Interoceptive Signals Bias Decision Making in Rhesus Macaques

Authors

Michael A. Cardenas[1, 2], Ryan P. Le[1], Tess M. Champ[1], Derek O'Neill[1], Andrew J. Fuglevand[1, 2], Katalin M. Gothard[1, 2]

Affiliation

[1]Department of Physiology, College of Medicine, The University of Arizona, Tucson, AZ, USA.
[2]Graduate Interdisciplinary Program in Neuroscience, College of Science, The University of Arizona, Tucson, AZ, USA.

Corresponding Author

Katalin M. Gothard
kgothard@arizona.edu

Author Contributions

MAC: designed the experiments, collected the data, analyzed the data, and wrote the manuscript.
RPL: helped train the animals, helped analyzed the data.
TMC: set up and implemented the first version of the experiments, trained the animals.
DO: trained the animals, designed and implemented the arm restraint, helped collect the data.
AJF: helped design the experiments, helped analyzed the data, and wrote the manuscript.
KMG: designed the experiment, oversaw data collection and analyses, and wrote the manuscript.

Competing interest statement

The authors declare no competing interests.

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Abstract

Several influential theories have proposed that interoceptive signals, sent from the body to the brain, contribute to neural processes that coordinate complex behaviors. Using pharmacological agents that do not cross the blood-brain barrier, we altered interoceptive states and evaluated their effect on decision-making in rhesus monkeys. We used glycopyrrolate, a non-specific muscarinic (parasympathetic) antagonist, and isoproterenol, a beta-1/2 (sympathetic) agonist, to create a sympathetic-dominated physiological state indexed by increased heart rate. Rhesus monkeys were trained on two variants of an approach-avoidance conflict task, where they chose between enduring mildly aversive stimuli in exchange for a steady flow of rewards, or cancelling the aversive stimuli, forgoing the rewards. The delay to interrupt the aversive stimuli and the reward were used as a measure of the cost-benefit estimation that drove the monkeys' decisions. Both drugs altered approach-avoidance decisions, substantially reducing the delay to interrupt the aversive stimuli. To determine whether this autonomic state lowered tolerance to aversive stimuli or reduced the subjective value of the reward, we tested the effects of glycopyrrolate on a food preference task. Food preference was unaltered, suggesting that the sympathetic dominated state selectively reduces tolerance for aversive stimuli without altering reward-seeking behaviors. As these drugs have no direct effect on brain physiology, interoceptive afferents are the most likely mechanism by which decision making was biased toward avoidance.

Significance statement

Influential theories have proposed that the organs of the body send information to the brain and these signals contribute to higher cognitive functions including emotion. Concomitantly, the brain adjusts body physiology to the behavioral agenda of the organism. Empirical support for these ideas, however, has been limited because of the difficulty of dissociating the contributions of brain circuits and body physiology to cognitive processing. Here we selectively manipulated the autonomic state of the body using drugs that do not cross the blood-brain barrier while macaques performed complex decision-making tasks. Drugs that induced sustained peripheral sympathetic activity significantly altered decision making. These findings suggest that ascending, interoceptive signals play a critical role in shaping behavior.

Introduction

At the 2022 Academy Awards, which honors cinematic excellence and attracts millions of viewers, actor Will Smith suddenly walked on stage and slapped comedian Chris Rock following an offhand joke about Smith's wife. The abruptness and intensity of Smith's actions left the audience in shock and disbelief. How could a light-hearted comment evoke such a dramatic response? One possibility is that the joke was perceived by Smith as deeply offensive, and the emotion he experienced caused both increased sympathetic activation and poor impulsivity control. Alternatively, Smith's body might have already been in a physiological state that hindered emotional regulation and biased his decision making toward impulsivity and aggression. In this scenario, the affective state that biased his behavioral propensity was caused by his body physiology. The joke was only the spark that ignited action. These alternatives reflect an age-old, and still unresolved, debate about the role of visceral factors in shaping mental/emotional states (Bard, 1928; Bard & Rioch, 1937; Barrett, 2017; Bechara & Damasio, 2005; Cannon, 1931; Damasio, 2012; Damasio, 1996; Dewey, 1894; Ekman et al., 1983; James, 1890; Lange & Haupt, 1922; Papez, 1937; Schachter & Singer, 1962).

Indeed, in the canonical brain-body dialogue, the brain continuously adjusts the functional state of the body to the organism's behavioral needs through efferent autonomic control (Dum et al., 2019). The resultant visceral changes are reported back to the brain via spinal and vagal interoceptive afferents that close a loop (Chen et al., 2021). Such closed-loop dynamics hinder the ability to isolate the role of interoception alone in shaping the activity of neural circuits that control affect and decision making. The main challenge is to alter physiological states with manipulations that solely target the internal organs and measure the subsequent changes in brain states or behavior (Berntson & Khalsa, 2021).

New approaches have shown promise in overcoming this challenge. Hsueh et al. (Hsueh et al., 2023), for example, showed that experimentally induced tachycardia in mice elicits affective states that manifest in exaggerated avoidance behaviors. Likewise, tachycardia induced in humans by isoproterenol, a beta receptor agonist that does not cross the blood-brain barrier, caused feelings of anxiety (Khalsa et al., 2009; Verdonk et al., 2024). The questions left unanswered by these approaches is whether interoceptive states are sufficient to bias affective behavior in more complex tasks (Bechara & Damasio, 2005).

Approach-avoidance conflict tasks provide a fertile platform to explore the influence of interoception on decision making as they require judicious balancing of costs and benefits (Amemori & Graybiel, 2012; Aupperle et al., 2011; Kirlic et al., 2017; Vogel et al., 1971). We trained non-human primates to perform different versions of approach-avoidance conflict tasks and leveraged pharmacological agents with limited blood-brain barrier penetrance (Carnovale et al., 2023; Chabicovsky et al., 2019; Olesen et al., 1978) to isolate the causal effect of interoception on decision making. We found that application of these peripherally-restricted drugs had substantial effects on decision making, demonstrating the potency of bodily signals on brain processes that coordinate complex behaviors.

Results

Pharmacological manipulations

We identified three drugs that alter the sympathetic/parasympathetic balance in the signaling of the autonomic nervous system (henceforth, autonomic state) but do not cross the blood-brain barrier. Two of the drugs, glycopyrrolate (a non-specific muscarinic receptor antagonist) and isoproterenol (a beta-receptor agonist), shift autonomic state toward sympathetic dominance. The third drug, atenolol (a cardioselective beta receptor blocker), blunts the heart's ability to respond to sympathetic inputs. In healthy adult macaques, glycopyrrolate increased heart rate relative to saline control (**Figure 1**) by 20.5 ± 6.7 (mean ± SD) beats per minute (BPM) (Wilcoxon rank-sum test $p < 0.01$). Likewise, isoproterenol (**Figure 1**) increased heart rate by 11.3 ± 6.0 BPM

(Wilcoxon rank-sum $p < 0.01$). atenolol caused a large decrease in heart rate in monkey A (- 26.1 ± 5.2 BPM) but had no effect in money S (3.7 ± 4.5 BPM).

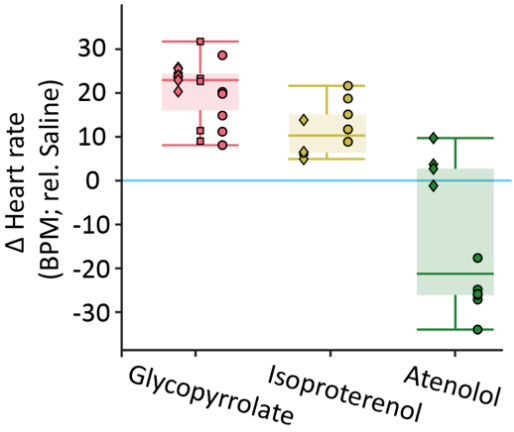

***Figure 1**: Effects of glycopyrrolate, isoproterenol and atenolol on heart rate in two male (A and S) and one female (P) adult macaques (monkey S = diamond, monkey P = squares, monkey A = circle). glycopyrrolate (n = 21 sessions) and isoproterenol (n = 10 sessions) led to significant increases in heart rate ($p < 0.01$, Wilcoxon rank-sum test) compared to saline injection. atenolol (n = 10 sessions) decreased heart rate in one monkey but not in the other ($p = 0.065$ Wilcoxon rank-sum test).*

Approach-avoidance conflict task

We designed a novel approach-avoidance conflict task in which animals chose between two options: (1) endure a hot but non-painful stimulus in exchange for a steady flow of fruit juice, or (2) turn off the heat by touching a bar instrumented with a switch, forgoing the juice reward. A thermode (a Peltier machine that rapidly heats and cools) was attached to a shaved region of the monkeys's arms (**Figure 2A**). On each trial, the temperature was set either to remain at 35°C, a neutral temperature (referred to as no-heat trials), or to ramp up over 1s to a pre-determined hot but not painful temperature ranging between 46 and 48°C depending on the tolerance of the monkey (referred to as heat trials). While the heat remained on (for a maximum of 20 s), monkeys received a 0.2 ml drop of fruit juice at a rate of 1 drop/s. At any point after the heat began to increase, monkeys could interrupt heat and juice delivery by touching the metal bar for 600 ms (**Figure 2A)**. The latency to turn off the heat served as a measure of the animal's tolerance to the mildly aversive heat stimulus in exchange for receiving the juice reward. Each experimental session consisted of a block of 15 - 50 no-heat trials and a block of 30 - 50 heat trials. The order of the blocks was randomized across sessions. As the animals were not water restricted, the number of trials in each session varied with the subjects' level of satiation. The three animals tested on this task rarely stopped the no-heat trials but stopped the majority of heat trials (Chi-Squared test of proportions, $p<0.001$ **Figure 2B**), indicating that they were distinguishing between the two trial types.

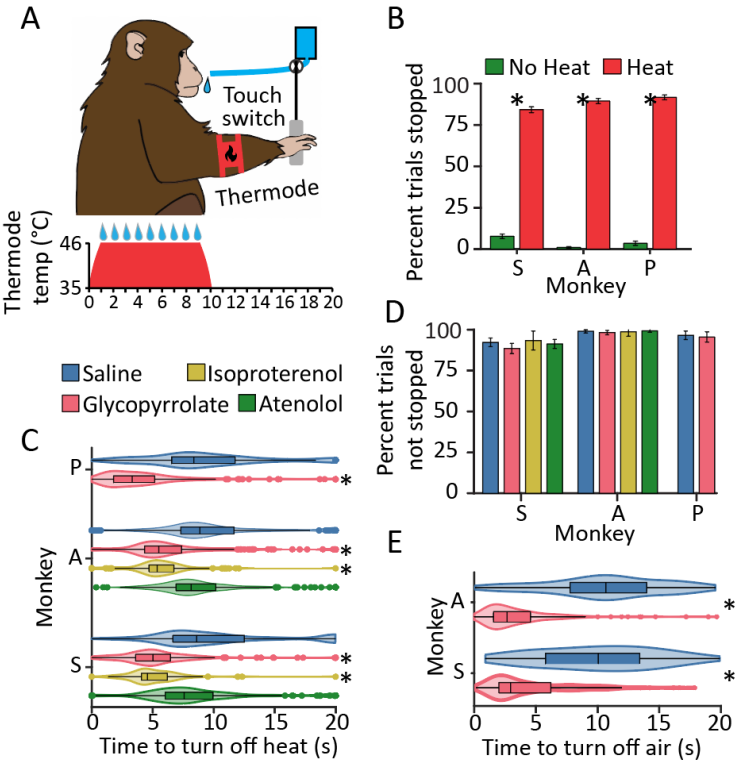

***Figure 2**. Effects of pharmacological manipulations on behavior during approach-avoidance conflict. **A**: Cartoon of the thermal approach-avoidance conflict task. A Peltier device was attached to the monkey's arm. At the start of a trial, the Peltier device rapidly heated up to a predetermined temperature and remained at that temperature for a maximum of 20 s. A drop of juice was delivered every second while the heat was on (red trapezoid). The monkey could interrupt the heat stimulus by touching a switch, but*

this also halted juice delivery (in this caricature trial the monkey interrupted the heat at ~9s). ***B****: Proportion of trials stopped by the monkeys during heat and no heat trials when no drugs were delivered (Chi-squared test of proportions p<0.001).* ***C****: Latency of heat deactivation during heat trials under different drug conditions (number of trials: monkey A: saline = 400, glycopyrrolate = 400, isoproterenol =150, atenolol = 400; monkey S: saline = 400, glycopyrrolate = 400, isoproterenol = 150, atenolol = 400; monkey P: saline = 300, glycopyrrolate = 300). Stars represent significant change in behavior relative to Saline (Wilcoxon rank-sum test p<0.001).* ***D****. During no heat trials, monkeys rarely turned off the juice. There was no difference between the saline control and the drug conditions (Chi-squared test of proportions p>.05;number of trials: monkey A: Saline= 400, glycopyrrolate = 400, isoproterenol = 75, atenolol = 400; monkey S: saline = 400, glycopyrrolate = 400, isoproterenol = 75, atenolol = 400; monkey P: saline = 200, glycopyrrolate = 200), indicating that drugs did not change the value the monkey placed on receiving juice reward.* ***E****. Latency to deactivate airflow. Both monkeys performed a total of 200 trials in each of the Saline and glycopyrrolate conditions. Stars represent significant difference in behavior between glycopyrrolate and Saline (Wilcoxon rank-sum test p<0.001).*

A sympathetic-dominated state reduces tolerance for aversive stimuli

Under control (saline) conditions for heat trials, all monkeys tolerated the heat for 7 - 9 s (**Figure 2C**, blue violin plots, overall average 8.6 ± 3.6 s). Glycopyrrolate administration shortened heat tolerance to 5.1 ± 3.1 s (a 3.5 s or 40.7% decrease) (**Figure 2C**, pink violins; Wilcoxon rank-sum $p<0.001$; Cohen's d: monkey A: 0.8, monkey S: 1, monkey P: 1.5), indicating that blocking peripheral parasympathetic receptors reduced heat tolerance. To determine if the reduced heat tolerance was the result of a sympathetic-dominated autonomic state and not the specific result of a muscarinic blockade, we replaced glycopyrrolate with isoproterenol in monkeys A and S. Isoproterenol, like glycopyrrolate, reduced the duration of heat tolerance in both monkeys to 5.3 ± 2.1 s (a 3.3 s or 38.4% decrease) (**Figure 2C** yellow violins; Wilcoxon rank-sum $p<0.001$; Cohen's D: monkey A: 1 monkey S: 1.1). Thus, blocking parasympathetic muscarinic receptors or activating sympathetic beta receptors had the same effect of increasing heart rate and reducing heat tolerance. Next, we tested whether shifting sympathetic / parasympathetic balance toward parasympathetic dominance by blocking the sympathetic cardiac receptors with atenolol would have the opposite effect. While atenolol decreased heart rate in one monkey, it had no effect on approach-avoidance decisions in either subject (**Figure 2C**, green violins). Furthermore, as the animals did not stop the steady flow of juice in the *no heat* trials, it is unlikely that the drugs altered reward-seeking behavior or devalued the reward (**Figure 2D**; Chi-squared test of proportions compared to saline control, $p>0.05$).

To determine if the effects of isoproterenol and glycopyrrolate were specific to thermal stimuli, or generalize to other aversive stimuli as well, monkeys A and S performed an airflow version of the approach-avoidance conflict task. In this case, for each trial, monkeys either endured a continuous flow of room-temperature air directed at their nose and muzzle for 20 s while receiving a juice reward or could turn off the airflow and forgo the juice reward by touching the switch. Airflow pressure was set to a fixed value of ~ 65 Pa. Here too, the latency to turn off the airflow served as a measure of the animal's tolerance to the mildly aversive stimulus. Eight experimental sessions were performed by both animals (four each for saline and glycopyrrolate) and each experimental session consisted of 50 trials. As in the case of thermal stimuli, glycopyrrolate markedly reduced both subjects' tolerance to continuous airflow directed at their face, from 10.3 ± 4.7 s with saline to 4.0 ± 3.4 s with glycopyrrolate, a 61.2 % drop (**Figure 2E**, blue and red violins; Wilcoxon rank-sum $p<0.001$; Cohen's d: monkey A: 1.3; monkey S: 1.7). Collectively, these results suggest that a sympathetic-dominated autonomic state in the periphery reduces tolerance to aversive outcomes, regardless of the nature of the stimulus.

A sympathetic-dominated state does not alter decision making related to non-aversive stimuli

To explore whether sympathetic-dominated states alter decision making in non-aversive tasks, we evaluated the effects of glycopyrrolate on a task where the subjects chose between appetitive stimuli only. On each trial, monkeys were offered two foods randomly selected from a pool of four possible foods. Pictures of the offered foods were presented on a screen and the monkeys made their selection by fixating for 500 ms on the picture of their preferred food. Two monkeys (monkeys C and P) performed this food preference task before and after receiving a glycopyrrolate injection (36 pre-administration and 36 post-administration trials). Each monkey participated in 8 sessions. We limited the number of trials within a session to minimize satiation effects. We hypothesized that if decision-making was generally disrupted by glycopyrrolate, the monkeys might change their food preference. Glycopyrrolate did not alter absolute or ordinal food preference (**Figure 3**; Friedman's test $p>0.05$). Thus, a sympathetic-dominated peripheral autonomic state has little effect on decision making regarding appetitive stimuli compared to aversive stimuli.

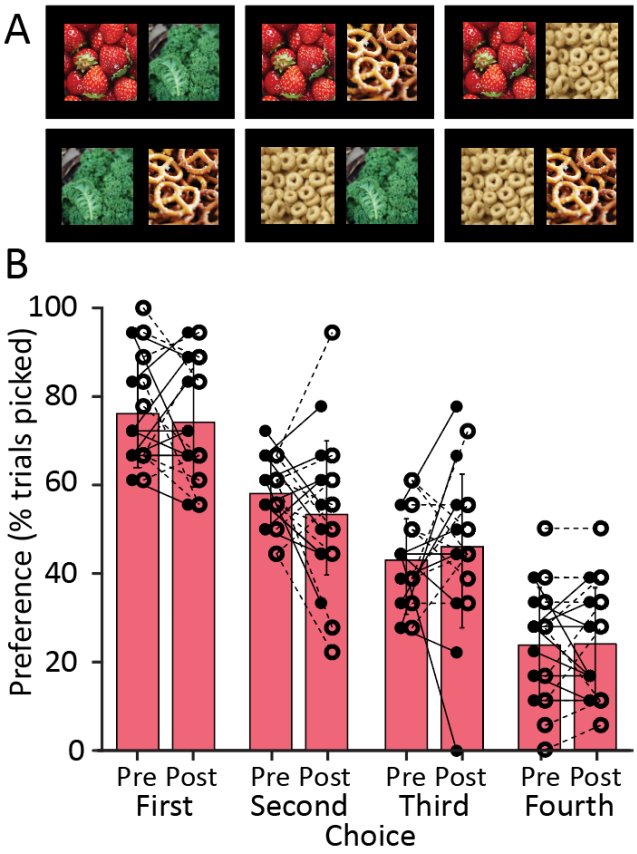

*Figure 3: Glycopyrrolate did not change ordinal food preference. **A**. Monkeys were shown pairs of images depicting the foods available on a given trial. All six pairs of the four available food items were tested. The monkey indicated which food they prefer by fixating on the image for 500 ms, after which the image of non-preferred food disappeared from the monitor and a human handler delivered the chosen food into the monkey's mouth using long forceps. **B**. The bar plots depict the monkeys' combined food preferences pre and post glycopyrrolate injection. Each pair of dots connected by a line are the choices from the same session. Filled dots and lines are for monkey P while circles and dashed lines are for monkey C. Monkeys' food preference before injection were ordinally ranked with the leftmost bars indicating the most preferred food and rightmost bars the least preferred food. Error bars are standard deviation. glycopyrrolate administration did not lead to a change in food preference (Friedman's test p>.05).*

Discussion

Here we show that decision-making in two versions of an approach-avoidance task can be biased by peripheral manipulations of autonomic signaling. Despite acting on different receptors, glycopyrrolate and isoproterenol caused both a relative dominance of sympathetic tone, as indexed by increased heart rate. This peripheral autonomic state caused a large decrease in tolerance for aversive stimuli while leaving responses to appetitive stimuli intact. Interestingly, atenolol had no discernable effect on approach-avoidance conflict behavior even for the one monkey in whom heart rate was reduced by 25 BPM with the drug. The reason for this asymmetry in responses (i.e., diminution in tolerance with enhanced sympathetic drive and absence of change in tolerance with reduction in sympathetic drive) is not clear. In addition, it is important to emphasize that the animals were not food or water restricted, so the decision to tolerate the heat or the airflow was not informed by a homeostatic

imperative such as thirst or hunger. Such homeostatic drives are known to induce changes in internal state that subsequently alter behaviors (Allen et al., 2019; Livneh et al., 2020).

The drugs used in this study have low penetrance of the blood brain barrier (Chabicovsky et al., 2019; Olesen et al., 1978). Thus, it is unlikely that they directly influenced brain circuits that controlled performance in the approach-avoidance tasks. None of the drugs we used are known to cause changes in the perception of heat or tactile stimuli. Information about the autonomic states induced by these drugs was likely transmitted to the brain via interoceptive pathways. These pathways originate in the internal organs and are transmitted through spinal and vagal afferents to the nucleus of the solitary tract and other brainstem nuclei that carry out the initial processing of visceral inputs (Berntson & Khalsa, 2021; Craig, 1995, 2002, 2003; Craig et al., 1994). While these inputs serve homeostatic regulation in the brainstem and the hypothalamus, they are known to propagate further to cortical and subcortical circuits and may contribute to various cognitive functions(Benarroch, 1993; Berntson & Khalsa, 2021). Cortically processed interoceptive inputs give rise to interoceptive awareness, that can be assessed through verbal reports in human adults (for a comprehensive review, see Azzalini *et al*., 2019(Azzalini et al., 2019)) and through non-verbal tasks in human infants and monkeys (Charbonneau et al., 2022; Maister et al., 2017).

Interoceptive signals, particularly those generated on the timescale of the cardiac cycle, have been shown to modulate the processing of external stimuli (Critchley et al., 2005; Dunn et al., 2010; Ekman et al., 1983; Garfinkel et al., 2014; Gray et al., 2012; Gray et al., 2009). The changes in decision making reported here, however, persisted throughout an experimental session, suggesting that interoceptive signals contributed to a sustained change in cognitive processing. Similarly, recent work in which prolonged tachycardia was induced by optogenetic stimulation of the heart led to a persistent increase in avoidance behavior to anxiogenic stimuli (Hsueh et al., 2023).

Beyond decision making, the present findings contribute empirical support for influential theories suggesting that bodily states modulate other complex functions of the brain, such as affect and emotion (Bard, 1928; Barrett, 2017; Cannon, 1931; Craig, 2009; Damasio, 2012; Damasio, 1996; Dewey, 1894; James, 1890; Khalsa & Feinstein, 2018; Lange & Haupt, 1922; Papez, 1937; Schachter & Singer, 1962). As the substrate of emotional behavior evolved from ancestral circuits that regulate homeostasis and defensive behaviors (Buzsáki & Tingley, 2023; Cisek, 2022; LeDoux, 2012), these circuits retain the closed-loop connections by which the internal organs and the brain co-regulate their functions. Future studies are needed to explore the neural mechanisms by which interoceptive signals can alter cognitive, perceptual, and emotional operations in the brain.

Methods

Subjects and Living Arrangements

All protocols were approved by the University of Arizona Institutional Animal Care and Use Committee and carried out in accordance with the US National Institutes of Health guidelines. All procedures were performed at the University of Arizona.

Two female (monkeys C and P, 16 and 12 years old, respectively) and two male (monkeys A and S, both 8 years old) adult rhesus monkeys (*Macaca mulatta*) weighing, on average 11.7 ± 2.1 kg were housed in standard indoor cages in temperature-controlled rooms with automatically regulated lighting (12 h light/dark cycle with lights on at 7:00 AM and off at 7:00 PM). All monkeys were housed with a cage mate of the same sex, were allowed access to water *ad libitum* in cage, and fed once daily (Tekklad 2050) in accordance with veterinary directions. Plastic toys, mirrors, and other objects were provided in-cage for enrichment. Nightly enrichment

including foraging boxes, vegetables, ice cubes, etc., was provided alongside their standard diet. Monkeys A and S were cage mates, as were monkeys C and P. Monkeys A, S, and P were trained on the thermal approach-avoidance conflict task and monkeys P and C were trained on the food preference task.

Thermal approach-avoidance conflict task.

Monkeys were acclimated to custom-made primate chairs and trained to place their arms in custom made arm restraints built from PVC tubes attached to a rail system. The restraint for the right arm had an opening on its left side for the monkey to move their wrist freely and interact with a metal bar instrumented with a switch that was mounted on the arm restraint. The left arm restraint had an opening that snuggly fit the 30 x 30 mm head of a Medoc TSA-II Thermode (Ramat Yishay, Israel). Elastic Velcro straps were attached to keep the thermode head in contact with the skin of the dorsal proximal forearm. Care was taken not to put excessive pressure on the thermode head that might restrict blood flow or numb the skin.

Automated stimulus and juice delivery was coordinated using custom NIMH MonkeyLogic scripts (Hwang et al., 2019). The thermal stimulus was delivered using a MEDOC TSA-2 thermal sensory device. This is a Peltier device approved by the FDA for human use. Custom scripts were written to allow MonkeyLogic to interface with the MEDOC software package to control the heat in accordance with MEDOC external control documentation (TSA-2 manual appendix K). For reproducibility, we used the external control mode and created custom versions of the *TSA – 2 42 ramp and hold* demo program where the baseline was set to 35°C and the target heat was different for each condition (heats ranging from 46 to 48°C). The ramp time from baseline to target was 1 s. The metal bar was attached to a battery powered PCB gadgets CP100 Capacitive Switch with the sensor connected to a metal bar and the output signal was sampled by MonkeyLogic and data acquisition systems.

After being trained to tolerate arm restraints, monkeys learned to hold the metal bar for a solid food reward when cued. They then learned to touch the bar to turn off a heat when set to 46°C. Next, we trained the monkey to associate touching the bar with stopping juice delivery in the absence of heat. When the monkey started to avoid touching the bar while juice was being delivered, they moved to the full task by combining the heat and juice trials together.

Once trained on the task, we adjusted heat tolerance for each animal by gradually increasing the heat delivered over 10-20 trials/heat level and assessing stopping behavior. Once an animal stopped the heat on average between 7 and 10 seconds, we did not increase the heat further. We then assessed the stopping behavior on the heat for three days (50 trials/day) before beginning experimental manipulation.

Once a consistent stopping behavior for a given temperature was established, the experiments were initiated. On each trial, the heat ramped to the pre-determined temperature without a cue. Monkeys received juice at a rate of 1 drop/s beginning at the start of the ramp until the instrumented bar was touched for 600 ms or 20 seconds had elapsed. Intertrial interval was 10 seconds.

Airflow approach-avoidance conflict task.

Monkeys A and S, who had already been trained on the thermal approach-avoidance conflict task, were arm restrained as in the thermal version of the task so that they could operate the touch switch. Airflow was delivered to the face through lock line hose (Crist Instruments) placed about 12 cm from the muzzle by a compressed air system containing a solenoid. The pressure applied at the muzzle was estimated to be ~ 65 Pa (pressure at the nozzle was 60 PSI). Airflow was automatically turned on in conjunction with juice delivery by a

custom script made in NIMH MonkeyLogic. The task parameters were otherwise identical to that of the thermal approach-avoidance conflict task.

Heart rate measurements

Raw electrocardiogram (ECG) was collected via three 2.5 x 1 cm self-adhesive electrodes attached to the monkey's back and optimized with conductive gel and amplified through a Grass amplifier. ECG was sampled at 1000 Hz using either a Spike2 (Cambridge Electronics Design) or Plexon Omniplex recording system. Raw signals were filtered using a 3 – 35 Hz bandpass filter. The timing of R-waves was identified using template matching or cluster analysis from which instantaneous heart rate (BPB) was determined.

Pharmacological manipulations

Saline, isoproterenol (0.0001mg/kg), and glycopyrrolate (0.008mg/kg) were administered by subcutaneous injection to a shaved and cleaned (with isopropyl alcohol) region on the back of the monkey. All monkeys were trained to tolerate the injection. Atenolol (6-7mg/kg) was delivered orally as a crushed powder in a peanut butter filled tortilla. Monkeys P did not accept this route of administration, likely due to the bitter taste of the powder, and therefore did not participate in the atenolol experiments. All drugs were approved by the University of Arizona's Institutional Animal Care and Use Committee and by the veterinary team. Atenolol and glycopyrrolate were procured from MWI Animal Health (Boise, ID) and isoproterenol was generously provided by Dr. William Stauffer at the University of Pittsburgh.

For glycopyrrolate, at the beginning of each session, monkeys were pseudo-randomly administered either the drug or saline subcutaneously allowed to rest in the chair 30 minutes before testing. This rest period was needed to allow glycopyrrolate to take effect. For isoproterenol, because it is rapidly metabolized and cleared, experiments began directly after injection. For atenolol, the drug was given orally each of eight consecutive testing days 30 minutes before testing.

Statistical analysis

Statistical analysis was done using MATLAB version 2023a. Non-parametric tests were performed across all data. Heart rate recordings were excluded if signal was lost in at least 30% of trials. This could happen as a result of the monkey shaking their body or pads falling off during the recording.

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