

# Offset analgesia is mediated by activation in the region of the periaqueductal grey and rostral ventromedial medulla

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## ABSTRACT

Interrupting a continuous noxious heat by a greater noxious heat causes rapid and disproportionate pain reduction when the original noxious heat returns. This reduction in pain experience, known as offset analgesia, is believed to be the consequence of active descending inhibitory control of pain originating in the periaqueductal grey (PAG) and rostral ventromedial medulla (RVM). To test this possibility, brain activation was measured using fMRI in twelve healthy controls during an offset procedure. Each subject experienced six second periods of noxious heat followed by an equal period of more intense heat before returning to the original temperature for a further 6 s. Subjects were also scanned during control trials involving continuous, unchanging, noxious heat for 18 s or involving 6 s of noxious heat followed by an equal period of more intense heat before returning to the non-noxious baseline for a further 6 s. Brain activation during the final 6 s of each trial was compared with activation during the first 6 s and this difference was contrasted across trials. PAG/RVM activation was observed during the final 6 s of offset trials but not during either of the control trials and this difference across trials was significant. Activation throughout the pain neuromatrix was inhibited during the final 6 s of the offset trials and was comparable to the activation observed when the heat returned to a non-noxious baseline. These findings provide strong evidence that offset analgesia engages an endogenous inhibitory mechanism originating in the PAG/RVM region, which inhibits pain experience and activation of the pain neuromatrix.

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## Introduction

An increased noxious heat followed by a less noxious heat reduces pain experience significantly more than reductions due to stimulus adaptation (Grill and Coghill, 2002; Derbyshire and Osborn, 2008). Pain intensity ratings fall by 30–100% (i.e., towards an experience of no pain) when low noxious intensity follows high noxious intensity and this reduction in ratings of pain intensity has been described as offset analgesia. Offset analgesia is distinct from adaptation or primary afferent fatigue that occurs during prolonged or repeated noxious stimulation within or across sessions (LaMotte and Campbell, 1978; LaMotte et al., 1983; Gallez et al., 2005).

Offset analgesia is a potent example that stimulus manipulations can induce adaptive plastic changes within the nociceptive system. This has been demonstrated consistently using concurrent noxious stimuli to produce “diffuse noxious inhibitory control” (DNIC) (Willer 1977; Le Bars et al., 1979a; 1979b; Talbot et al., 1989; Andersen et al., 2001; Motohashi and Umino, 2001; Lautenbacher et al., 2007). DNIC occurs when a noxious stimulus at one body site reduces pain from a noxious stimulus at another body site. Both

offset analgesia and DNIC demonstrate that noxious stimuli can generate endogenous inhibitory processes as well as excitatory processes, although not necessarily by the same mechanism.

Several decades ago it was demonstrated in rats that electrically stimulating the periaqueductal grey (PAG) allows surgery to advance without further analgesia or anaesthesia (Reynolds, 1969). Further anatomical studies of descending modulation of pain determined that the analgesia relied on relays in the medulla, specifically the rostroventral medulla (RVM) (Gebhart et al., 1983; Sandkuhler and Gebhart, 1984). The PAG–RVM–spinal cord pathway comprises an essential neural circuit supporting opioid-based pain inhibition (Basbaum and Fields, 1978; 1984; Basbaum et al., 1978). Administering opioid agonists directly into the PAG produces naloxone-reversible analgesia and naloxone-reversible excitation of RVM neurons (Yaksh and Rudy, 1978; Cheng et al., 1986; Morgan et al., 1992). The rapidity of offset analgesia and the magnitude of its effect suggest that offset analgesia engages this descending PAG/RVM mediated inhibition (Grill and Coghill, 2002; Derbyshire and Osborn, 2008) but this is yet to be demonstrated and peripheral changes might alternatively explain the offset analgesic response.

Although we favour an explanation based on brain mechanisms, it has been demonstrated that peripheral fibres alone can provide the information necessary to detect noxious stimuli and to make magnitude judgements of pain elicited by fluctuating noxious sti-

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muli (Robinson et al., 1983). Here we examine brain activation during offset analgesia directly using fMRI. If offset analgesia does engage descending inhibitory controls then increased activity in the PAG/RVM region should be observed during the offset period.

## Methods

### Subjects

Twelve right handed females (mean age 23 years) participated in the study. Each subject received a brief demonstration of the thermal probe prior to testing to familiarise them with the sensation and to allay any initial anxiety. Subjects were informed that the heat induced pain would not cause tissue damage and that they were free to withdraw from the experiment at any time. Ethical approval was provided by the local ethics committee and all subjects provided written consent. All were otherwise naive of the purpose of the experiment. No subject had a history of neurological, psychiatric or pain disorder. Subjects were paid £15 for completing the fMRI procedure.

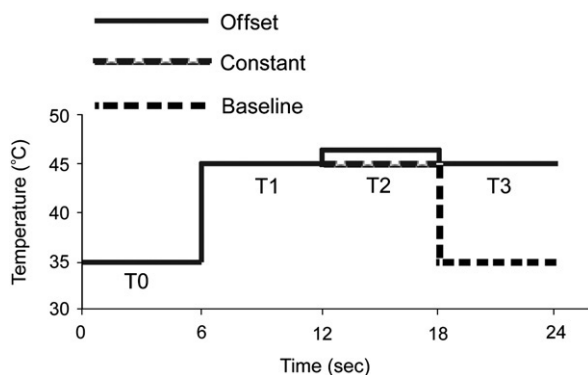
### Apparatus

Thermal stimuli was administered to the volar surface of both forearms using a 27 mm diameter peltier thermode with rise and fall rates of 30 °C/s using the CHEPS system (Medoc Advanced Medical systems; Ramat Yishia, Israel). The probe was attached to the forearm using a Velcro strap and set to a baseline temperature of 35 °C. An online sliding scale was used to continuously assess pain intensity from 0 (no pain) to 100 (maximal pain). The probe was moved after each run to minimise sensitisation.

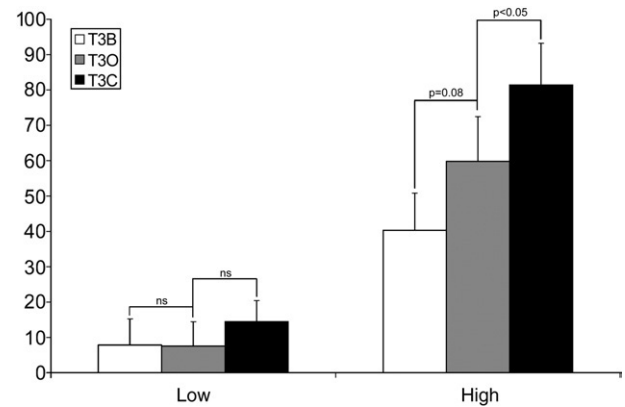
### Procedure

All subjects were tested using three different sets of heat trials: offset, constant and baseline (illustrated in Fig. 1) that were interleaved during the same scanning run. Each run involved either a low (41 °C) or high (45 °C) temperature delivered to the right or left volar forearm. The order of temperature and arm was counterbalanced across subjects and each subject provided 2–6 runs of data depending on their tolerance for the procedure.

All trials began with an initial 6 s innocuous stimulus of 35 °C (T0). Offset trials continued with the low (41 °C) or high (45 °C) noxious stimulus (T1, 6 s), followed by a 1 °C increase (T2, 6 s) and then a 1 °C decrease (T3, 6 s) to the original T1 temperature. Baseline trials were the same as offset trials but at T3 the temperature



**Fig. 1.** The time course of the offset, constant and baseline trials. Each trial consisted of four contiguous phases: T0, an initial innocuous heat stimulus (35 °C); T1, an initial noxious heat stimulus; T2 a second noxious heat stimulus 1 °C greater than T1 (offset and baseline trials) or equal to T1 (constant trials); and T3, a third heat stimulus equivalent to T1 (offset and constant trials) or to T0 (baseline trials).



**Fig. 2.** The average pain intensity ratings reported during the T3 period for the baseline (T3B), offset (T3O) and constant (T3C) trials. Constant trials produced a significantly higher T3 pain rating than offset trials for the high noxious temperature.

returned to the innocuous baseline temperature of 35 °C. Constant trials continued with the same low (41 °C) or high (45 °C) noxious stimulus throughout T1, T2 and T3. A jittered interval was implemented between trials ranging from 2–4 s to facilitate estimation of the BOLD response.

### fMRI data acquisition

We used a Phillips 3 T Achieva system to acquire blood oxygenated level dependent (BOLD) contrast weighted echoplanar images (EPI) for the functional scans (repetition time TR=3000 ms, echo time TE=2000 ms, FoV=220 mm, 40 slices, 2.75 isotropic voxels). Images were acquired using an eight channel phase array coil with a sense factor of 2. For each functional run, 96 dynamics were acquired from the AC-PC line. Structural images were acquired using T1TFE technique (TR=8.4, FoV=232 mm, flip angle=60° 288×288 matrix, 175 slices).

### Data analysis

Data analysis was performed using the FMRIB Software Library (FSL release 4.1-Oxford Centre for Functional Magnetic Resonance Imaging of the Brain), described in detail elsewhere (Smith et al., 2004). In summary, head movement between scans was corrected by aligning all subsequent scans with the first. Each re-aligned set of scans from every subject was coregistered with his or her own hi-res structural MRI image, with the non-brain components edited out, and reoriented into the standardized anatomical space of the average brain provided by the Montreal Neurological Institute (MNI). To increase the signal to noise ratio and accommodate variability in functional anatomy, each image was smoothed in X, Y and Z dimensions with a Gaussian filter of 6 mm (FWHM).

A box-car model with a hemodynamic delay function was fitted to each voxel generating a statistical image corresponding to condition. Baseline drifts were removed by applying a high-pass filter. Brain regions with a large statistic correspond to structures whose BOLD response shares a substantial amount of variance with the induced changes in the participant's experience. Critically, direct contrast of the brain response during T3 with that during T1 provided an estimate of brain activation during the critical offset analgesia, baseline and constant periods. Contrasts across these conditions revealed areas of activation during offset analgesia greater than that during the equivalent periods for the baseline and constant trials. The multiple comparison problem of simultaneously assessing all the voxel statistics was addressed via cluster based thresholding. Clusters of voxels that exceeded a Z-score>2.3 and  $p<0.05$  (corrected for multiple comparisons) were considered

statistically significant. The use of a combined height and cluster threshold is a standard and generally accepted method to solve the problem of multiple comparisons with functional imaging data (Friston et al., 1992; Worsley et al., 1992) and has been used extensively by other pain researchers (e.g., Lloyd et al., 2006; Morrison et al., 2004). For display purposes, images are thresholded at  $Z > 2.3$  and  $k > 3$ .

## Results

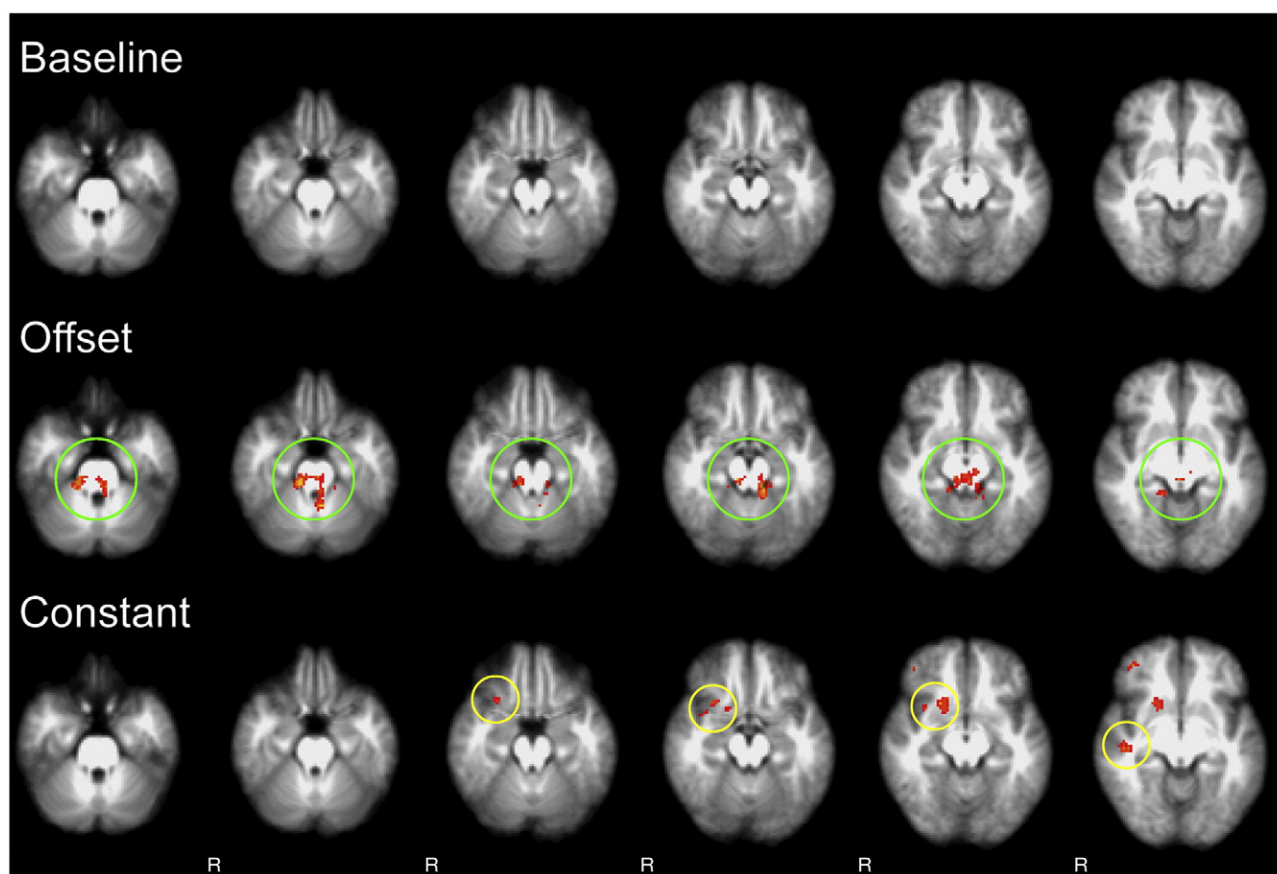
Pain ratings recorded during T3 for the low (41 °C) and high (45 °C) noxious trials are illustrated in Fig. 2. During the high trials, T3 offset periods were rated as significantly less painful than T3 constant. The difference between T3 offset and T3 baseline trended towards significance. For the low trials the pattern was similar but no differences were significant or approached significance. These findings replicate previous behavioural findings from our laboratory (Derbyshire and Osborn, 2008).

BOLD activation measured with fMRI during T3 was compared with the activation during the initial six second innocuous delivery for the baseline, offset and constant trials. Fig. 3 demonstrates no activity for this comparison during baseline trials but significant activity in the PAG/RVM region during offset trials (peak voxel 18, −30, −30,  $z = 4.4$ ,  $k = 538$ ). This contrast during constant trials resulted in no significant activity in the PAG/RVM region but significant activation of the posterior (peak voxel 40, −20, 2,  $z = 3.8$ ,  $k = 594$ ) and anterior (peak voxel 42, 24, 14,  $z = 3.9$ ,  $k = 2,901$ ) insula extending into the region of the putamen and prefrontal cortex.

Across trial differences were also assessed to compare T3 constant and T3 baseline with T3 offset trials. Fig. 4 shows significantly reduced activation in the PAG/RVM region for the T3 constant and baseline comparisons with offset indicating that the PAG/RVM region was significantly more active during offset trials. There is considerably more activation during T3 constant trials than T3 offset trials in regions of the brain typically associated with pain including the thalamus, midcingulate cortex (MCC), S1 and S2. Differences between T3 baseline and T3 offset are much less apparent. Significant differences are listed in Table 1.

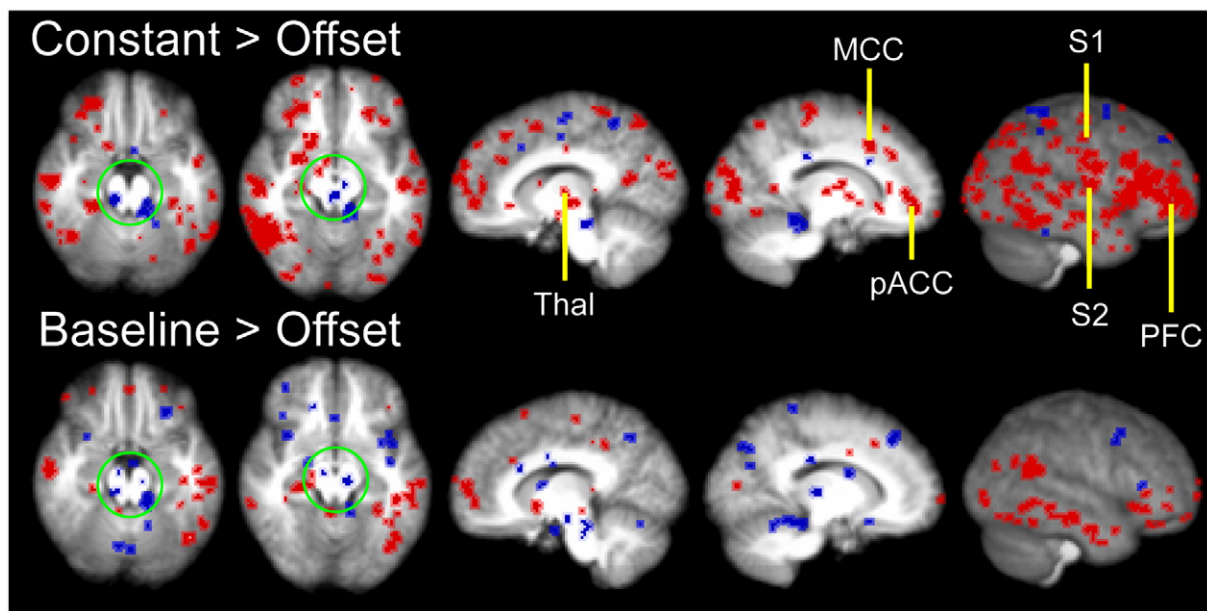
## Discussion

The present study suggests that the disproportionate reduction in pain that follows a more noxious stimulus, known as offset analgesia (Grill and Coghill, 2002; Derbyshire and Osborn, 2008), is mediated by activation in the PAG/RVM region. PAG/RVM activation was significantly increased relative to a continuous delivery of constant noxious heat and relative to a return to an innocuous baseline heat. Furthermore, the increased activation of the PAG/RVM during offset analgesia was accompanied by reduced activity of the insula and other regions commonly activated during pain (Apkarian et al., 2005). Comparison of the T3 constant periods with the T3 offset periods demonstrated significantly greater responses throughout the pain neuromatrix during T3 constant. This finding is consistent with the pain being greater during T3 constant despite exactly the same noxious stimulus being delivered during T3 constant and T3 offset. In contrast, comparison of the T3 baseline periods with the T3 offset periods



**Fig. 3.** Activation during T3 compared with the innocuous baseline is shown for the baseline, offset and constant trials. Significant PAG–RVM activation is highlighted (green circles) during the offset trials. No significant PAG/RVM response was observed during the constant and baseline trials and this difference between trials was significant (see Fig. 4). Significant activation of the anterior and posterior insula is highlighted (yellow circles) during the constant trials.





**Fig. 4.** Activation during T3 constant compared with T3 offset (top) and during T3 baseline compared with T3 offset (bottom). Significantly greater (blue scale) PAG–RVM response is highlighted (green circles) during the offset trials compared to both constant and offset. Significantly greater activation (red scale) of the thalamus, MCC, S1, S2 and PFC during the constant compared with offset trials is highlighted and not evident or attenuated during the baseline compared with offset trials.

demonstrated attenuated differences consistent with a smaller difference in pain experience. Overall, these findings provide good evidence that offset analgesia engages an endogenous inhibitory mechanism originating in the PAG/RVM region, which inhibits pain experience and activation of the pain neuromatrix (Tracey and Mantyh, 2007).

The precise mechanism for this inhibition remains uncertain but it is generally accepted that the PAG projects to the RVM and the RVM projects along the dorsolateral funiculus to the dorsal horn where nociceptive transmission is inhibited (Vanegas and Schaible, 2004). Two types of neuron in the RVM, known as on and off cells, have been described as facilitating or inhibiting nociceptive transmission (Fields, 2004). Noxious inputs trigger both descending facilitation and inhibition of nociceptive transmission and, presumably, changing from a highly noxious to a less noxious stimulus alters the balance of activity towards inhibition.

**Table 1**

The regions with greater or lesser (*italics*) BOLD response during the T3 constant and T3 baseline period compared with T3 offset.

T3 constant>T3 offset			T3 baseline>T3 offset		
Brain area (x, y, z coordinates) (region)	Side	Z-score	Brain area (x, y, z coordinates) (region)		Z-score
PAG/RVM (2, -26, -10)	R	-3.2	(14, -30, -24)		-3.4
Thalamus (14, -24, 0)	R	3.2	(16, -26, 8)		2.8
MCC (-14, 16, 42)	L	3.0	(-2, 24, 32)		3.2
pACC (-14, 46, 4)	L	3.3	(-2, 28, 10)		3.3
Inf. Parietal Cortex (-38, -54, 22) (BA 39/40)	L	3.9	(-60, -54, 20)		3.0
S1 (-14, -26, 68)	L	3.0	No observable response		–
(36, -20, 54)	R	3.1			
S2 (-32, -12, 2)	L	3.8	No observable response		–
Prefrontal Cortex	R	4.1	(16, 68, 12) (BA 10)		2.9
(42, 20, 18) (BA 45/46)					

The areas are tabulated in terms of the brain region, as illustrated in Fig. 4, and their approximate cytoarchitecture (BA = Brodmann's area). The x, y, z coordinates plot each peak (defined as the pixel with the highest Z-score within each tabulated region) according to the MNI coordinate system (negative is left, posterior and inferior). MCC = midcingulate cortex; pACC = perigenual cingulate cortex; S1 = primary somatosensory cortex; S2 = secondary somatosensory cortex.

Descending facilitatory processes have been suggested as contributing to the development and maintenance of hyperalgesia and contributing to chronic pain states such as fibromyalgia (Woolf, 2004). It is also plausible that a failure of descending inhibition contributes to the experience of chronic pain. Until recently it has been difficult to directly assess these possibilities but a recent study has demonstrated that patients with active DNIC identified before surgery are protected against chronic pain (Yarnitsky et al., 2008). This finding is important as it indicates that active endogenous analgesic circuits may be protective against developing chronic pain. Offset analgesia is a technique that might be used in a similar way to assess the likelihood of postsurgical pain, or other acute pains, transitioning from acute to chronic. Offset analgesia might also be used to assess endogenous analgesia in patients with functional pain such as fibromyalgia. Assessments of offset analgesia, with or without functional imaging, may be a useful part of a pain susceptibility profile that can be used to predict and treat pain.

These optimistic comments should be tempered by the need to replicate Yarnitsky et al. (2008) and the current study. Chronic pain is a notoriously difficult condition to predict or treat and it is unlikely that any single mechanism will provide an entire solution (Borsook and Becerra, 2006). Chronic brain stimulation that involves just the PAG, for example, typically does not relieve chronic pain and even simultaneous stimulation of the thalamus and PAG only provides useful benefit for a small minority of patients (Hamani et al., 2006). It is also possible that sensitisation effects contributed to the observed RVM/PAG activation instead or in addition to any effects of offset analgesia (Lee et al., 2008). Although we moved the probe as a precaution against sensitisation, and the offset trials produced greater RVM/PAG response compared with the other noxious trials, the higher offset temperature may have generated additional sensitisation. Future studies may employ imaginative designs to assess the possible contribution of sensitisation in addition to offset effects.

In summary, this study suggests that the plasticity of pain revealed by offset analgesia is mediated by activity in the PAG/RVM. Similar mechanisms may also underlie other plastic changes within the

nociceptive system including the dysfunctional changes that may partly cause or maintain chronic pain syndromes.

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