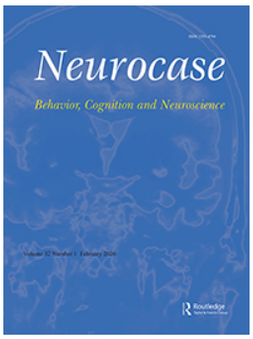

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Stimulation of the human periaqueductal gray induces threat bradycardia: a case report

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ABSTRACT

The ability to recognize and anticipate potential danger is crucial for survival across species. The midbrain periaqueductal gray (PAG) is implicated in regulating threat-anticipatory responses, including heart rate deceleration (threat bradycardia) which is typically observed during freezing. Although animal studies have provided causal evidence that the PAG regulates threat-anticipatory bradycardia, causal evidence does not exist in humans. To address this translational gap, we performed a single-case study to elucidate the causal role of the human PAG in threat-anticipatory bradycardia using deep brain stimulation (DBS). We report on a participant who received PAG DBS for chronic pain treatment. The participant performed an instructed fear task during which cues were presented signaling either threat of electrical shock or safety. During the task, we applied DBS in the PAG, no DBS, or DBS at a control site bordering the PAG. Deep brain stimulation in the PAG significantly increased threat bradycardia responses (i.e. reduced heart rate for threat vs. safety) compared to no stimulation, whereas control-site stimulation bordering the PAG did not significantly affect threat bradycardia. Together, this single-case report provides causal evidence that the human PAG regulates threat bradycardia responses, furthering our understanding of the neural circuit underlying defensive reactions in humans.

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Periaqueductal gray (PAG); deep brain stimulation (DBS); threat bradycardia; defensive reactions; instructed fear

Introduction

To adaptively cope with environmental threat, humans and other animals show a variety of defensive reactions (e.g., freezing) that are characterized by distinct physiological (e.g., cardiac) response profiles (Bolles, 1970). Threat-anticipatory freezing is one of the most common defensive reactions, characterized by bodily immobility and a reduction in heart rate (threat bradycardia) in response to cues signaling threat. Freezing reactions serve to reduce the likelihood of detection and to prepare the organism for subsequent action (fight/flight reactions; Bolles, 1970; Roelofs, 2017). Fight/flight reactions, in turn, are associated with cardiac acceleration (tachycardia) and function to deal with acute and proximate danger (Kozlowska et al., 2015; Lang & Davis, 2006). Animal studies have shown that threat bradycardia is regulated by the midbrain periaqueductal gray (PAG; Carrive, 1993; Signoret-Genest et al., 2023; Tovote et al., 2016). Thanks to its extensive (sub)cortical and brainstem connections with the central nucleus of the amygdala, the hypothalamus, and medullary relay centers (that in turn project to spinal cord and vagus nerve), the PAG can orchestrate bradycardia, immobility, and concurrent suppression of nociceptive stimuli (analgesia) in the anticipation of threat (Carrive, 1993; Fields, 2004; Silva & McNaughton, 2019). However, as of yet, causal evidence that threat bradycardia in humans depends on the PAG remains absent. Confirming such a causal role for the PAG in defensive reactions in humans is important to identify future directions for investigating the neural mechanisms underlying psychopathology. Indeed, associative threat conditioning – the main experimental model for fear learning and exposure therapy – relies on our understanding of the

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physiological correlates of fear and anxiety, such as threat bradycardia (Battaglia et al., 2023; Beckers et al., 2023; Haaker et al., 2019). We sought to address this knowledge gap by using deep brain stimulation (DBS) to investigate the causal role of the human PAG in threat-induced cardiac deceleration.

The PAG consists of several interconnected subregions, organized as columns around the midbrain aqueduct, which together orchestrate the behavioral and physiological (e.g., cardiac) responses associated with defensive freeze/fight/flight reactions. Over the past decades, studies using animal models have characterized the PAG as the regulation center of these defensive reactions and associated responses (Carrive, 1993; Rizvi et al., 1991; Silva & McNaughton, 2019; Walker & Carrive, 2003; Zhang et al., 1990). Specifically, electrochemical stimulation of the dorsal portion of the PAG has been shown to trigger fight/flight-like reactions and tachycardia, but can similarly evoke anticipatory behavioral freezing instead (Brandão et al., 2008; Fadok et al., 2017; Tovote et al., 2016; Vianna et al., 2001). Also in humans, correlational evidence suggests that the PAG may be involved in the regulation of defensive reactions. Studies using fMRI for instance suggest that PAG activity increases with higher threat imminence (Mobbs et al., 2007; Murty et al., 2023), and that threat-related bradycardia during freezing is associated with increased PAG activity and stronger amygdala-PAG connectivity (Hashemi et al., 2019; Hermans et al., 2013; Lojowska et al., 2018; Schipper et al., 2019; see Kragel et al., 2019; Satpute et al., 2013; Wang et al., 2022; Weis et al., 2022 for functional involvement of human PAG (subregions) in emotional processing and cognitive control). Seminal first studies have successfully applied deep brain stimulation (DBS) to human PAG to study the effects of PAG stimulation on blood pressure (Green et al., 2006) and heart rate variability (Pereira et al., 2010) outside the context of threat. However, whether the human PAG is causally involved in threat-induced responses – such as threat bradycardia – is not clear.

Here we report on a unique case study using deep brain stimulation (DBS) to elucidate the causal role of the human PAG in threat bradycardia responses during an instructed fear task. We show that stimulation inside the PAG (and not stimulation bordering the PAG) increased threat bradycardia responses to cues that signal threat compared to safety, providing evidence that the human PAG regulates defensive cardiac responses.

Materials and methods

This study was approved by the local ethics committee (Ethical Reviewing Board METC [Institutional Research Review Board] Oost-Nederland, CMO 2022/13854), and was conducted according to these guidelines and regulations (i.e., medical/scientific research), and in accordance with the Declaration of Helsinki.

Case description

The participant included in this case report is a 53-year-old man with a long history of severe progressive, treatment-resistant pain in his right shoulder, arm, and hand, caused by a motorcycle accident-related right-sided brachial plexopathy at 25 years old. Various types of high-dose medications such as paracetamol, non-steroidal anti-inflammatory drugs, amitriptyline, gabapentin, pregabalin, and tramadol were not able to reduce the pain. Transcutaneous electrical nerve stimulation, multiple dorsal root entry zone lesioning surgeries at the cervical levels C4-C7, and cervical spinal cord stimulation neither offered long-term relief.

At the age of 45 years, the participant was offered compassionate-use, off-label deep brain stimulation treatment (Pereira & Aziz, 2014). During this awake stereotactic procedure, a DBS electrode with four 1.5 mm circumferential contacts separated by 0.5 mm interspaces (model 3389, Medtronic Inc) was implanted in the left-sided periaqueductal gray matter (dorsal to the aqueduct; Figure 1(a), upper two panels). Test stimulation in this area (at 3 mA, 30 Hz, 450 µs) elicited a pleasant, warm feeling in the right shoulder, arm, and hand. Subsequent implantation of the subcutaneous extension cable and implantable pulse generator (model Activa SC, Medtronic Inc) was done under general anesthesia. Programming and optimization during various outpatient clinic visits showed that DBS at 0.2 - 0.5 V (30 Hz, 450 µs) at the most ventral contact point was most efficacious in long-term pain reduction (located 0.5 mm lateral, 7.7 mm posterior, and 7.1 mm inferior relative to the posterior commissure; Figure 1(a), lower two panels). To prevent stimulation tolerance (i.e., the gradual decrease in stimulation efficacy over time), DBS was applied in a cyclic setting, with DBS ON for 10 seconds followed by DBS OFF for 5 minutes (see e.g., Pereira & Aziz, 2014).

Procedure

For the present study, the participant came to the lab for two separate sessions. For both sessions, the participant came to the lab with the deep brain stimulation electrode already turned OFF for at least 12 hours. This way, we avoided potential carry-over effects from stimulation ON to stimulation OFF conditions, which is relevant as the exact wash-out time of PAG stimulation effects

remains unknown. In the first session, the participant first read and signed the screening form and gave written informed consent. The participant then filled in a set of questionnaires, including the State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983) and the Beck Depression Inventory (BDI; Beck et al., 1996). Next, the participant was attached to all measurement and stimulation equipment, and was instructed about the shock work-up procedure (see below) and the instructed fear task (IFT; see below). Before and after performing the IFT, the participant again performed the State questionnaire of the STAI. Additionally, immediately before starting the IFT, the participant provided VAS (visual analogue scale) ratings of alertness and fear (0 – 10). Next, the participant performed another experimental task that is not reported here. After a 30-minute break, the DBS electrode was turned on, after which the participant performed the entire procedure again (shock work-up, questionnaires and ratings, and IFT). The second session was performed approximately 2 months after the first session and followed exactly the same procedure, except for the stimulation location during the second half of the experiment. In session 1, the DBS electrode stimulated at a contact point located in the dorsomedial PAG (further referred to as experiment 1), whereas in session 2 the DBS electrode stimulated at a 2.4 mm more dorsal/2.3 mm more left lateral/2 mm more anterior contact point (located just on the border of the PAG; experiment 2). In both experiments, the stimulation parameters were identical (amplitude of 0.5 V, frequency of 30 Hz, pulse width of 450 µs). After each experiment, the stimulation parameters were reset to treatment settings. The participant did not report any side effects or inconvenience of the applied protocols (neither before, during, nor after the experiment). However, because there are instances where deep brain stimulation of the PAG has been associated with altered conscious and emotional states (Nashold et al., 1969) we explicitly assessed subjective state changes. This confirmed that across experiments and stimulation conditions subjective ratings of alertness were consistently high, while subjective ratings of fear and state anxiety were consistently low (see Supplemental Table S1).

Instructed fear task

We used an instructed fear task (IFT) to investigate threat bradycardia responses (Klumpers, Raemaekers, et al., 2010). This task consisted of the interleaved presentation of two pictures with neutral male faces on a blue vs. orange background to make them clearly distinctive (face stimuli were obtained from the NimStim dataset; Tottenham et al., 2009). The participant was informed that they could receive shocks at any time during the presentation of one picture (the CS+) whereas they never would receive a shock during the presentation of the other picture (CS-). The participant was explicitly instructed to rest in between picture presentations via onscreen presentation of the Dutch word for rest ("RUST") during the inter-trial interval (ITI). The task consisted of a single run of 28 trials (approx. 12 minutes) presented in a semi-random order, with no more than 3 consecutive repetitions of the same stimulus (CS+ vs CS-). Face pictures were presented for 10 – 24 seconds, whereas the ITI duration ranged from 8 – 12 seconds. Shocks were administered during four pre-specified trials; during the first, third, and fourth CS+ presentation (at 3 seconds), and during the ninth CS+ presentation (at 5 seconds). The task was programmed using Presentation software (Neurobehavioral Systems, Inc., Berkeley, CA).

Peripheral stimulation and measurement

As the participant did not suffer from chronic pain symptoms in his left arm and hand, electric shocks were delivered to the distal phalanges of the fourth and fifth left-hand fingers, using a 9 V MAXTENS 2000 shocker machine and standard 10 mm Ag/AgCl electrodes. Physiological responses were recorded using a BrainAMP EXG MR 16 channel amplifier, an EXG aux device, and BrainVisionRecorder software. To measure heart rate, we recorded an electrocardiogram (ECG) using a three-lead system with standard 3 M Red Dot 2249 Ag/AgCl measurement electrodes (6.1 cm diameter). Two electrodes were placed just below the right collar bone and around the lowest left rib (i.e., diagonally across the heart) and a third ground electrode was placed just below the left collar bone (i.e., approximately above the heart). We also recorded electrodermal activity and respiration which was not analyzed for this report.

Shock work-up procedure

We used a standardized shock work-up procedure to set the intensity of the electrical stimulation to a level that was rated as uncomfortable but not painful (see also de Voogd et al., 2018; Klaassen et al., 2024; Klumpers, Raemaekers, et al., 2010). Shock durations were 200 ms (consisting of a train of 250 µs pulses at 150 Hz), delivered at an intensity ranging from 0–40 V/0–80 mA divided in 10 pre-defined steps. During the work-up procedure, the participant received and rated exactly five shocks (starting at intensity level 2), with the aim to converge at an intensity that was rated as of 4 out of 5 (where 1 = "not painful at all" and 5 = "very painful").

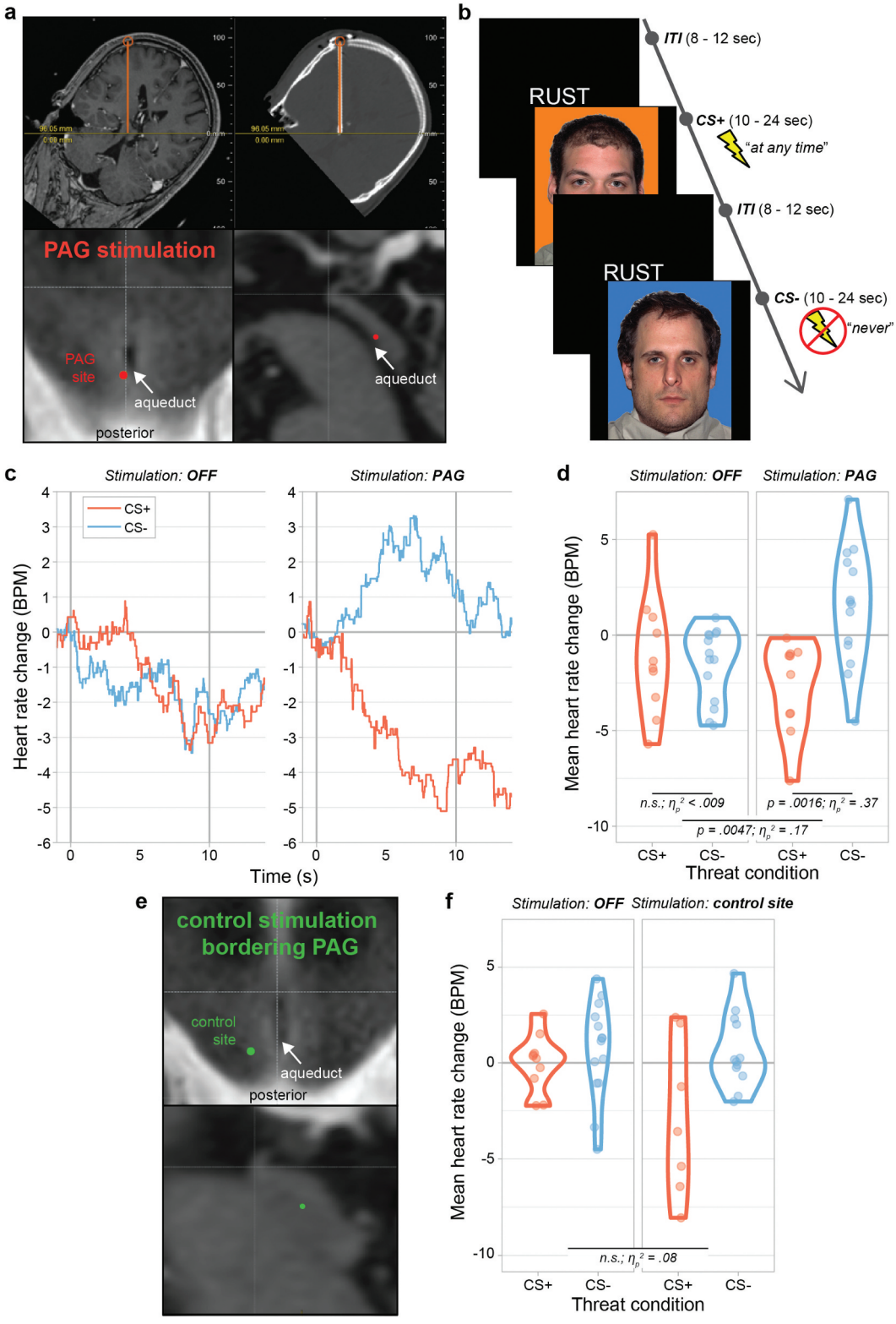

Figure 1. Experiment overview. a) DBS electrode positioning. Planned trajectory of PAG DBS electrode on preoperative semi-coronal T1-weighted MRI (upper left); DBS electrode on postoperative CT (upper right); Anatomical location of PAG stimulation (in red) on axial T2-weighted (bottom left) and sagittal T1-weighted MR image (bottom right), at 0.5 mm lateral, 7.7 mm posterior and 7.1 mm inferior relative to the posterior commissure (PC). b) Example trial sequence of the instructed fear task (IFT). During the IFT, the participant was presented with an interleaved sequence of two pictures of faces, during which they could either receive a shock at any time (CS+) or could never receive a shock (CS-). In between face picture presentations, the participant was explicitly instructed to rest. c) Mean heart rate change in beats per minute (BPM) across time relative to 1-second pre-trial baseline window is plotted separately for CS+ and CS- conditions, and separately for no deep brain stimulation (electrode OFF) and PAG stimulation (ON). d) Threat bradycardia, reflected by

Heart rate signal preprocessing

Raw ECG data was preprocessed using in-house software for visual artifact correction and peak detection (https://github.com/can-lab/brainampconverter, https://github.com/can-lab/hera). For 14 trials, missing peaks were interpolated by positioning new peaks exactly between two good-quality neighboring peaks. After data cleaning, six remaining trials with bad data quality (i.e., unintelligible r-peaks; 5.3% of the dataset) were excluded from data analysis. Cardiac signals were filtered (high-pass at 0.01 Hz, low-pass at 2.5 Hz), after which we computed inter-beat intervals (IBIs) between all peaks. Trial-by-trial IBIs were subsequently transformed to heart rate time series in beats per minute (BPM = 60/IBI), which were baseline corrected with respect to the average heart rate during the 1 second pre-trial baseline window. Trial-by-trial heart rate values used for statistical analysis were computed by taking the average (baseline-corrected) heart rate across a 0 – 10 second time window (relative to trial onset). One additional trial was removed due to outlying baseline values (i.e., exceeding 3 standard deviations from the mean baseline across trials). Reinforced CS+ trials (4 per run) were analyzed separately. The final data set (after removing trials with bad data quality and separating shock-reinforced trials) consisted of 91 trials.

Heart rate statistical analysis

To statistically test the effect of deep brain stimulation of the PAG on threat bradycardia, we used linear regression to model trial-by-trial average heart rate change responses across a 0 – 10 second time window (dependent variable) as a function of threat condition (CS+/CS-, sum-to-zero coded), stimulation (sum-to-zero coded), and their interaction (threat-by-stimulation). Note that since all observations are contained within a single individual and are not nested across multiple individuals, trials were treated as independent observations in the regression analysis. Threat bradycardia was operationalized as a relative difference in heart rate between the CS+ and CS- conditions (i.e., a significant threat effect), such that heart rate responses to the CS+ were significantly lower compared to the CS-. To emphasize this operationalization, we sometimes refer to this effect as "relative threat bradycardia." In contrast, we use "general bradycardia" to refer to a general heart rate deceleration across time (relative to the pre-trial baseline). Categories for the stimulation condition were OFF/PAG for experiment 1, and OFF/control-site for experiment 2. Thus, we performed separate linear regressions for experiment 1 and experiment 2. To aid statistical inference, we report standardized effect sizes: partial eta squared (η_p^2) for regressions, and Cohen's d (*d*) for post-hoc one-sample t-tests. Data analysis was performed using R (v4.1.3) and RStudio.

Results

Experimental paradigm

In the present study, the participant performed an instructed fear task (IFT; see Klumpers, Raemaekers, et al., 2010) in which the participant could receive an electrical shock at any time during presentation of one face (CS+) and never during presentation of the other face (CS-; Figure 1(b)). The participant performed two runs of this sequence (±12 minutes each); first with the deep brain stimulation (DBS) electrode turned OFF and subsequently again with DBS ON at the most ventral contact point, located dorsomedial to the aqueduct. This stimulation site was chosen because it was most treatment-efficacious and was therefore deemed most likely to affect the PAG circuit.

PAG stimulation induces threat bradycardia

To test whether PAG DBS influences threat bradycardia responses, we performed linear regression of the average heart rate change (i.e., baseline corrected per trial, see Methods) as a function of threat condition (CS+/CS-), stimulation (OFF/ON), and their interaction. Through the threat-by-stimulation interaction, we test the effect of DBS on threat bradycardia relative to the preceding OFF stimulation condition to account for baseline effects.

First, the task elicited the typical threat bradycardia response, namely a significant main effect of threat condition. This effect indicates a stronger deceleration in

relative reduction in mean heart rate for CS+ vs CS- during the 0–10 second time window, was significantly more pronounced for PAG stimulation compared to no stimulation (OFF). e) Anatomical location of control-site stimulation (in green) on axial T2-weighted (left) and sagittal T1-weighted MR images (right), at 2.8 mm lateral, 5.7 mm posterior, and 4.7 mm inferior relative to the PC. f) Threat bradycardia was not significantly different for control-site stimulation bordering the PAG ('control site') compared to no stimulation ('OFF'). Dots in d and f reflect individual trials. n.s. not significant; η_p^2 effect size.

mean heart rate (HR) during CS+ relative to CS- trials (Figure 1(c, d); B = −0.96, 95% CI [−1.72, −0.15], t(44) = −2.39, $p = .021$, $\eta_p^2 = .12$) (Klumpers et al., 2017; Lang & Davis, 2006). Importantly and in line with our expectations, threat bradycardia was stronger during PAG stimulation (ON) than during no stimulation (OFF). This was indicated by a significant threat-by-stimulation interaction effect (B = −1.16, 95% CI [−1.94, −0.37], t(44) = −2.97, $p = .0047$, $\eta_p^2 = .17$). Post-hoc tests revealed that this interaction was driven by a significant threat effect (CS + versus CS-) in the stimulation ON condition and no significant threat effect in the OFF condition (ON: Figure 1(c, d), right panels; B = −2.09, 95% CI [−3.29, −0.88], t(22) = −3.59, $p = .0016$, $\eta_p^2 = .37$, M_{diff_ON} = −4.17; OFF: Figure 1(c, d), left panels; B = 0.23, 95% CI [−0.85, 1.29], t(22) = 0.44, $p = .668$, $\eta_p^2 < .009$, M_{diff_OFF} = 0.45). In the stimulation ON condition, HR to the CS+ was significantly below zero (t(9) = −3.57, $p = .006$, $d = -1.13$) and HR to the CS- was numerically but not significantly above zero (t(13) = 1.79, $p = .09$, $d = 0.48$). In the stimulation OFF condition, although we observed no relative threat bradycardia (i.e., no HR difference between CS+ and CS- conditions), a significant negative intercept did indicate general bradycardia across time (B = −1.30, 95% CI [−2.37, −0.23], t(22) = −2.52, $p = .0196$; confirmed by a separate one-sample t-test: t(23) = −2.67, $p = .014$, d = −0.55). Follow-up analysis confirmed that PAG stimulation (compared to no stimulation) significantly increased heart rate responses to the CS- (B = 1.49, 95% CI [0.51, 2.49], t(26) = 3.11, $p = .005$, $\eta_p^2 = .27$), but left general bradycardia to the CS+ preserved (B = −0.81, 95% CI [−2.13, 0.50], t(18) = −1.3, $p = .21$, $\eta_p^2 = .09$). Finally, there was no significant main effect of stimulation (OFF vs. PAG) on heart rate responses during the task (B = 0.34, 95% CI [−0.44 1.12], t(44) = 0.87, $p = .387$, $\eta_p^2 = .02$). Together, these findings suggest a causal role for the PAG in threat bradycardia responses.

Active control-site stimulation does not induce significant threat bradycardia

To investigate the extent to which the observed stimulation effects on threat bradycardia are specific to the stimulated region, we repeated the experiment in a second session (approximately 2 months after the first experiment) with an active control stimulation site. This second session consisted of the same experimental procedure and task as the first session, except that the stimulation protocol was this time applied at a different contact point on the DBS electrode positioned 2.4 mm more dorsal, 2.3 mm more left lateral, and 2 mm more anterior relative to PAG stimulation (Figure 1(e)). Since the PAG typically has a radius of about 4 – 5 mm, this contact point is positioned at or just outside of the border of the PAG matter (Linnman et al., 2012). Indeed, this contact point was not used for the participant's neuropathic pain relief treatment. It is thus likely that this contact point does not effectively target the PAG, making it an optimal control stimulation site for our paradigm. Given the location of this contact point relative to the PAG, we anticipated that stimulation at this control site could still lead to threat bradycardia (through spread of the current) but to a lesser extent than stimulation of the contact point located in the PAG (experiment 1).

Similar to the first experiment, the task induced the typical cardiac response pattern with significant lower heart rate for threat compared to safe conditions across stimulation conditions (i.e., threat bradycardia; B = −1.04, 95% CI [−1.84, −0.24], t(39) = −2.63, $p = .0122$, $\eta_p^2 = .15$). However, threat bradycardia was not significantly different during control-site stimulation (ON) relative to no stimulation (OFF), as shown by a non-significant threat-by-stimulation interaction (B = −0.71, 95% CI [−1.51, 0.09], t(39) = −1.79, $p = .0806$, $\eta_p^2 = .08$). While this interaction effect did not reach statistical significance, as anticipated, control-site stimulation did seem to induce threat bradycardia to some extent. That is, the mean heart rate difference between threat conditions (CS+ minus CS-) in the control-site stimulation condition was numerically larger compared to the OFF condition (Figure 1(f); $M_{diff\_control}$ = −4.80; M_{diff_OFF} = −0.66). There was again no significant main effect of stimulation on the mean heart rate change (B = −0.74, 95% CI [−1.54, 0.06], t(39) = −1.86, $p = .0703$, $\eta_p^2 = .08$).

Comparison of PAG-site and control-site stimulation effects on heart rate change

Next, we tested whether the effect on threat bradycardia was statistically different for PAG- vs. control-site stimulation. We first transformed t values of the corresponding threat-by-stimulation interaction effects to partial r values to allow statistical comparison of the two effects. By comparing the interaction effect between experiments rather than the threat main effect, we could test whether the effect of PAG vs. control-site stimulation on threat bradycardia was different while accounting for differences in the preceding OFF condition. A two-sided Fisher's z test on the resulting partial r values was not significant, suggesting that the two interaction effects did not significantly differ from each other (r_{PAG} = −0.41, $r_{control}$ = −0.28, n_{PAG} = 48, $n_{control}$ = 43; z = −0.6807, $p = .496$). Therefore, even though PAG stimulation significantly induced threat bradycardia (relative to no stimulation), this stimulation effect was not

significantly stronger than the effect of PAG-bordering control-site stimulation on threat bradycardia (relative to no stimulation).

The latter finding is not surprising, because low-level stimulation at a control site bordering the PAG is likely to still reach the PAG itself, albeit to a lesser extent. To investigate this possibility, we performed simulations to inspect the volume of activated tissue (VTA) for both applied stimulation conditions (PAG- and control-site stimulation). These simulations revealed that while the VTAs for PAG- vs. control-site stimulation were substantially different, there was still some minimal overlap (Supplemental Figure S1). This minimal amount of overlap in the VTAs may have caused the non-significant difference between PAG- and control-site stimulation.

Finally, we assessed whether PAG- and control-site stimulation affected the mean heart rate response to the shock. Regression analyses on the reinforced trials revealed that the mean heart rate response (time-locked to the shock onset) did not significantly differ between ON and OFF stimulation conditions in either experiment (OFF vs. PAG: B = 0.45, 95% CI [−1.26, 2.17], t(6) = 0.65, p = .541, η_p^2 = .07; OFF vs. control-site: B = −0.54, 95% CI [−1.60, 0.52], t(4) = −1.41, p = .23, η_p^2 = .33; note that these effect sizes may be less reliable due to the small number of reinforced trials per experiment). This result suggests that the processing of nociceptive stimuli was not affected by PAG or control-site stimulation (relative to no stimulation).

Discussion

In this single-case report, we provide causal evidence that the human periaqueductal gray (PAG) regulates defensive cardiac responses. We investigated how deep brain stimulation of the PAG affects threat bradycardia, a common defensive response observed across species characterized by a reduced heart rate for threat relative to safe cues. Deep brain stimulation of the PAG significantly increased threat bradycardia compared to the no stimulation condition. Identical stimulation at a nearby control site bordering the PAG did not significantly increase threat bradycardia, although the absence of the effect of control-site stimulation was statistically indistinguishable from PAG stimulation. Hence, this report provides proof-of-principle causal support for the idea that the human PAG regulates threat bradycardia responses, substantiating the relevance of this region for past and future human neuroimaging studies into defensive responding.

Our main finding is that PAG stimulation significantly increased threat bradycardia relative to the no stimulation condition. This result is in line with studies showing that the PAG regulates bradycardia responses commonly observed during freezing-like threat reactions using animal models (Hermans et al., 2013; Signoret-Genest et al., 2023; Walker & Carrive, 2003). Typically, neural instantiation of threat bradycardia is attributed to the ventral/ventrolateral column of the PAG rather than the dorsal column (Walker & Carrive, 2003) which was primarily targeted in this study. However, there have also been reports where dPAG stimulation in rodents induced freezing-like reactions (Brandão et al., 2008; Vianna & Brandão, 2003; Vianna et al., 2001), although it is unclear if this stimulation also induces bradycardia to threat cues. How might dPAG stimulation induce threat bradycardia? It is possible that threat bradycardia due to dPAG stimulation could occur through stimulation of dmPAG circuits that are innervated by the central nucleus of the amygdala (CeA; Carrive, 1993; Kozlowska et al., 2015; Linnman et al., 2012). The CeA indeed plays an important role in regulating threat bradycardia responses, although its connections to the dPAG have mostly been thought to regulate active fight/flight reactions and concurrent tachycardia rather than bradycardia (Healy & Peck, 1997; Lang & Davis, 2006). It is therefore perhaps more likely that dPAG stimulation induced threat bradycardia indirectly through anatomical and functional connections to other structures in the defense circuit and to the vPAG. Indeed, threat-related cardiac responses likely emerge from coordinated interactions across distributed brain-body systems (Battaglia et al., 2024; DiGregorio et al., 2024; Klein et al., 2021; Roelofs & Dayan, 2022). For example, dPAG stimulation might indirectly activate the hypothalamus, which can in turn generate bradycardia responses via projections to cardiovascular centers of the medulla (Hardy, 2001; Hermans et al., 2013; Kozlowska et al., 2015). Similarly, the applied stimulation might have activated the intrinsic GABAergic network which involves the whole PAG but primarily terminates on glutamatergic neurons in the ventral columns (Reichling, 1991; Silva & McNaughton, 2019). It is possible that stimulation of this circuit at the dorsal PAG caused concurrent disinhibition of glutamatergic vlPAG neurons. In non-human animals, such disinhibition has been shown to evoke conditioned bradycardia- (Signoret-Genest et al., 2023) and immobility responses (Tovote et al., 2016) via projections to the (rostral ventrolateral and magnocellular nucleus of the) medulla and the vagus nerve (Carrive, 1993; Walker & Carrive, 2003; see Roelofs & Dayan, 2022 for a review). The latter interpretation would be in keeping with existing accounts positing that dPAG primarily regulates tachycardia and vPAG regulates bradycardia, and suggests that our findings may be driven by a combination of direct and indirect effects of dPAG stimulation on conditioned

heart rate responses. Indeed, simultaneous (direct) dPAG activation and (indirect) vPAG disinhibition could explain why (d)PAG stimulation in experiment 1 seemed to increase heart rate responses to the CS- while general bradycardia to the CS+ appeared to be preserved. Together, while the exact contribution of the respective PAG columns to defensive reactions and associated physiological responses remains to be fully elucidated, our findings support that the human PAG, like rodent PAG, mediates threat bradycardia responses.

Notably, while threat bradycardia (i.e., the CS+ vs. CS- heart rate difference) numerically increased during DBS at a more dorsal and lateral positioned control site bordering the PAG (relative to no stimulation), this effect was not significant. However, this effect of control-site stimulation on threat bradycardia was in turn not significantly different from PAG-site stimulation. We have identified two potential explanations for this finding. First, it might be that larger threat bradycardia during the stimulation conditions was due to order effects, since the no-stimulation condition always came first and the (PAG and control-site) stimulation conditions came after. This condition order was chosen to prevent potential wash-out effects of the stimulation to subsequent no-stimulation conditions. As such, it is an unavoidable limitation of our design that learning, habituation, or expectancy effects may have contributed to the observed effects of PAG-DBS on CS differentiation. However, previous work has shown highly stable CS differentiation for instructed fear experiments across sessions (Klumpers, Van Gerven, et al., 2010), suggesting that order effects may not completely explain our findings. A second, more likely, explanation is that control-site stimulation still activated nearby PAG tissue or downstream areas involved in threat bradycardia. Our simulations of the volume of activated tissue indeed suggest that low-level stimulation from the control-site might have reached the PAG. To some extent this was to be expected, as anatomical images already suggested that the control stimulation site was located at or just outside the PAG border (see Figure 1(e)). Nonetheless, PAG stimulation affected threat bradycardia significantly, whereas control-site stimulation increased threat bradycardia only marginally. Therefore, these results do suggest that modulation of threat bradycardia in this study was specific to PAG stimulation and cannot be fully attributed to general stimulation effects independent of the PAG circuit. Still, the lack of a clear distinction between PAG- and control-site stimulation may weaken the causal claim that specifically the dorsal PAG regulates threat bradycardia. However, to offer even more precise and stronger causal evidence, future studies would need to include patients with DBS electrodes in other additional brain areas, such as the vlPAG, anterior cingulate cortex, or thalamus (Fontaine et al., 2025), and such cases may be rare.

The finding that PAG and control-site stimulation did not result in significantly different effects on threat bradycardia (compared to no stimulation) raises the question how the applied DBS affects the underlying neural circuitry. First, while our simulations suggested that there might have been some current spread from the control site to the PAG, the applied stimulation may still have mostly targeted the dorsal portion of the PAG. This is supported by the fact that PAG and control-site stimulation had no effect on the heart rate response to administration of electrical shocks, suggesting that nociception – which is mediated by opioidergic neurons in the ventral PAG (Fields, 2004) – remained unaffected. It might however be possible that control-site stimulation bordering the PAG still activated the GABAergic circuit described above, thereby inducing the marginal threat bradycardia effect in experiment 2. Secondly, and relatedly, one open question is whether the type of stimulation that we applied inhibits or excites the underlying neural circuitry. While the effect of DBS on the underlying neural circuitry is still under debate, it has been suggested that low-frequency PAG DBS (as was applied here) stimulates rather than inhibits the targeted area (Chiken & Nambu, 2016). Together, we conclude that while our applied DBS may have affected more than just the targeted region, our results suggest that stimulation – rather than inhibition – of the PAG induces threat bradycardia. Moreover, these effects appear to be independent of nociception.

Although we found a significant main effect of threat on bradycardia in both experiments (across stimulation conditions), there was surprisingly no significant threat bradycardia when the DBS electrode was turned off in experiment 1 (pre PAG stimulation). The absence of a threat bradycardia effect cannot be attributed to low levels of subjective fear and anxiety, since fear- and anxiety ratings were consistent across conditions whereas threat bradycardia responses were not. Rather, this finding might be due to individual differences in the magnitude and occurrence of threat bradycardia responses. For example, while some individuals show clear bradycardia to threat stimuli, other individuals may show no differentiation or even tachycardia (Battaglia et al., 2024; Jaswetz et al., 2025). These distinct responses are tied to differences in sympathetic vs. parasympathetic autonomic arousal, and might reflect different tendencies of individuals to engage in active (fight/flight) or passive (freezing) defensive reactions (de Echegaray & Moratti, 2021; DiGregorio & Battaglia, 2024; Jaswetz et al., 2025; Livermore et al., 2021).

Finally, it is worthy to note that, since this is a single-case study, our findings need to be interpreted with some caution. This report includes data from a single individual, and replication, ideally in a larger sample, is needed to generalize to the population. Indeed, we cannot be certain that other individuals would show similar effects. In addition, a larger sample would allow better control of potential order effects, since one could then counterbalance the stimulation condition order across participants (which was not possible in this single-case report). However, DBS-PAG cases are rare (Shaheen et al., 2023) and not all individuals will be able to participate in experiments, which limits the feasibility of such replication studies. Therefore, in this study, we have attempted to maximize the reliability of our findings by including sufficient observations per condition and by employing trial-by-trial analyses. Overall, our general heart rate and threat bradycardia effects are similar to what was observed in previous studies (Jaswetz et al., 2025; Klumpers et al., 2017), supporting the reliability of our findings. Thus, until replication studies are possible, the current study serves as a valuable proof-of-principle (Crowe et al., 2011).

To conclude, in this single-case report we find that deep brain stimulation of the periaqueductal gray induces threat bradycardia responses during an instructed fear task. We provide proof-of-principle causal evidence that the human PAG regulates threat bradycardia responses, substantiating the relevance of this region for human neuroimaging studies and existing animal models. Additionally, this finding furthers our knowledge of the neural circuit mediating threat-anticipatory cardiac responses, which may inform our understanding of defensive reactions such as freezing and fight/flight reactions.

Author contributions

CRediT: **Felix H. Klaassen:** Conceptualization, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing; **Lycia D. de Voogd:** Conceptualization, Methodology, Software, Supervision, Writing – original draft, Writing – review & editing; **Anneloes M. Hulsman:** Methodology, Software, Writing – review & editing; **Floris Klumpers:** Conceptualization, Methodology, Software, Writing – review & editing; **Sarah M. Farrell:** Conceptualization, Writing – review & editing; **Alexander L. Green:** Conceptualization, Writing – review & editing; **Pepijn van den Munckhof:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing; **Karin Roelofs:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing.

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Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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