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Ventral periaqueductal grey stimulation alters heart rate variability in humans with chronic pain

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ABSTRACT

Background: The midbrain periaqueductal grey (PAG) area is important for both pain modulation and cardiovascular control via the autonomic nervous system (ANS). While changes in blood pressure dependent upon dorsal or ventral electrode positioning have been described with PAG deep brain stimulation (DBS), little is known mechanistically about the relationships between pain and cardiovascular regulation in humans. Heart rate variability (HRV) is an established measure of cardiovascular regulation, and an index of autonomic function.

Methods and results: 16 patients undergoing DBS of the rostral PAG for chronic neuropathic pain were investigated post-operatively to determine whether PAG stimulation would alter HRV, and the subjects' perception of pain. Mean heart rate together with HRV, time and frequency domain measures, low frequency (LF) and high frequency (HF) power components of heart rate and the ratio of LF to HF were calculated before and during DBS. Ventral but not dorsal PAG DBS significantly decreased the ratio of LF to HF power ($p<0.05$, $n=8$) with HF power significantly increased. Changes in LF/HF ratio correlated significantly with subjective reporting of analgesic efficacy using a visual analogue score (VAS; $\gamma^2=0.36$, $p=0.01$, $n=16$). Diffusion tensor imaging and probabilistic tractography of 17 normal controls' seeding voxels from the mean ventral and dorsal PAG stimulation sites of the 16 patient cohort revealed significant differences between rostral tract projections and separate, adjacent projections to ipsilateral dorsolateral medulla.

Conclusions: Ventral PAG DBS may increase parasympathetic activity to reduce pain via anatomical connections distinct from dorsal PAG DBS, which may act by sympathetic mechanisms.

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Introduction

The periventricular and periaqueductal grey region (PAG) is a structure optimally sited anatomically to integrate interoceptive function, both from adjacent mesencephalic cardiovascular centres and more distal pain processing areas. Its autonomic effects have been well studied in animals (Bandler et al., 1991; Behbehani, 1995; Carrive, 1993; Rossi et al., 1994) and changes noted with deep brain stimulation (DBS) (Young and Rinaldi, 1997). Animal experiments have suggested that ventral PAG stimulation augments parasympathetic effects, concomitant with opioid mediated analgesia, whereas dorsal PAG stimulation is sympathomimetic and non-opioid mediated (Bandler and Carrive, 1988; Depaulis et al., 1994), responses intimately related to affective changes such as fear are also described in humans (Fanselow, 1991; Nashold et al., 1969). The 'freezing' and 'fight or flight' responses suggested to be mediated by ventral and dorsal PAG comprising the 'passive' and 'active' coping responses.

DBS of the PAG area has been used to ameliorate chronic pain refractory to pharmacotherapy for half a century (Hosobuchi et al., 1977; Richardson and Akil, 1977). It remains effective in the long term in appropriately selected patients, in particular following amputation, plexopathies, anaesthesia dolorosa and after stroke (Pereira et al., 2009). We have demonstrated a positive correlation between the degree of analgesia in patients receiving PAG DBS and the magnitude of blood pressure reduction and shown that whereas dorsal PAG stimulation can acutely elevate blood pressure, ventral stimulation

Abbreviations: DBS, deep brain stimulation; DTI, diffusion tensor imaging; HRV, heart rate variability; PAG, periaqueductal/periventricular grey; RRI, R–R interval; RVLM, rostral ventrolateral medulla; VAS, visual analogue score.

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reduces it (Green et al., 2006a,b, 2005; Pereira et al., 2010). Autonomic changes, such as tachycardia, hypertension, diaphoresis and lacrimation, although non-specific, are also established signs of pain or inadequate analgesia. Such findings advance investigations for objective markers of chronic pain and also potential selection of patients who may respond best to PAG DBS.

The rostral ventrolateral medulla (RVLM) contains nuclei that control the sympathetic system (Aicher et al., 1996; Hancock, 1988), whereas the nucleus ambiguus and dorsal motor nucleus of the vagus drive the parasympathetic system (McAllen and Spyer, 1976). Cardiovascular regulation depends on the balance of activity between these brain regions. Heart rate variability (HRV) is thought to provide a quantitative index of this balance and is easily measured.

In this study, therefore, we investigated adaptive effects on the autonomic regulation of the cardiovascular system during DBS for chronic pain to assess firstly whether objective changes in HRV correlate with the degree of subjective analgesia and secondly whether such changes differ between dorsal and ventral PAG stimulation. Diffusion tensor imaging (DTI) was then used to assess whether dorsal and ventral PAG have different anatomical connections. DTI enables study of macroscopic axonal organisation in the brain through fitting of a diffusion tensor to obtain the fractional anisotropy (FA) of neuronal tissue (Mori and Zhang, 2006).

Methods

Patients and surgery

Sixteen patients with chronic neuropathic pain undergoing DBS of the PAG areas were recruited to the study. Patients were excluded if they 1) had an irregular heart rhythm; 2) had heart disease, including previous myocardial infarction or valvular disease; 3) were taking medication that might affect autonomic response, e.g., β-blockers or antidepressants; or 4) had any other disease, such as Parkinson's disease or alcoholism, that might affect the autonomic response. The study was performed according to the Declaration of Helsinki and approved by the Oxford local ethics committee (OxREC number 05/Q1605/47), and informed written consent was obtained from all patients. Subject demographics including age, sex and aetiology are summarized in Table 1. 13 of the 16 subjects were male and three were female. Age ranged from 37 to 74 years old. Eight patients had post-stroke pain, three pain after amputation (including stump and phantom pain), one pain after brachial plexus injury and the others pain after trauma or malignancy. All patients had contralateral PAG DBS, one had bilateral PAG DBS for bilateral amputation pain and four had additional thalamic DBS which was switched off during the study. The surgical technique and neuroprostheses used are detailed elsewhere (Bittar et al., 2005; Pereira et al., 2007a,b).

Protocol

ECG recordings were obtained during the first week after the initial implantation of the DBS electrode. All sessions were carried out on a single day at least 3 h after any meal at constant room temperature of 22 °C. DBS was initially turned off for 30 min.

First, the awake subject sat for 10 min with the stimulator turned off, while recording ECG signals. Then, randomly selected, the stimulator was turned on or off for six 8-min periods. Between each of these 'on' or 'off' sessions, there was a 30-min rest period with stimulation off to allow the ECG to return to baseline. During the recordings, both the experimenter and the subject were blind as to whether the stimulator was on or off. The subject was asked to record his pain level on a visual analogue score (VAS; 0–10, 0 = no pain, 10 = worst pain ever experienced) to estimate pain severity during each session. DBS parameters were set so as to provide optimal analgesia for each individual and are detailed in Table 1. Efficacious stimulation frequencies ranged from 5 to 50 Hz, amplitudes from 1.0 to 5.0 V, and pulse widths from 60 to 450 μs.

HRV measurements

Lead II ECGs were recorded from left and right forearms using adhesive disposable Ag/AgCl surface electrodes (H27P; Kendall-LTP, Mansfield, MA), amplified 1000 times (CED 1902; Cambridge Electronic Design, Cambridge, UK). The recordings were digitized at 4000 Hz with 16-bit resolution (CED 1401 Mark II, Cambridge Electronic Design, Cambridge, UK) using Spike II software (Version 5.0, Cambridge Electronic Design, Cambridge, UK). Each ECG data set was linearly down-sampled at 1000 Hz using Spike II software and fed into a custom-designed program to extract the HRV signal. The extraction method incorporated a peak detection algorithm that found the time of occurrence for every QRS complex in the ECG signal. The durations

Table 1
Summary of patient demographics, pain duration and aetiology, deep brain stimulation parameters, distances of the stimulated periventricular grey electrode contact from deep brain anatomical landmarks (MCP, mid-commissural point) and whether the contact studied is dorsal (D) or ventral (V).

Patient	Sex	Age at surgery (years)	Pain duration before surgery (years)	Pain aetiology	Amplitude (v)	Frequency (Hz)	Pulse width (μs)	Bipolar (B) or monopolar (M)	Distance posterior to MC point (mm)	Distance from red nucleus (mm)	Dorsal (D) or ventral (V)
1	M	38	13	Amputation (trauma)	1.0	5	210	B	13.5	7.3	V
2	M	37	4	Stroke (cortical)	3.5	5	390	B	12.0	7.1	V
3	M	74	20	Stroke (subcortical)	2.0	30	330	B	15.0	8.3	V
4	M	50	24	Stroke (subcortical)	1.6	40	450	B	13.0	8.5	V
5	M	61	5	Stroke (brainstem)	2.0	20	450	B	12.0	7.6	V
6	M	61	5	Stroke (brainstem)	4.9	30	450	B	12.0	7.6	V
7	M	60	19	Stroke (brainstem)	2.0	15	210	M	13.0	8.5	V
8	F	34	3	Scalp (schwannoma resection)	5.0	20	160	B	11.5	4.9	V
9	M	53	8	Amputation (trauma)	5.8	35	450	B	17.0	10.2	D
10	F	56	6	Amputation (ischaemia)	4.9	40	450	B	14.5 (left) 17.5 (right)	8.5 (left) 10.9 (right)	D
11	M	67	4	Stroke (cortical)	1.6	50	450	M	30.5	23.7	D
12	M	69	9	Stroke (thalamic)	3.0	20	150	B	17.5	24.6	D
13	M	29	6	Brachial plexus avulsion	5.0	30	450	B	16.0	9.8	D
14	M	34	14	Scalp (trauma)	2.8	20	450	B	19.0	12.0	D
15	M	56	2	Malignancy	1.1	20	60	M	17.0	8.9	D
16	F	37	9	Spinal cord injury	2.8	30	210	B	21.0 (left) 18.0 (right)	14.1 (left) 12.5 (right)	D

between successive peak locations were calculated to produce a time series of R–R intervals (RRI). All of the RRI time series were firstly edited automatically, after which careful manual editing was performed by visual inspection. The RRI time series were then cubically interpolated with a sampling interval of 0.5 s, and saved for further analysis.

Electrode location assessment

Post-operatively, each patient had either a computerized tomography (CT) or a T1 weighted magnetic resonance (MR) brain scan to determine the location of the stimulating electrode (Fig. 1). An imaging software program (MRIcro version 1.38, Chris Rorden) was used to determine the relative positions of the electrodes to each other and in relation to the mid-commissural point and midbrain red nucleus (Table 1) using additional information from the pre-operative MRI if required. This technique is detailed elsewhere (Green et al., 2006a,b). Patients were grouped into those with stimulating electrode contacts in eight more ventral locations in the PAG and eight more dorsal locations, shown in Fig. 2, and comparison made between ventral and dorsal PAG DBS.

Signal processing

HRV was detrended using a method based on the 'Smoothness Priors' approach which operates like a time-varying finite-impulse response high-pass filter. This was performed to detrend the HRV before analysing the power spectrum. Details of the method are described elsewhere (Tarvainen et al., 2002). In this study, the cutoff frequency was 0.02 Hz.

Spectral HRV power was calculated in accordance with previously published standards (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), yielding the two frequency domain measures: LF power at 0.04–0.15 Hz and HF power at 0.15–0.4 Hz. Power frequency (Hz) was converted to ms^2 by auto-regressive power spectral analysis employing 1024 points and a model order of 16, using custom software. Normalized LF and HF powers were computed as the integral of the power bands. The ratio of the logarithm of the LF to HF power was also calculated. Signal processing was performed in MATLAB® (v6.5; Math Works Inc., Natick, MA, USA).

Statistical analysis

To assess whether the HRV changed during the three 'stimulation off' compared with the three 'stimulation on' sessions, a one sample paired *t*-test was used. To assess whether the HRV was related to changes in VAS pain scores, linear regression analysis and Pearson correlation were performed. Before analyses, raw values of all variables were examined for deviations from normality by the Kolmogorov–Smirnov test. The level for significance was set at $p<0.05$. Statistical analyses were performed in SPSS (v13, SPSS Inc., Chicago, IL, USA). Graphs were plotted using Origin (v7.5776, Northampton, MA, USA). All *p* values are two-tailed.

Diffusion tensor imaging

Diffusion weighted data was acquired for 17 healthy subjects on a Philips Achieva 1.5 Tesla Magnet using echo planar imaging, (70×2 mm thick slices, f.o.v. $=320\times264\times128$; images reconstructed on a 176 matrix; TE 70 ms, TR 17497 ms; voxel size isotropic at $2\times2\times2$ mm; 8 channel Head RF coil; 32 directions of diffusion weightings). Images were analysed using tools from The Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) software library (www.fmrib.ox.ac.uk/fsl/). Diffusion data was analysed using the FMRIB Diffusion Toolbox (FDT) detailed elsewhere

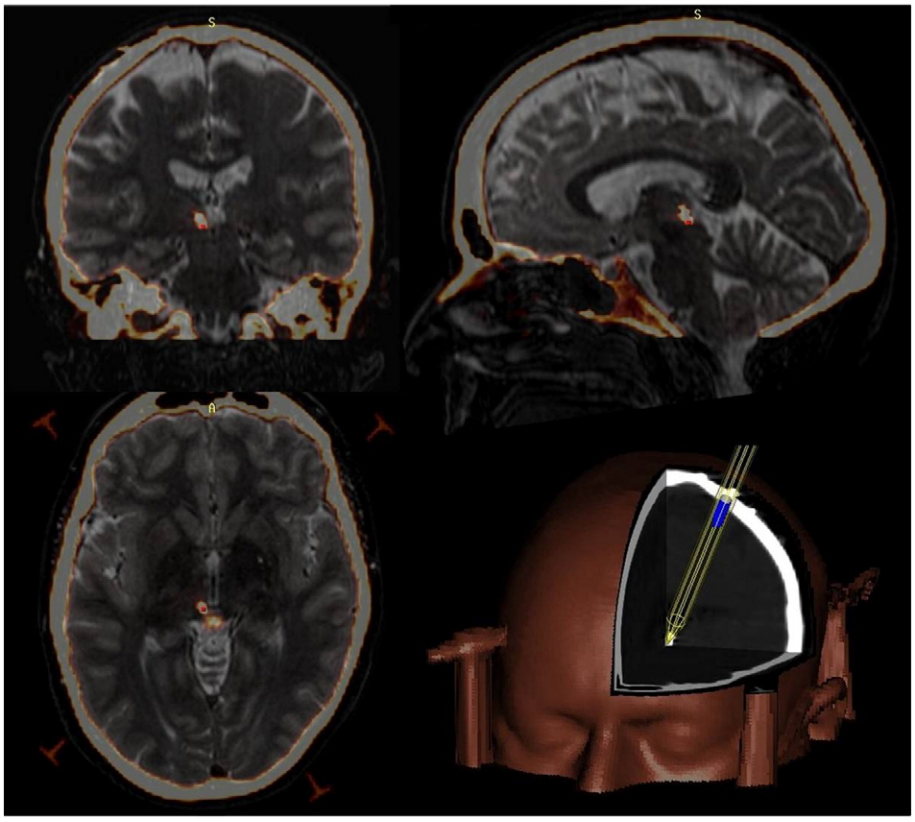

Fig. 1. Fused pre-operative MRI and post-operative CT images highlighted using heat mapping (axial, coronal and sagittal clockwise from bottom left) showing left PAG electrode placement. Three-dimensional reconstruction of electrode trajectory is shown bottom right.

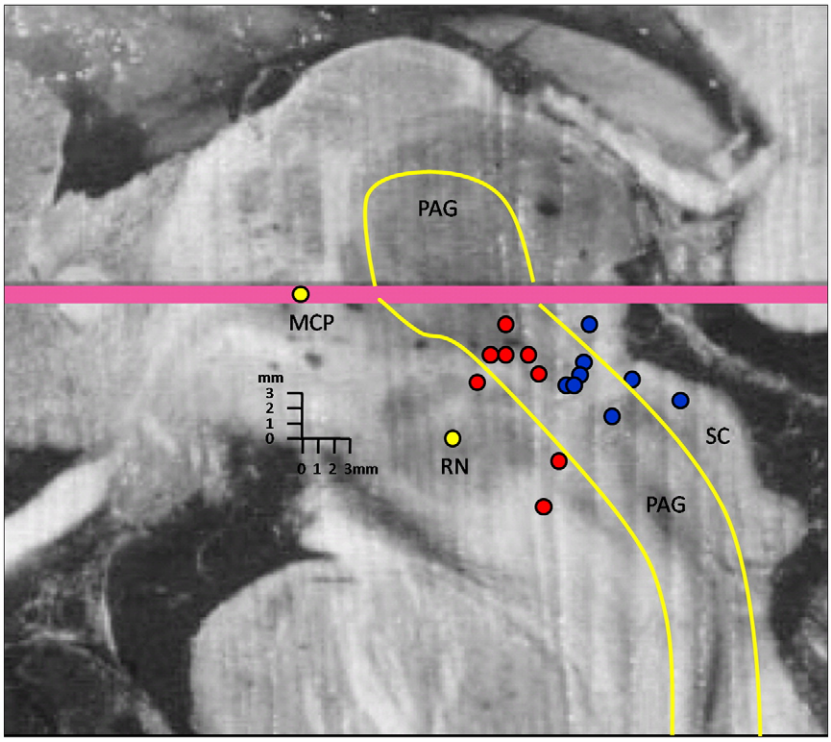

Fig. 2. Sagittal positions of all dorsal (blue) and ventral (red) PAG electrode active contacts relative to the mid-commissural point (MCP) and red nucleus (RN). Periaqueductal grey (PAG) is outlined in yellow. SC, superior colliculi. (background reprinted from Mai et al. Atlas of the human brain. San Diego: Academic Press, 1998, with permission from Elsevier).

(Behrens et al., 2003). Probabilistic tractography was then implemented by FDT. The structural MRI was registered to the DTI using the FMRIB Linear Registration Tool to produce a co-registered image where seed voxels could be placed accurately.

Active DBS electrode contact positions were identified for each patient. For each DTI subject, two seed voxels were chosen, each representing the mean electrode positions of the ventral (13.1 mm posterior, 5.9 mm lateral and 7.1 mm inferior to the mid-commissural point; 7.6 mm distance from the centre of the red nucleus) and dorsal (19.3 mm posterior, 3.2 mm lateral and 5.6 mm inferior to the mid-commissural point; 12.5 mm from the centre of the red nucleus) PAG electrodes of the patients from data shown in Table 1 and depicted in Fig. 2. Probabilistic tractography was performed in each normal subject for both seed voxels and results averaged and placed onto a Montreal Neurological Institute standard brain template, where tracts were thresholded at a minimum of 10 streamlines and a maximum of 1000 streamlines through each voxel for analysis. The mean ventral and dorsal positions were loaded into a stereotactic planning computer (Radionics, MS, USA).

Results

Whole group HRV changes with PAG stimulation

Mean heart rate without stimulation of the PAG was 76.82 (SD 13.34) beats per minute (bpm) compared to 76.75 (SD 14.08) bpm with stimulation. This slight reduction was not statistically significant ($p=0.485$, $t=0.19$, $n=16$). Spectral analysis revealed the dominant frequency components of the heart rate between the on and off conditions. Fig. 3 provides an example of frequency changes in two patients — one with a more ventral and the other with a more dorsal electrode location in the PAG. In the ventral example, stimulation reduced the LF component but increased the HF component. We compared the normalized power of both the LF and the HF components between stimulation on and off conditions. Taking all patients, the mean LF component 'off' stimulation was 1.72 ms^2 (SD 0.18) versus 1.75 ms^2 (SD 0.15) 'on' stimulation. This change was not significant ($p=0.19$, $t=1.37$, $n=16$). Similarly, HF showed no significant difference between the two conditions; 'off' = 1.52 ms^2 (SD 0.29) versus 'on' = 1.54 ms^2 (SD 0.28; $p=0.54$, $t=0.65$, $n=164$). However, LF/HF significantly reduced overall with stimulation, from 1.23 to 1.16 ($p<0.05$, $t=2.23$, $n=16$).

Ventral versus dorsal PAG stimulation

Differences were seen between the eight subjects with the most ventral and the eight with the most dorsal PAG electrodes (Fig. 4). Although their initial heart rates were similar ($p=0.21$, $n=8$) and did not change significantly on stimulation ($p=0.83$ in dorsal and $p=0.70$ in ventral groups, Fig. 4A), LF/HF reduced significantly in the ventral PAG group during DBS ($p=0.02$, $n=8$; Fig. 4D) but not in the dorsal PAG group ($p=0.93$, $n=8$). This HRV ratio change associated with ventral PAG stimulation was caused mainly by increased HF power ($p=0.01$, $n=8$) which was not seen in the dorsal PAG group ($p=0.67$, $n=8$, Fig. 4C). In contrast, LF power showed no significant differences between dorsal and ventral PAG groups ($p=0.31$, $n=8$ for DBS 'off', $p=0.97$, $n=8$ for DBS 'on'; Fig. 4B).

HRV and analgesic efficacy

Visual analogue scores (VAS) of pain were recorded before and during stimulation. Stimulation significantly reduced VAS overall with a mean 'off stimulation VAS' of 7.52/10 compared to a mean 'on stimulation VAS' of 4.34/10, an 84% reduction ($p<0.01$, $n=16$). These changes with stimulation were compared to the changes in LF and HF HRV within the same patients. Both HF power changes ($\gamma^2=0.27$, $p=0.04$, $n=16$, Fig. 5B) and the LF/HF power ratio ($\gamma^2=0.36$, $p=0.01$, $n=16$; Fig. 5C) correlated well with pain reduction and hence analgesic efficacy, whereas LF power changes did not (Fig. 5A).

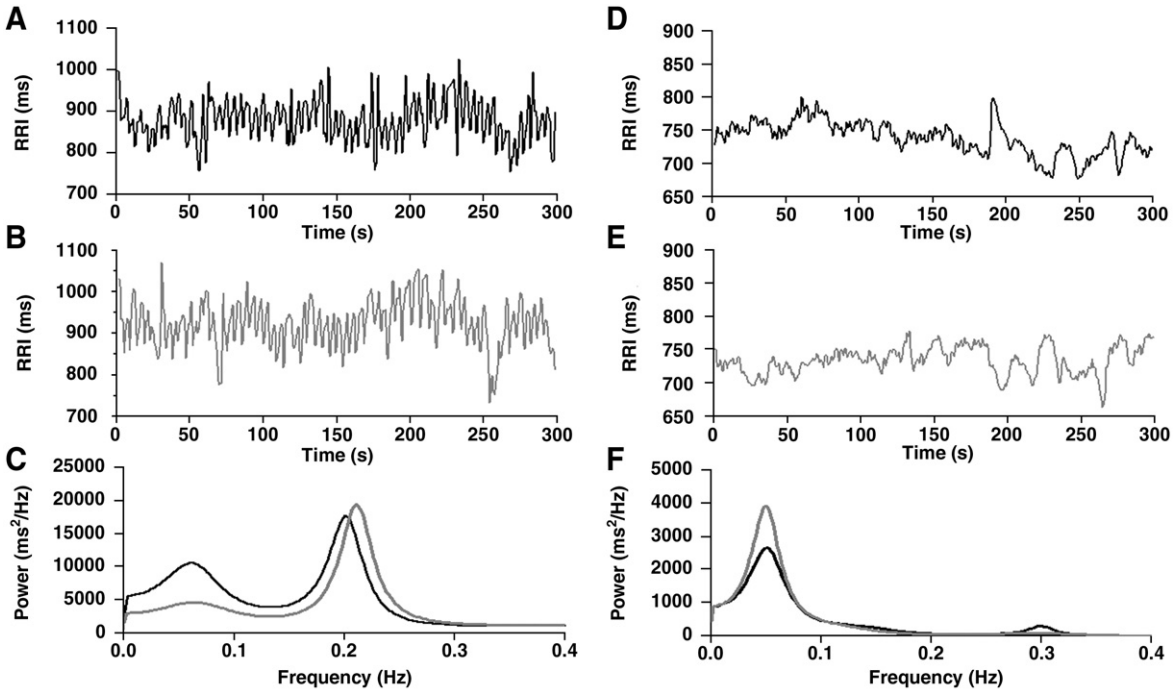

Fig. 3. RR interval time series (A, B, D, E) and their power spectra (C, F) in two patients. Left: ventral PAG DBS; right: dorsal PAG DBS. Black line: off DBS; grey line: during DBS. These example frequency changes show increased high frequency (HF) and decreased low frequency (LF) components with ventral stimulation.

Diffusion tensor imaging

Probabilistic tractography of 17 normal subjects with tracts thresholded from 10 to 1000 streamlines through each voxel demonstrated distinct cortical connections from ventral and dorsal PAG seed points and separate, adjacent connections to dorsolateral medulla (Fig. 6). Dominant ventral PAG connections included amygdala, nucleus accumbens, anterior cingulate cortex and ventromedial prefrontal cortex whereas prominent dorsal PAG connections included ventral posterior thalamus and primary somatosensory cortex.

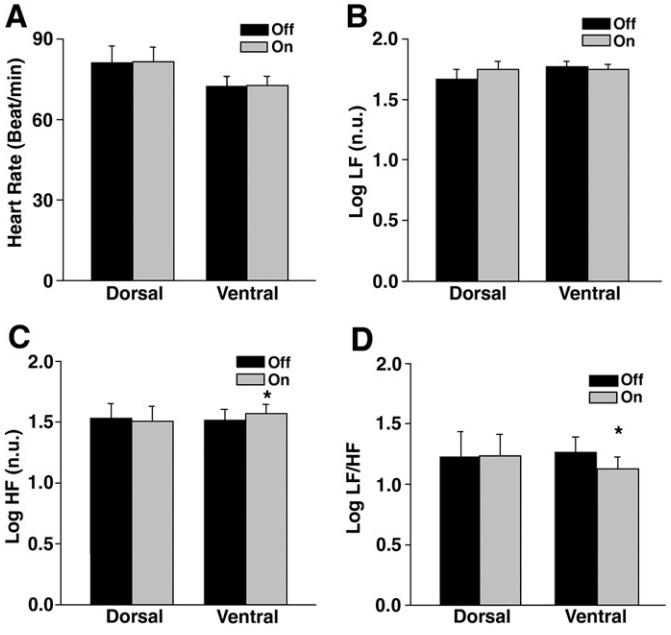

Fig. 4. Dorsal and ventral group heart rate and heart rate variability (HRV) changes before and during stimulation. (A) Mean heart rate (+/−1 s.e.), (B) HRV low frequency component (0.04–0.15 Hz); (C) HRV high frequency component (0.15–0.4 Hz); (D) LF/HF ratio. Heart rates were similar ($p = 0.21$) and changed little on stimulation (dorsal $p = 0.83$; ventral $p = 0.70$). LF/HF ratio reduced significantly with ventral ($p = 0.02$) but not dorsal stimulation ($p = 0.93$), with significantly increased HF power in the ventral group ($p = 0.01$).

Discussion

The new findings discussed below in this study are firstly that ventral but not dorsal PAG stimulation significantly increased the HF power and decreased the LF/HF ratio of HRV, suggesting that it may increase parasympathetic activity. Secondly, such changes in LF/HF ratio correlated significantly with reported analgesia. Thirdly, diffusion tensor imaging and probabilistic tractography revealed significant differences between cortical tract projections from the different PAG regions and separate, adjacent projections to ipsilateral dorsolateral medulla. The observations together suggest that ventral PAG DBS may increase parasympathetic activity to reduce pain via anatomical connections distinct from dorsal PAG DBS.

High frequency (0.15–0.4 Hz) HRV power has been shown experimentally to be a marker of vagal parasympathetic control (Kamath and Fallen, 1993; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Low frequency (0.04–0.15 Hz) HRV power was initially thought to be related only to sympathetic cardiovascular activity, but recent evidence suggests that it is affected by both vagal and sympathetic tones (Pagani et al., 1997; Pumprla et al., 2002; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Under conditions of minimum postural or physiological stress, the parasympathetic influence on LF is thought to be greater than that of the sympathetic nervous system. Thus the LF component is abolished during pharmacological vagal blockade (Martinmaki et al., 2006). However, despite the dominance of parasympathetic activity on HRV, changes in the LF/HF ratio may still provide some indication of the balance between the sympathetic and parasympathetic effects upon the heart. Changes in LF/HF have been found in various disease states including

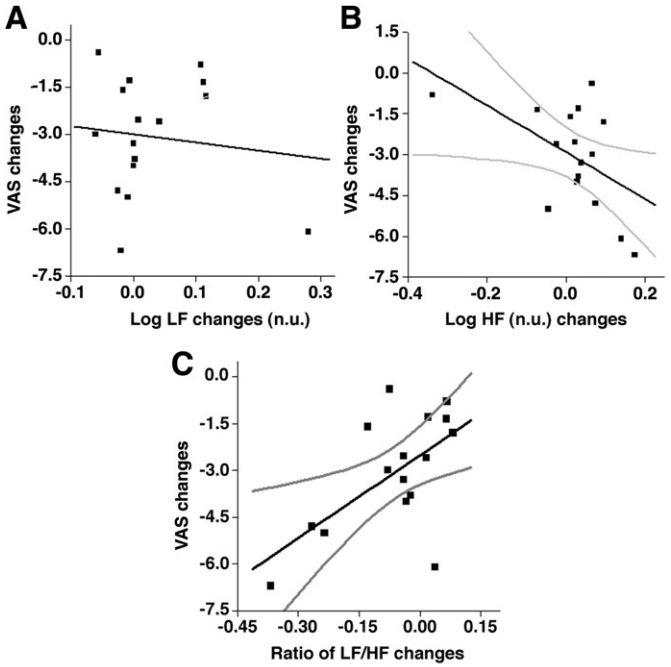

Fig. 5. Pearson correlation between changes in heart rate variability (HRV) and visual analogue scores of pain (VAS). (A) Low frequency power ($\gamma^2 = 0.02$); (B) high frequency power ($\gamma^2 = 0.27$); (C) LF/HF ratio ($\gamma^2 = 0.36$). Solid line: linear regression; outer dashed line: 95% confidence intervals.

cardiac failure, diabetes mellitus and myocardial infarction (Kuch et al., 2004; Mestivier et al., 1997; Saul et al., 1988).

The relationship between the autonomic nervous system and pain is a complex one that has not been fully elucidated. It is known that sympathetic activity is increased in complex regional pain syndromes with alteration of the normal sympathetic vasoconstrictor reflex (Birklein et al., 1998). Similarly pain after amputation is frequently associated with complex regional pain syndromes and referred to as reflex sympathetic dystrophy. Furthermore the roles of higher brain centres in controlling HRV are poorly understood. There is evidence that HRV is linked to emotion (Appelhans and Luecken, 2006). This is consistent with the notion that affective states are related to physiological arousal (Hagemann et al., 2003). Thus an affective state, such as pain, will promote arousal and this will be mediated by autonomic activation, which may manifest with changes in HRV. The polyvagal theory (Porges, 1997) suggests that the rapid, myelinated, vagal system allows mammals to inhibit autonomic arousal, for example from pain, to produce a state of calmness. Theories of neurovisceral integration (Thayer and Brosschot, 2005) characterise this vagal influence in a wider neural network subserving homeostatic responses. These theories predict, and it has been demonstrated (Appelhans and Luecken, 2008), that a reduction in low frequency HRV is seen in subjects with less emotional pain valence and higher pain thresholds given similar nociceptive stimuli. Thus, descending vagal control may modulate analgesic efficacy.

Investigations into HRV changes and preliminary findings from ambulatory blood pressure monitoring that such blood pressure changes are sustained (Pereira et al., 2008, 2010) may provide objective somatic measures that correlate to subjective rating scales of efficacy as found in this study. Detailed autonomic testing utilising such equipment as tilt-tables, Portapres (TNO, Amsterdam, The Netherlands) combined with real-time VAS recording would further elucidate both mechanisms and the validity and limitations of such correlative measures.

It seems that ventral PAG DBS may engage analgesia commensurate with increased vagal output and passive coping behaviour whereas dorsal PAG DBS may involve 'fight or flight' analgesia, perhaps with associated sympathomimetic effects (Green et al., 2006a,b). Either may act via an opioid mediated mechanism, suggested by animal experiments revealing stimulation produced analgesia reversed by naloxone (Akil et al., 1976) and human studies that also showed elevated levels of cerebrospinal fluid enkephalins and endorphins with DBS (Akil et al., 1978; Hosobuchi et al., 1977, 1979). However, the cerebrospinal fluid measures were artefactual (Dionne et al., 1984; Fessler et al., 1984) and double blinded investigation in humans has revealed no cross-tolerance between DBS and morphine and similar reversibility between naloxone and saline placebo (Young and Chambi, 1987), confirming others' findings. Whether PAG DBS acts via endogenous opioid release therefore remains unclear and worthy of further investigation.

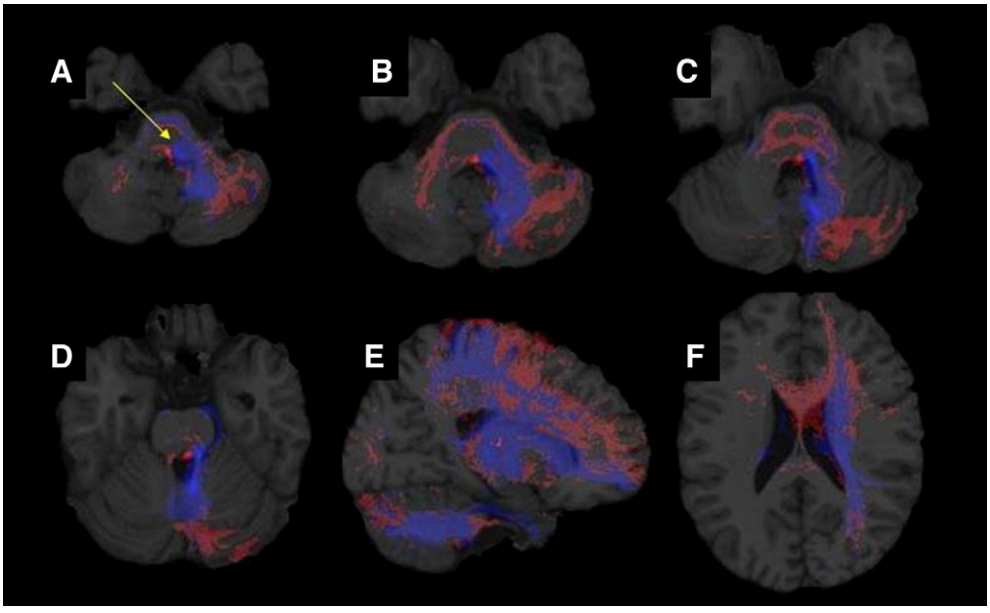

Fig. 6. Probabilistic tractography displayed in standard space showing tracts from the dorsal PAG (blue) and ventral PAG (red). (A–D) Axial sections through the medulla, lower pons, upper pons, and midbrain showing separate projections through the brainstem and cerebellum. Notable are separate but adjacent projections of both seeds along the ipsilateral dorsolateral medulla (arrow). (E and F) Sagittal and axial sections showing separate tracts, whole brain and cortical connectivity.

Our results show that stimulation of ventral PAG caused a significant increase in the HF component of HRV and a significant reduction in the LF/HF ratio. The magnitude of these HRV changes correlated positively with the amount of analgesia reported. These results fit with both polyvagal and neurovisceral integration theories of pain, suggesting that analgesia with DBS in chronic pain is associated with increased vagal parasympathetic activity. How much of this vagal activity is a direct result of PAG DBS, and whether this leads to or is a result of the analgesia, requires further study. It is also worth noting that our ventral PAG group electrodes were on average 2.7 mm more lateral than our dorsal group electrodes, and therefore perhaps contributed more to 'autonomic inhibition', showing homology with ventrolateral versus dorsomedial columnar organisation well characterized in rodent PAG (Carrive, 1993).

Also unknown, is whether higher brain regions are involved in the changes in HRV. We have used single photon emission computed tomography (SPET) and magnetoencephalography (MEG) to image changes in brain activity during PAG DBS. These showed that anterior cingulate cortex and insula are activated, which are medial components of the pain neuromatrix and are proposed to attribute emotional valence to pain (Kringelbach et al., 2007; Pereira et al., 2007a,b; Ray et al., 2009). Conducting such investigations alongside autonomic measures seem appropriate further studies. DTI and probabilistic tractography of normal control subjects have demonstrated strong connections to different cortical brain regions from ventral and dorsal PAG and separate, adjacent dorsolateral medullary connections. This suggests distinct ascending and descending functional neuroanatomic pathways from ventral and dorsal PAG likely to be involved in HRV and pain modulation.

PAG DBS for chronic pain is undertaken in specialist centres experienced in the technique in appropriately selected patient refractory to pharmacotherapy. Challenges to long-term efficacy and tolerance phenomena pervade DBS, spinal cord stimulation, motor cortex stimulation and other neurostimulatory treatments for this challenging patient group. At present, patient selection is guided by aetiology, clinical expertise and qualitative aspects of the chronic pain as determined by the McGill pain questionnaire (Pereira et al., 2009). HRV presents a promising, simply undertaken and swiftly analysed non-invasive tool that may help to identify patients most responsive to ventral PAG DBS, augmenting current patient selection methods and assisting post-operative DBS parameter titration with an objective somatic marker of analgesic efficacy. Larger studies and complementary autonomic experiments to evaluate its utility should be undertaken in this regard.

An obstacle yet to be surmounted in the quest to understand the mechanisms of analgesic stimulation is the lack of adequate animal models of chronic pain (Blackburn-Munro, 2004; Oliveras and Besson, 1988). In addition to their limited homology in chronic pain paradigms, the smaller brains of rodent models increase targeting inaccuracies, in particular for small brainstem structures like the PAG. This study emphasizes the important opportunities presented by translational research into DBS in human subjects for studying mechanisms underlying efficacious analgesia and advancing our understanding of central control of autonomic function.

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References

Aicher, S.A., Saravay, R.H., Cravo, S., Jeske, I., Morrison, S.F., Reis, D.J., Milner, T.A., 1996. Monosynaptic projections from the nucleus tractus solitarii to C1 adrenergic neurons in the rostral ventrolateral medulla: comparison with input from the caudal ventrolateral medulla. J. Comp. Neurol. 373, 62–75.

Akil, H., Mayer, D.J., Liebeskind, J.C., 1976. Antagonism of stimulation-produced analgesia by naloxone, a narcotic antagonist. Science 191, 961–962.

Akil, H., Richardson, D.E., Hughes, J., Barchas, J.D., 1978. Enkephalin-like material elevated in ventricular cerebrospinal fluid of pain patients after analgetic focal stimulation. Science 201, 463–465.

Appelhans, B.M., Luecken, L.J., 2006. Heart rate variability as an index of regulated emotional responding. Rev. Gen. Psychol. 109, 229–240.

Appelhans, B.M., Luecken, L.J., 2008. Heart rate variability and pain: associations of two interrelated homeostatic processes. Biol. Psychol. 77, 174–182.

Bandler, R., Carrive, P., 1988. Integrated defence reaction elicited by excitatory amino acid microinjection in the midbrain periaqueductal grey region of the unrestrained cat. Brain Res. 439, 95–106.

Bandler, R., Carrive, P., Zhang, S.P., 1991. Integration of somatic and autonomic reactions within the midbrain periaqueductal grey: viscerotopic, somatotopic and functional organization. Prog. Brain Res. 87, 269–305.

Behbehani, M.M., 1995. Functional characteristics of the midbrain periaqueductal gray. Prog. Neurobiol. 46, 575–605.

Behrens, T.E., Woolrich, M.W., Jenkinson, M., Johansen-Berg, H., Nunes, R.G., Clare, S., Matthews, P.M., Brady, J.M., Smith, S.M., 2003. Characterization and propagation of uncertainty in diffusion-weighted MR imaging. Magn. Reson. Med. 50, 1077–1088.

Birklein, F., Riedl, B., Neundorfer, B., Handwerker, H.O., 1998. Sympathetic vasoconstrictor reflex pattern in patients with complex regional pain syndrome. Pain 75, 93–100.

Bittar, R.G., Burn, S.C., Bain, P.G., Owen, S.L., Joint, C., Shlugman, D., Aziz, T.Z., 2005. Deep brain stimulation for movement disorders and pain. J. Clin. Neurosci. 12, 457–463.

Blackburn-Munro, G., 2004. Pain-like behaviours in animals — how human are they? Trends Pharmacol. Sci. 25, 299–305.

Carrive, P., 1993. The periaqueductal gray and defensive behavior: functional representation and neuronal organization. Behav. Brain Res. 58, 27–47.

Depaulis, A., Keay, K.A., Bandler, R., 1994. Quiescence and hyporeactivity evoked by activation of cell bodies in the ventrolateral midbrain periaqueductal gray of the rat. Exp. Brain Res. 99, 75–83.

Dionne, R.A., Mueller, G.P., Young, R.F., Greenberg, R.P., Hargreaves, K.M., Gracely, R., Dubner, R., 1984. Contrast medium causes the apparent increase in beta-endorphin levels in human cerebrospinal fluid following brain stimulation. Pain 20, 313–321.

Fanselow, M.S., 1991. The midbrain periaqueductal gray as a coordinator of action in response to fear and anxiety. In: Depaulis, A., Bandler, R. (Eds.), The Rat Brain Periaqueductal Gray Matter: Functional, Anatomical and Neurochemical Organization. Plenum Press, New York, pp. 1–8.

Fessler, R.G., Brown, F.D., Rachlin, J.R., Mullan, S., Fang, V.S., 1984. Elevated beta-endorphin in cerebrospinal fluid after electrical brain stimulation: artifact of contrast infusion? Science 224, 1017–1019.

Green, A.L., Wang, S.Y., Owen, S.L.F., Xie, K.N., Liu, X.G., Paterson, D.J., Stein, J.F., Bain, P.G., Aziz, T.Z., 2005. Deep brain stimulation can regulate arterial blood pressure in awake humans. Neuroreport 16, 1741–1745.

Green, A.L., Wang, S., Owen, S.L., Paterson, D.J., Stein, J.F., Aziz, T.Z., 2006a. Controlling the heart via the brain: a potential new therapy for orthostatic hypotension. Neurosurgery 58, 1176–1183 discussion 1176–1183.

Green, A.L., Wang, S., Owen, S.L., Xie, K., Bittar, R.G., Stein, J.F., Paterson, D.J., Aziz, T.Z., 2006b. Stimulating the human midbrain to reveal the link between pain and blood pressure. Pain 124, 349–359.

Hagemann, D., Waldstein, S.R., Thayer, J.F., 2003. Central and autonomic nervous system integration in emotion. Brain Cogn. 52, 79–87.

Hancock, M.B., 1988. Evidence for direct projections from the nucleus of the solitary tract onto medullary adrenaline cells. J. Comp. Neurol. 276, 460–467.

Hosobuchi, Y., Adams, J.E., Linchitz, R., 1977. Pain relief by electrical stimulation of the central gray matter in humans and its reversal by naloxone. Science 197, 183–186.

Hosobuchi, Y., Rossier, J., Bloom, F.E., Guillemin, R., 1979. Stimulation of human periaqueductal gray for pain relief increases immunoreactive beta-endorphin in ventricular fluid. Science 203, 279–281.

Kamath, M.V., Fallen, E.L., 1993. Power spectral analysis of heart rate variability: a noninvasive signature of cardiac autonomic function. Crit. Rev. Biomed. Eng. 21, 245–311.

Kringelbach, M.L., Jenkinson, N., Green, A.L., Owen, S.L., Hansen, P.C., Cornelissen, P.L., Holliday, I.E., Stein, J., Aziz, T.Z., 2007. Deep brain stimulation for chronic pain investigated with magnetoencephalography. Neuroreport 18, 223–228.

Kuch, B., Parvanov, T., Hense, H.W., Axmann, J., Bolte, H.D., 2004. Short-period heart rate variability in the general population as compared to patients with acute myocardial infarction from the same source population. Ann. Noninvasive Electrocardiol. 9, 113–120.

Martinmaki, K., Rusko, H., Kooistra, L., Kettunen, J., Saalasti, S., 2006. Intraindividual validation of heart rate variability indexes to measure vagal effects on hearts. Am. J. Physiol. Heart Circ. Physiol. 290, 640–647.

McAllen, R.M., Spyer, K.M., 1976. The location of cardiac vagal preganglionic motoneurones in the medulla of the cat. J. Physiol. 258, 1976.

Mestivier, D., Chau, N.P., Chanudet, X., Bauduceau, B., Larroque, P., 1997. Relationship between diabetic autonomic dysfunction and heart rate variability assessed by recurrence plot. Am. J. Physiol. 272, 1094–1099.

Mori, S., Zhang, J., 2006. Principles of diffusion tensor imaging and its applications to basic neuroscience research. Neuron 51, 527–539.

Nashold, B.S., Wilson, W.P., Slaughte, D.G., 1969. Sensations evoked by stimulation in the midbrain of man. J. Neurosurg. 30, 14–24.

Oliveras, J.L., Besson, J.M., 1988. Stimulation-produced analgesia in animals: behavioural investigations. Prog. Brain Res. 77, 141–157.

Pagani, M., Montano, N., Porta, A., Malliani, A., Abboud, F.M., Birkett, C., Somers, V.K., 1997. Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. Circulation 95.

Pereira, E.A., Green, A.L., Bradley, K.M., Soper, N., Moir, L., Stein, J.F., Aziz, T.Z., 2007a. Regional cerebral perfusion differences between periventricular grey, thalamic and dual target deep brain stimulation for chronic neuropathic pain. Stereotact. Funct. Neurosurg. 85, 175–183.

Pereira, E.A., Green, A.L., Nandi, D., Aziz, T.Z., 2007b. Deep brain stimulation: indications and evidence. Expert Rev. Med. Devices 4, 591–603.

Pereira, E.A., Dobbs, T., Kringelbach, M.L., Paterson, D.J., Aziz, T.Z., Green, A.L., 2008. Sustained blood pressure changes with periventricular grey but not posterior hypothalamic deep brain stimulation. Acta Neurochir. 150, 933.

Pereira, E.A., Moir, L., Green, A.L., Aziz, T.Z., 2009. Deep brain stimulation for chronic pain. In: Krames, E., Peckham, P.H., Rezai, A.R. (Eds.), Neuromodulation, pp. 499–506.

Pereira, E.A., Wang, S., Paterson, D.J., Stein, J.F., Aziz, T.Z., Green, A.L., 2010. Sustained reduction of hypertension by deep brain stimulation. J. Clin. Neurosci. 17, 124–127.

Porges, S.W., 1997. Emotion: an evolutionary by-product of the neural regulation of the autonomic nervous system. Ann. N. Y. Acad. Sci. 807, 62–77.

Pumprla, J., Howorka, K., Groves, D., Chester, M., Nolan, J., 2002. Functional assessment of heart rate variability: physiological basis and practical applications. Int. J. Cardiol. 84, 1–14.

Ray, N.J., Jenkinson, N., Kringelbach, M.L., Hansen, P.C., Pereira, E.A., Brittain, J.S., Holland, P., Holliday, I.E., Owen, S., Stein, J., Aziz, T., 2009. Abnormal thalamocortical dynamics may be altered by deep brain stimulation: using magnetoencephalography to study phantom limb pain. J. Clin. Neurosci. 16, 32–36.

Richardson, D.E., Akil, H., 1977. Long term results of periventricular gray self-stimulation. Neurosurgery 1, 199–202.

Rossi, F., Maione, S., Berrino, L., 1994. Periaqueductal gray area and cardiovascular function. Pharmacol. Res. 29, 27–37.

Saul, J.P., Arai, Y., Berger, R.D., Lilly, L.S., Colucci, W.S., Cohen, R.J., 1988. Assessment of autonomic regulation in chronic congestive heart failure by heart rate spectral analysis. Am. J. Cardiol. 61, 1292–1299.

Tarvainen, M.P., Ranta-aho, P.O., Karjalainen, P.A., 2002. An advanced detrending method with application to HRV analysis. IEEE Trans. Biomed. Eng. 49, 172–175.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. Eur. Heart J. 17, 354–381.

Thayer, J.F., Brosschot, J.F., 2005. Psychosomatics and psychopathology: looking up and down from the brain. Psychoneuroendocrinology 30, 1050–1058.

Young, R.F., Chambi, V.I., 1987. Pain relief by electrical stimulation of the periaqueductal and periventricular gray matter. Evidence for a non-opioid mechanism. J. Neurosurg. 66, 364–371.

Young, R.F., Rinaldi, P.C., 1997. Brain Stimulation. Springer-Verlag, New York.