving an aversive heat stimulus while simultaneously receiving a consistent reward of juice, or 2) turning off the heat but giving up the juice. The reaction time to turn off the heat was measured and compared before and after the glycopyrrolate. Monkeys also performed in a reward-only task and their food preferences were analyzed before and after glycopyrrolate. We found that a negative outcome in a sympathetic-dominated state increases avoidance, as glycopyrrolate shortened the time the monkey tolerated the heat. On the other hand, reward preference and seeking were not affected during a sympathetic-dominated state. These findings conclude that altering the internal state of the body will subsequently alter decision-making.

Introduction

Interoception, the ability to sense the internal state of the body, is critical to maintaining homeostasis. Interoceptive signals, which communicate the state of the body to the brain, are used by the brain to coordinate various physiological processes like heart rate, digestion, and breathing (Sherrington, 1906). The brain acts as a conductor, ensuring that all the different organs of the body work together in harmony to maintain the overall well-being of the organism. Although most interoceptive signals do not reach our conscious awareness, the degree to which one is aware of signals like one's own heartbeat is associated with the intensity of emotional experiences (Critchley et al., 2004).

The brain-to-body component of brain-body communication involves the brain sending signals to receptors on the organs via autonomic and somatic efferent neural pathways through the spinal cord and vagus nerve. The reciprocal circuit, made up of ascending pathways through the same structures transmits interoceptive information from the body, or visceral systems, to the brain (Sherrington, 1906). Once reaching the brain, interoceptive signals are transmitted to brains areas associated with emotional state such as the insula and another and anterior cingulate cortex (ACC) (Tranel et al., 2009). During heartbeat detection, for example, neural activity in the right anterior insula predicts the accuracy of detecting one's own heartbeat (Critchley et al., 2004). Breakdown in the processing of these signals is associated with mental disorders like depression and anxiety. (Paulus & Stein, 2010). One goal of current research on interoceptive processing is to understand how changes in the information sent to the

brain by the body, the ascending or afferent component, alters the activity of neurons in the brain. One major challenge in doing this is to change organ activity without directly influencing brain physiology and observe how these changes inform behavioral outcomes.

Animal models are an ideal way to study the effects of changes in interoception on behavior in the lab and non-human primates (NHPs) are unique in this regard as they share many similarities with humans in social behavior and brain structure (Kalin & Sheltona, 2006). For example, there are primate-specific interoceptive pathways in the spinal cord (Craig, 2003). Furthermore, when stimuli are presented asynchronously or synchronously with a NHP's heartbeats visual attention increased towards asynchronous stimuli (Charbonneau et al., 2022). This effect is also present in human infants, suggesting similar levels of interoceptive awareness (Maister et al., 2017). These types of studies, which compare animal and human behavior, allow us to draw conclusions about how the human brain works from observing animals.

Our lab is interested in understanding how emotions are represented in the brain, and how they modify decision making. Approach-avoidance conflict is a type of decision making where subjects evaluate multiple conflicting factors reflecting the tradeoff between approaching what is desired and avoiding what is not (Kirlic & Aupperle, 2017). Previously, translational animal models, like rodents, have been used to support approach-avoidance as a tool for investigating and understanding mental illnesses (Kirlic & Aupperle, 2017). Animals and humans perform similarly on the same

behavioral tasks when seeking reward and avoiding danger, as both can learn to predict which stimuli will result in reward or negative outcome (Aupperle et al., 2011). NHPs, for example, can understand the varying amounts of reward and aversion, showing similar patterns to humans, like choosing a reward when the risk or punishment is low (Sierra-Mercado, et al., 2015).

We designed an approach-avoidance conflict task where the monkey had two choices: leave on a heat stimulus (aversive stimulus) attached to their arm in exchange for a constant flow of juice (reward), or turn off the heat stimulus and forgo the juice reward. Using a drug (glycopyrrolate) that does not cross the blood-brain barrier, we selectively altered the internal state of the body without influencing brain physiology (Chabicovsky et al., 2019). Glycopyrrolate is an anticholinergic drug that causes the inhibition of cholinergic neurotransmission at muscarinic receptor sites. Muscarinic receptors are the main target of the parasympathetic nervous system on various organs. Glycopyrrolate has short-term effects on the cardiovascular system such as an increase in heart rate (Seebri Breezhaler, INN-glycopyrronium, 2012). It also offers minimal penetration of the blood-brain barrier, meaning it does not have an effect on brain physiology (Chabicovsky et al., 2019). Without the effects on the central nervous system, we can induce in the body a sympathetic-dominated visceral state by decreasing parasympathetic activity (e.g., increasing heartrate) (Mirakhur et al., 1980). An observed change in behavior, such as a higher preference to avoid rather than approach, after an altered internal state would suggest that interoceptive signals directly interact with the process of decision-making.

Methods

Training and experiments were performed in compliance with the guidelines of the National Institute of Health (NIH) for the use of non-human primates in research and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Arizona.

Subjects

We analyzed the behavior in two female rhesus macaque monkeys (Macaca mulatta): Monkey B (11 years old) and Monkey C (15 years old). Each monkey was housed with a bonded partner in a colony of 4-8 monkeys; Monkey B and Monkey C were kept in separate pairs and rooms. Monkeys were kept on a 12-hour light/dark cycle, provided water ad libitum and were fed in the evenings alongside daily enrichment of toys and food.

Thermode Task Overview

For each session, monkeys were brought up to the lab in their bonded pair, each seated in a custom-made metal NHP chair. Monkeys were placed in double arm restraints attached to the inside of their chair. Arm restraints were placed on the monkey's arm to maintain hand in reaching distance of the button and thermode device. The thermode device, a Medoc TSA2 Advanced Thermosensory Analyzer, was programmed to heat to a desired temperature between 44-48 ˚C and attached to a shaved patch of the monkey's arm. After being habituated with the thermode placement, monkeys

performed sessions of two blocks of 50 trials (20 seconds per trial) in a seated position within their chairs.

Equipment

Automated stimulation and fluid delivery were coordinated using a NIMH Monkeylogic script (Hwang et al., 2019). Thermal stimulation was performed using a MEDOC TSA-2 thermosensory device, a Peltier device that has been approved by the FDA for human use. A custom script was written in MATLAB to allow Monkeylogic to interact with the MEDOC software package to manage columns according to the MEDOC documentation. We used an external control mode and created a custom version of the DEMO-RampTo45 parameter set where the baseline was set to 35 °C and the desired temperature for each condition was pre-programed (a temperature range of 42 °C to 48°C). Ramp rates were calculated as desired heat - base heat (change in heat/1 second), allowing for 1 second ramp and cooldown times in all conditions. Each heat condition was assigned a unique bit code using the external control window of the MEDOC software. These bit code start and stop commands were issued using the original script, described in the documentation. The Thermode was attached to the monkey's arm via Velcro on the back of the 16x16mm or 30x30mm thermode head to a custom PVC arm restraint. Care was taken not to apply pressure to the thermode head during fixation, and to keep the head close to the monkey's arm, to avoid compression of the skin or other injury/bruising. The button is an electrical capacitive switch whose sensor is connected to the metal bar by a single wire. The outputs were connected to

the recording system and Monkeylogic via BNC connectors via National Instruments boards and Plexon/spike2 analog channels.

Training:

1. Button introduction: Reward for button touch and reset

Monkeys were first familiarized to the button through sessions where a dry treat was provided as a reward for each touch of the button. Once the monkey confidently grasped the button, they were rewarded for releasing the button to reset for the next reward to discourage prolonged button holding. After 1-3 sessions of reward for touching the button, monkeys became comfortable with grabbing the button confidently, and releasing it in an appropriate amount of time.

2. Button-heat association: Deactivating heat with button

Next, the monkeys were trained to turn off the heat by learning to touch the button when the thermode heat ramped to between 44 ˚C and 48 ˚C. If needed, monkeys could be prompted with a dry treat to activate the button. Once the monkey stopped the heat within five seconds on >80% of trials, they were introduced to the juice.

3. Juice-button association: Reward for not deactivating heat

To reinforce the button-heat association, a juice reward replaced dry treats and the juice flow was stopped if the monkey touched the button. Once monkeys avoided button contact during >80% of trials, they were advanced to the final task where the juice and heat were combined.

4. Final step of training: Combining reward and heat for an approach-avoidance task

Our final task offered the monkeys two choices: (1) endure a hot but non-painful stimulus on their skin in exchange for a steady flow of juice, or (2) turn off the heat, forgoing the juice reward. While the heat remained on (maximum 20 s per trial), monkeys received juice at a rate of 1 drop per second. Throughout the presentation of the heat stimulus, monkeys could activate a button that turned off both the heat and the juice delivery. The latency to deactivate the thermode served as a measure of the animal's tolerance to the heat stimulus in exchange for receiving the juice reward. Monkeys underwent two blocks of trials a day, one with no-heat (35C) vs. high heat (48C), the blocks consisted of 50 trials of the same heat, order was randomized, and monkeys could undergo no-heat or heat first (Fig 1).

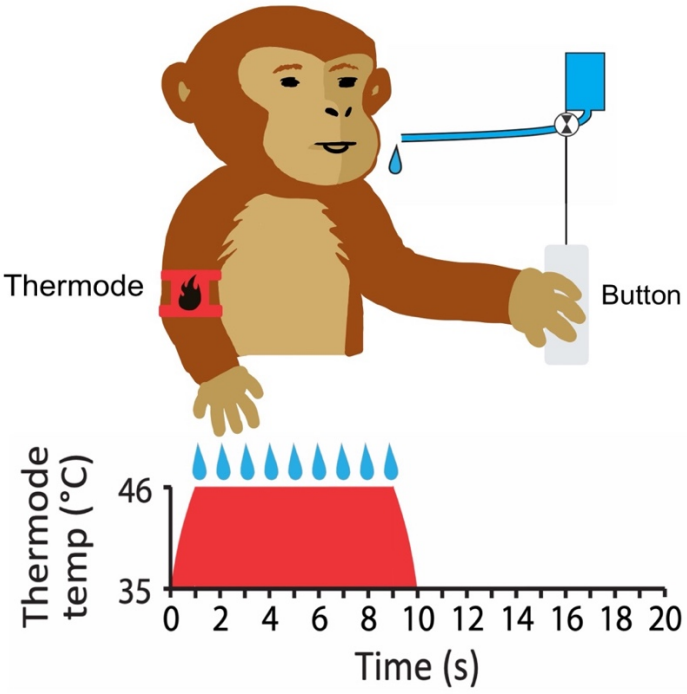

***Figure 1**. A) Monkey is wearing thermode device attached to the skin of the bicep area. A button sits in front of them within reach, as well as a juice tube that delivers 0.100 mL of juice reward per second. At the start of each trial, the thermode begins to heat and maintains a constant temperature of either 35 ˚C or 44-48 ˚C for 20 seconds. The thermode will remain on and juice will be delivered until the end of trial. B) If the monkey presses the button within the 20 seconds of the trial, the thermode heat and the reward turn off and the trial automatically ends. The next trial will begin once the monkey releases the button.*

Drug manipulations coupled with the thermode task

To test whether changing the state of the body is sufficient to influence decision-making, pharmacological manipulations were incorporated with the thermode task. Glycopyrrolate, also known as glycopyrronium, was used to manipulate the body state of our monkeys. Glycopyrrolate was administered subcutaneously to the lower back. Sessions were either saline control days or glycopyrrolate days. Before each session, monkeys were injected with 0.5 mL of pure saline or glycopyrrolate (8 μg/kg) the experimental session started 20 minutes after the time of injection.

Food preference task as a control for the approach-avoidance task.

The food preference task was designed as an approach-approach decision-making task to serve as a control for the thermode approach-avoid conflict task. Each session involved 60 trials of food choices with each trial including a choice between two different food items. There was a total of four food items varying in color, texture, and sweetness per session. During every session, each of the food items and their combinations with each other appeared equally during the session, and alternated sides of the screen to avoid side preference. After food choice was made, monkey was given a raisin-sized portion of their chosen reward by a trusted technician. Then the monkey would repeat the same steps for each trial after, presented with a new food choice. If the monkey did not make a choice on a trial, the food choice combination for the skipped trial would continue to repeat until the monkey made a choice. Like the thermode task, monkeys were injected with glycopyrrolate or saline and food preferences were recorded and compared.

Monkeys were offered two foods from a pool of four foods on each trial. Pictures of the two foods were presented on a screen and the monkey could select which food they would receive from a human handler by fixating on the picture of that food for 500 ms. In the two monkeys tested on this task, monkeys C and P, an ordinal preference hierarchy was established by presenting all food pairings from the four foods available five times (a total of 30 trials). After monkey-specific food preference was established on each day, the monkey was given a glycopyrrolate injection, waited 25 minutes, and performed the task again using the same foods.

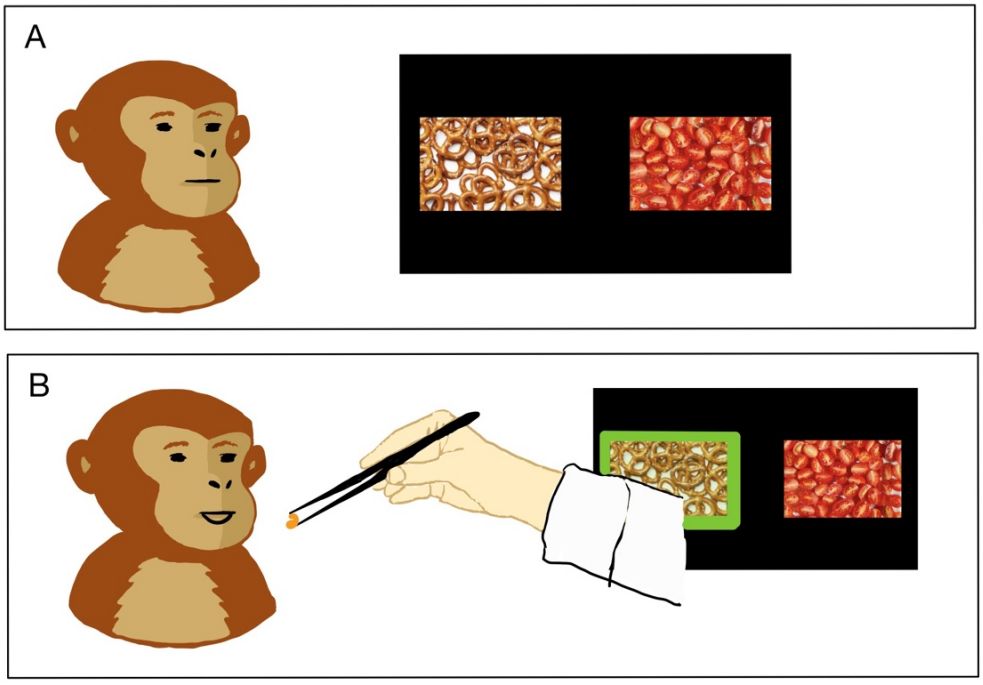

Figure 2. *Food preference task. A) Depiction of NHP looking at a screen containing two images of food choices (pretzels and tomatoes). NHP is given a left and right-side image of two separate food items on the screen. Monkey is given 5 seconds to make a food choice and complete the trial. B) Once food choice is made by the monkey, the trial is complete, and the monkey is rewarded. Monkey then moves on to the next trial to make a new food choice. For each food choice that is successfully completed, the monkey is rewarded in between trials with chosen food preference. Monkeys have the option to choose neither food, but they will not be rewarded and will be presented with the same choice in the next trial.*

Analysis

To analyze the effect of glycopyrrolate on heart rate, monkeys were fitted with EKG pads that continuously recorded during sessions. Once a session was complete, the data for a day was edited with the removal of noise through Plexon's Offline Sorter software. The daily beats per minute were then determined and graphed compared to the amount of glycopyrrolate a monkey received. Analysis was used with MATLAB program. We performed a two-way ANOVA for our statistical test which accounted for monkey identity and dose of glycopyrrolate.

To evaluate the amount of aversive stimuli a monkey was willing to tolerate for a reward, the time to stop the heat was deemed the "Latency". This stopping behavior was compared between sessions with saline control injections versus glycopyrrolate on board.

To determine the behavioral difference brought on by the desire to avoid, "heat" trial behavior with glycopyrrolate was compared to the "no-heat" trials (35 ˚C).

To ensure that glycopyrrolate's effect on heart rate was the reason for behavioral changes and not the effects of some other change to appetite, we compared the food-preference on the control approach-approach task in Excel. We also evaluated if the preference for certain foods was affected by the drug administration.

Results

Glycopyrrolate increases heart rate

Figure 3 is a dose-response curve (averaged from data recorded from 3 monkeys) that shows the change in heart rate produced by glycopyrrolate in a conscious monkey. We evaluated the effects of glycopyrrolate on heart rate by injecting subcutaneous doses ranging from 0 to 1 µg/kg. With glycopyrrolate, the maximum change in heart rate, 17 +/- 7 (error bars are standard error of mean) (range 5-20) beats per minute, was achieved with a dose of 7 µg/kg. Increasing the dose after 8 µg/kg did not result in any significant increase in heart rate. For all experimental manipulations, we used a dose of 8 µg/kg for the most consistent change in HR

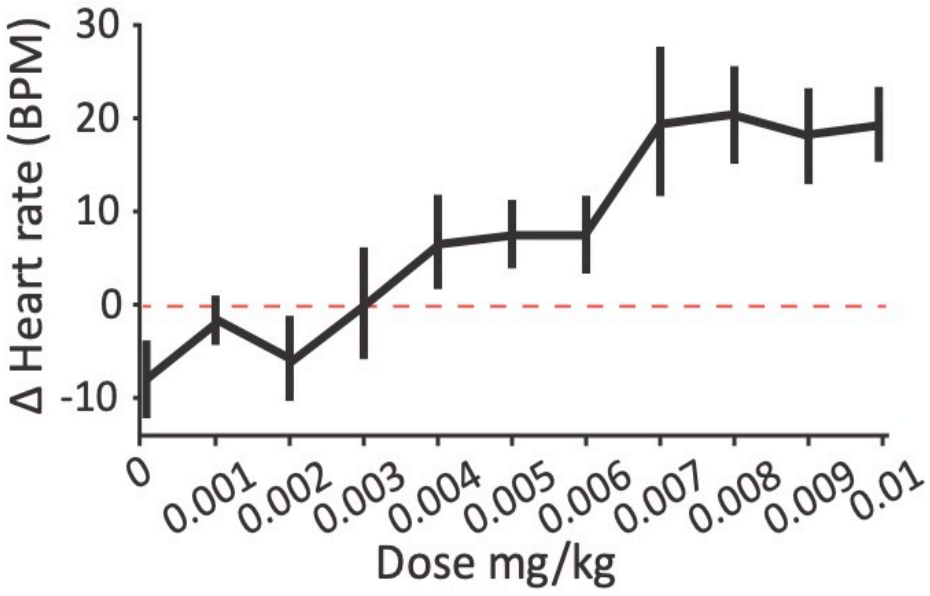

Figure 3 *Increase in heart rate following incremental doses of glycopyrrolate administered subcutaneously, 2 sessions per monkey per dose. Error bars are standard error of mean.*

Glycopyrrolate increases avoidance during conflict task

A sympathetic-dominated visceral state reduces the response time for turning off heat as shown in Figure 4 of response latencies for one female monkey (monkey B). When compared to saline, an uninterrupted interoceptive state, the monkey stopped the heat on average of 5.2 s earlier in the trial when glycopyrrolate was administered (Figure 4). The monkey did not develop a consistent stopping behavior with respect to trial time, but instead alternated between stopping the heat at any point in the trial, or not stopping it at all (Figure 5).

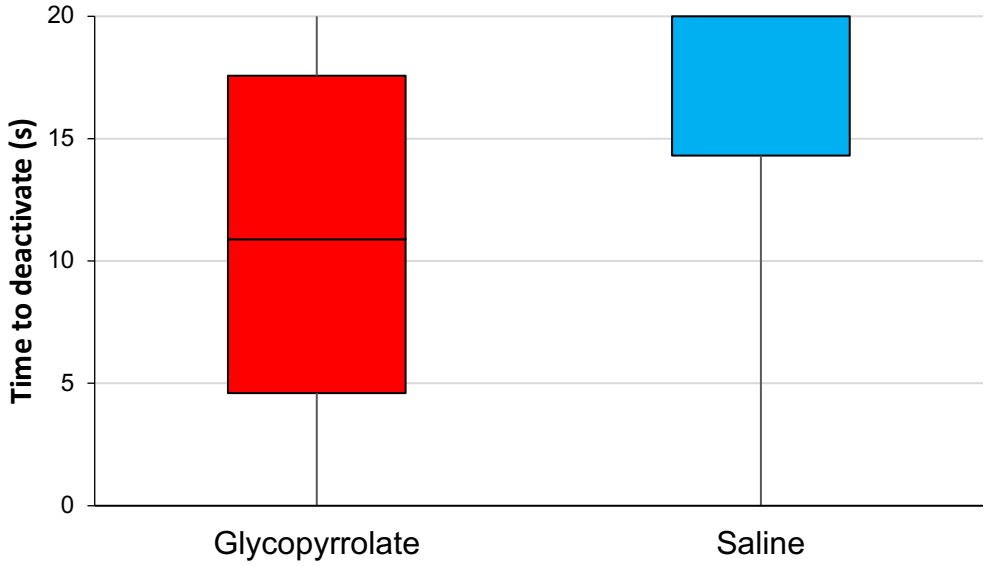

Figure 4 *Glycopyrrolate shortened the latency to turn off the heat during the approach-avoidance conflict task.*

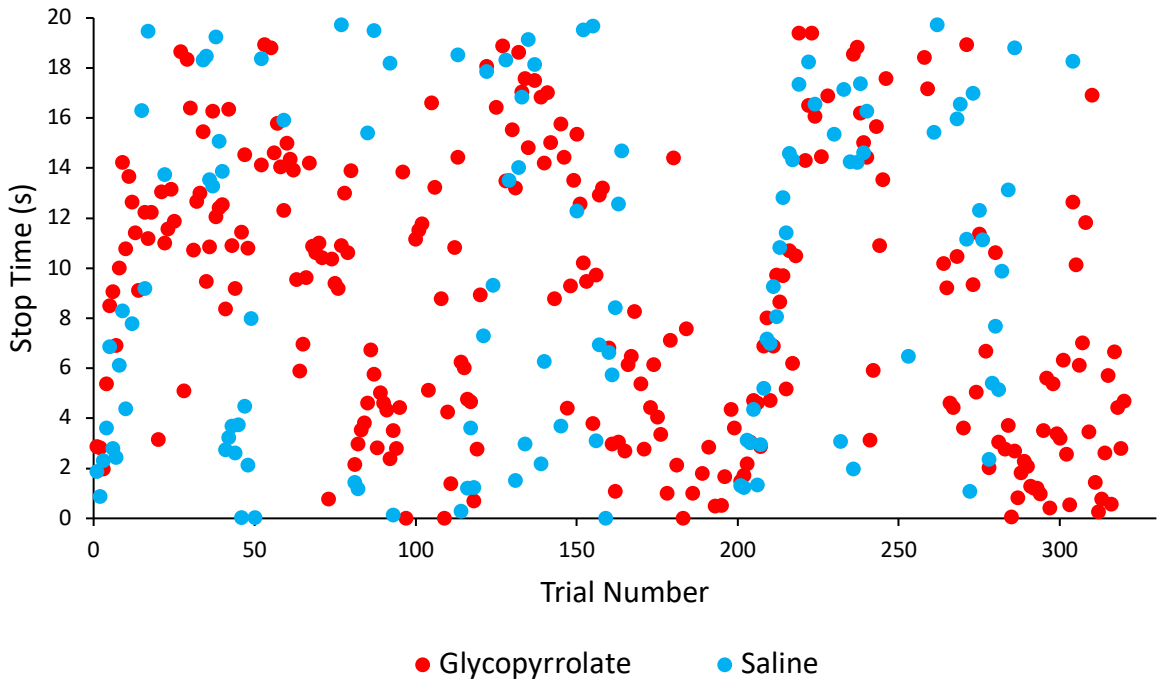

Figure 5 *The variation in response latency did not depend on the succession of trials.*

Glycopyrrolate does not influence reward-seeking

The possibility of glycopyrrolate influencing reward-seeking, such as devaluing the juice and reducing the motivation of the monkey to tolerate the heat, was tested by analyzing the behavior of the monkeys during non-heat trials (35 ˚C). The result was that glycopyrrolate did not increase the number of trials that the monkey stopped the juice flow in non-heat trials (Figure 6), indicating that glycopyrrolate does not change reward seeking in the absence of a negative stimulus. Furthermore, it supports the idea that glycopyrrolate only affects the behavior to avoid or approach in the presence of a negative outcome.

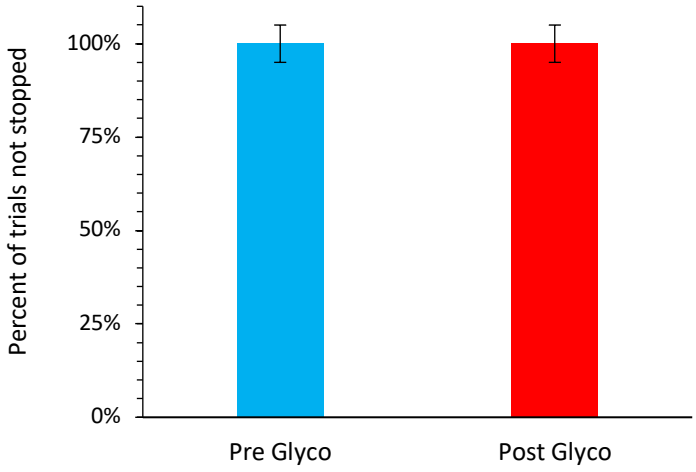

Figure 6. *There is no change in reward seeking during no heat trials before and after glycopyrrolate.*

No change in decision during an approach-only control

We evaluated behavior during a reward-only task to ensure that decision-making is attributed to the aversive component of the approach-avoidance conflict task. Across several food choices varying in monkeys' preference, the percentage of chosen options were consistent before glycopyrrolate administration and after (Figure 7). In this condition, eliminating the negative stimulus of heat and increasing the sympathetic visceral state using glycopyrrolate had no significant behavioral change in reward-seeking.

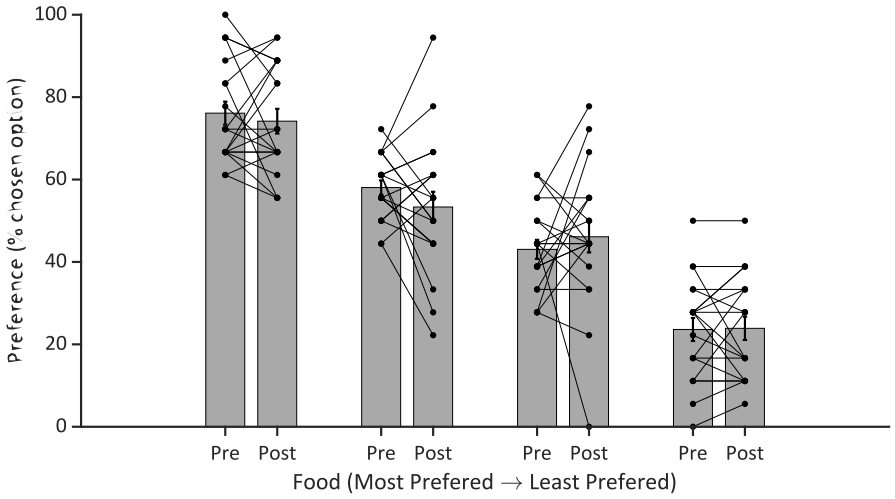

Figure 7 *Glycopyrrolate does not change food preference in the absence of a negative outcome.*

Discussion

In the presence of a negative outcome, a shift in the interoceptive state, such as a sympathetic-dominated visceral state established in our study, is sufficient to influence decision-making. Our results reveal that glycopyrrolate reduces monkey's tolerance for heat, but it did not affect their reward-seeking in the absence of heat. This indicates that a sympathetic-dominated visceral state increases avoidant behavior only in the presence of an aversive outcome. Furthermore, glycopyrrolate did not affect decision-making when picking between two positive outcomes. If decision-making was generally disrupted by glycopyrrolate administration, we expected monkeys to no longer exhibit a food preference and instead randomly select foods. However, glycopyrrolate did not influence food preferences (Figure 7), further demonstrating that decision-making is unaffected in the absence of an aversive outcome by a sympathetic-dominated visceral state. These findings together support the interaction between increased sympathetic tone (or decreased parasympathetic tone) and the decision to approach or avoid

negative outcomes. These results are consistent with a report of optogenetically-induced tachycardia increasing physiological and psychological states, or anxiety-like behaviors, in mice (Hsueh et al., 2023).

The interaction between interoceptive signals and decision-making are likely below the level of awareness, which can be evaluated when only 17.3% of human participants display accurate performance on self-detection of heartbeat (Wiens et al., 2000). Although our monkeys cannot self-report that these changes in interoceptive state are sensed by them, an altered visceral state is unlikely to be distracting to them during the task; otherwise, we would observe reduced reward-seeking and random food selection under the influence of glycopyrrolate.

Approach-avoidance conflict tasks are frequently used to assess anxiety-like behaviors in animals (Kirlic & Aupperle, 2017). As we are not able to collect self-reported data from our monkeys, we rely on such tasks to assess the behavioral correlates of emotional states. Consequently, we do not claim to have induced anxiety through glycopyrrolate. Instead, we suggest that the sympathetic-dominated visceral state that is transmitted through interoceptive afferents may be providing an error signal that influences the entire brain, as has been observed in hunger and thirst (Beutler et al. 2017) (Zimmerman et al., 2019). Therefore, we suggest that the observed behavioral changes of this study are the result of a change in internal state that is closer to anxiety than before the administration of glycopyrrolate.

The limitations of this current project include the limited sample size with only female monkey; however, previous work has shown no sex differences in rhesus macaque heat tolerance (Stevens Negus et al., 2004). We recognize this sample may not be representative of a larger population and more rigorous testing would need to be done before making much determination. Our lab is continuing to explore the use of glycopyrrolate and approach-avoidance with additional female and male monkeys.

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