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Central mechanisms of offset analgesia

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CENTRAL MECHANISMS OF OFFSET ANALGESIA

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ABSTRACT

Reduction from a more to a less noxious stimulus intensity produces a disproportionate but transient decrease in perceived pain. Although the relationship between the central nervous system and this offset analgesia has come under investigation using brain imaging, whether offset analgesia is primarily mediated by central rather than peripheral mechanisms has not been established. Here we investigate this question in healthy volunteers using thermal stimuli while recording continuous pain ratings. We constructed a composite stimulus using one Peltier thermode to deliver a constant painful test stimulus while a separate thermode coincidentally delivered a shorter but more intense conditioning stimulus at a distinct location. Three spatial configurations were investigated all delivering stimulation to the ventral forearm either proximally or distally from one another on the same forearm or with thermodes on opposing forearms. We demonstrate a decrease in test stimulus pain levels following offset of an ipsilateral but not contralateral conditioning stimulus. This decrease is comparable in magnitude to that observed during a single thermode classic offset analgesia stimulation. The manifestation of analgesia in one sensory field following cessation of stimulation in a distinct sensory field shows antinociceptive adaptation of primary afferent neurons is unnecessary to produce offset analgesia, and demonstrates central mechanisms are sufficient to achieve temporal filtering of nociceptive information during stimulus offset.

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LIST OF ABBREVIATIONS

BOLD.....Blood Oxygen Level Dependent

C.....Celsius

DNIC.....Diffuse Noxious Inhibitory Control

fMRI.....functional Magnetic Resonance Imaging

NMDA ..... N-methyl-d-aspartate

NS .....Not Significant

OA..... Offset Analgesia

PAG.....Periaqueductal Gray

SRD..... subucleus reticularis dorsalis

RVM .....Rostral Ventral Medulla

s.....seconds

SD .....Standard Deviation

SE.....Standard Error

VAS.....Visual Analog Scale

VTA .....Ventral Tegmental Area

INTRODUCTION

A disproportionate transient reduction in perceived pain intensity occurs following reduction of a painful stimulus in humans. This phenomenon is best illustrated by a subject rating the perceived pain intensity of a graded thermal stimulus delivered from an innocuous baseline. For instance, considering a 46-48-46C stimulus sequence, the increase in pain intensity when increasing temperatures from 46C to 48C (T1 to T2, figure 1) is not as great as the decrease in pain when decreasing stimulus intensity from 48C back to 46C (T2 to T3) a moment later (Grill & Coghill, 2002). Both small and large analgesic effects have been reported following conditioning by stimulus offset, but in all cases this conditioned effect is transient, lasting on the order of tens of seconds before subjects resume rating their pain at a level comparable to a test stimulus designed without a temperature offset (Nahman-Averbuch et al., 2014; Yelle, Rogers, & Coghill, 2008). Although pain is not simply a linear representation of a stimulus, and is known to be modulated by environmental and cognitive factors (Villemure & Bushnell, 2002), offset analgesia reliably occurs as a response to a specific stimulus feature without manipulation of any of these factors, and reflects an intrinsic characteristic of the neurophysiological processing that translates noxious stimuli to pain perception in healthy individuals.

Pain modulation occurs at multiple levels of the neuraxis, from spatial and temporal filtering at first order afferent neurons, in the spinal dorsal horn and in the brainstem, as well as more complex modulatory contributions provided at the level of the brain itself. For instance, C-fiber afferent nociceptors, which are thin and unmyelinated, are poorly suited for encoding fast and transient stimulus features, thus acting as low pass

filters of nociceptive information. Additionally, in the domain of increasing afferent inputs, repetitive firing of primary nociceptors sensitizes their central targets in the spinal dorsal horn, producing signal amplification and temporal sharpening (Dickenson & Sullivan, 1987). Finally, spatial sharpening of one nociceptive stimulus is achieved with inhibition of further heterotopic nociceptive signaling via interactions between the dorsal horn and brainstem nuclei (Le Bars, Dickenson, & Besson, 1979). Many similar examples could be cited at the risk of creating the impression that spatial and temporal filtering is unique to pain. Perhaps best known in visual input processing, where cortical circuits produce edge enhancement between dark and light surfaces (Daugman, 1985; Priebe & Ferster, 2012), spatiotemporal filtering is in fact ubiquitous throughout the nervous system. Nevertheless, temporal filtering in the domain of decreasing nociceptive stimuli remains poorly understood.

Offset analgesia highlights a form of edge enhancement in the temporal dimension which might help in escape behaviors and have important clinical and basic science implications. More so than any other sensory modality, pain is a motivating signal and achieves its biological utility by promoting diverse defensive and recuperative behaviors. Decreases in pain specifically carry positive hedonic value and serve a critical function in realizing this biological utility over the long term through reward learning (Navratilova et al., 2012). Conversely, persistent pain without relief serves no apparently useful function, but is paradoxically pervasive, burdening patients and society. Notably, in chronic neuropathic pain patients, analgesia following offset of an acute noxious stimulus appears diminished or absent, an observation which parallels findings of altered

hedonic response to noxious stimuli in chronic back pain (Baliki, Geha, Fields, & Apkarian, 2010). The same brain circuit underlying altered hedonic value in chronic pain moreover appears critical in the transition to chronic pain (Baliki et al., 2012). Offset analgesia may thus serve as a valuable model through which to better understand the method by which pain achieves its biological utility in health and becomes dysfunctional in disease. Although identifying the mechanism underlying this model would be a critical first step, of the several pointed attempts to test specific hypothetical mechanisms of offset analgesia all have so far missed the mark (Martucci, Eisenach, Tong, & Coghill, 2012; Martucci, Yelle, & Coghill, 2012; Nahman-Averbuch et al., 2014; Niesters, Dahan, et al., 2011). Here we aim to take a breadth-first approach and rule out the possibility that antinociceptive dynamics in first order afferents underlie the phenomenon, demonstrate the sufficiency of central mechanisms, and thereby narrow the search for such mechanisms.

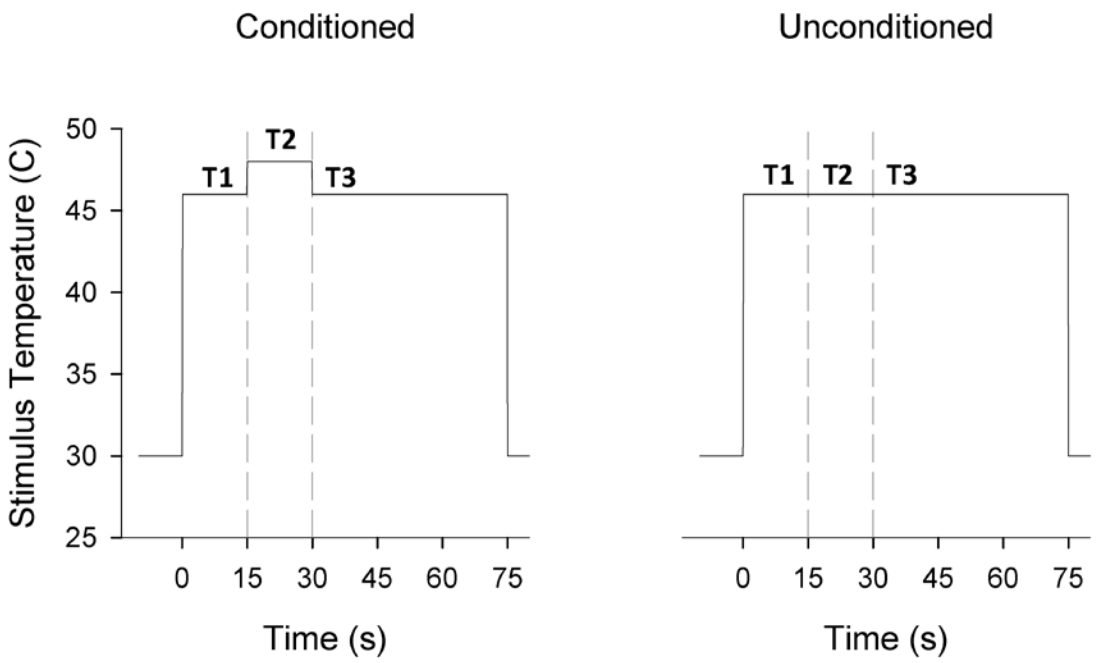

Figure 1 – A prototypical conditioned stimulus alongside its unconditioned counterpart. Pain during T3 after the conditioning offset from T2 has been shown to be lower than during T1 or an unconditioned T3 period.

Pathways from nociception to pain

Unlike the mechanoreceptors signaling touch, cochlear hair cells underlying audition, or photoreceptor cells detecting light, pain may not be as neatly coupled with a single peripheral sensory signaling pathway. For instance, daily experience demonstrates how overstimulation of any one of the five classic sensory systems under appropriate conditions can produce pain, and the phenomenological experience of pain evoked by bright lights, loud noises and intense mechanical pressure bears striking cross-modal qualitative similarity in some manner of its 'painfulness'. Although it appears afferent input from modality-specific neurons is necessary to elicit a sensation of pain from healthy tissue (Flores et al., 2015; Torebjork & Hallin, 1973), neurons which respond to specific kinds of tissue injury both exist and conversely are not sufficient to elicit pain.

Nociceptors were discovered by investigators searching for primary afferent neurons which activated in response to tissue injury (Burgess & Perl, 1967; Sherrington, 1906), and the most accurate manner of understanding nociception continues to be in terms of transmitting a protective signal to initiate corrective behavior, but this signal may produce corrective behavior without eliciting consciously discernible pain, and conversely tissue damage can be experienced without pain in genetically normal individuals. Offset analgesia can be exploited to produce painless but tissue damaging stimuli by follow a two-step-forward-one-step-back stimulation trajectory (Grill & Coghill, 2002), but offset analgesia is not the first phenomenon to seemingly demonstrate dissociation of pain from peripheral nociceptor stimulation, much more spectacular examples offer themselves. Soldiers who receive extensive physical trauma from battle (e.g. compound long bone fractures, chest, or abdominal penetrations, etc.) commonly do not experience pain upon arrival at field hospitals, with reports that greater than 70% claim moderate to no pain and decline offers of pain relieving medication (Beecher, 1946). This same phenomenon has been reported in more mundane situations (Melzack, Wall, & Ty, 1982). At the same time, individuals born with congenital nociceptive abnormalities provide an exceptional illustration of the need for persistent nociception in daily life. Unable to detect and thus avoid self-inflicted tissue injury these individuals may self mutilate by biting their tongues and lips, develop foot ulcerations due to gait disturbances, and damage their joints through hyperextension during routine activities. Due to the frequent accumulation of injuries and tissue damage, few survive to adulthood (Nagasako, Oaklander, & Dworkin, 2003). Although it thus appears frequent afferent

nociceptive feedback is needed to live a healthy and injury free life, it is conversely relatively uncommon to experience frequent and persistent pain, and contemporary society considers individuals who do experience persistent pain as abnormal, worthy of special accommodations and candidates for medical treatment. Thus, health must be characterized by a high level of nociceptive afferent activity but infrequent pain. The selection of and transformation of otherwise ubiquitous nociceptive signals into pain perception is a complex process, involving information from multiple sensory afferent fibers which are gated or modulated at different levels of the nervous system from the periphery to the brain.

Nociceptive afferents can be broadly classified into two categories based on response dynamics: fast acting, wide diameter myelinated Aδ fibers and slower acting thin unmyelinated C fibers. Aδ fibers have been implicated in the initial response to noxious stimuli, for instance the initial pricking sensation at the beginning of a thermal stimulus, while C fibers are responsible for delayed but more sustained afferent nociceptive signals following a noxious stimulus, often conveying a burning sensation (Torebjork & Hallin, 1973). Some of these afferents may be spontaneously active, and many must exceed some firing rate to elicit pain (Van Hees & Gybels, 1981). At the same time, after various sensitizing insults to the periphery other afferents which normally encode non-noxious stimuli are also able to elicit pain (Torebjork, Lundberg, & LaMotte, 1992). Although pain must necessarily be related to dynamic signaling from the periphery, and often is strongly correlated with this signaling, nociception at other times is only loosely related to pain which instead takes shape at a higher level of the nervous

system as a selective composition of peripheral afferent signaling. The site where this selective compositing first takes place is in the dorsal horn of the spinal cord at the convergence of Aδ, C, and other primary sensory fibers.

Afferent synapses in the dorsal horn encounter three broad classes of neurons which may be relevant for understanding offset analgesia: secondary afferent neurons which project to the brain, spinal interneurons that participate in local circuits and axons of efferent neurons from the brain which are implicated in modulatory control. Primary afferent nociceptive fibers project predominantly to the superficial layer of the spinal cord with an important minority of projections to deeper layers (Sugiura, Terui, & Hosoya, 1989; Woodbury & Koerber, 2003). Secondary nociceptive afferents in superficial layers of the spinal cord are nociception specific neurons with small receptive fields which allow for precise localization of noxious stimulation, but with few exceptions these neurons receive signals from multiple primary afferents of differing modalities (thermoreceptors, mechanoreceptors, etc), and so cannot individually convey specific sensory modality information to the brain. Additionally, nociception specific neurons are less precise in their stimulus coding than is needed to account for the intensity discriminative capacity of human pain perception. Projection neurons of deeper layers are much more sensitive, responding to changes as slight as 0.1C and appear to make up for the deficiency of nociception specific neurons (Maixner, Dubner, Bushnell, Kenshalo, & Oliveras, 1986), but these deep layer projection neurons have large receptive fields and a wide dynamic range, responding to both noxious and innocuous stimuli. It appears necessary for the brain to further decode composite afferent signals

from nociception specific and wide dynamic range secondary afferents to attain the spatial, intensity and modality specific information which are bound together in pain perception, although this is a topic of historical debate which continues to be revisited (Craig, 2003). Nevertheless the dorsal horn makes important contributions to pain perception by virtue of spinal interneurons and descending projections from the brain which contribute to pain modulation.

Interneurons of the spinal dorsal horn are both inhibitory and excitatory, and underlie a complex and still poorly understood circuitry. They constitute the majority of neurons present in the dorsal horn (Spike, Puskar, Andrew, & Todd, 2003), and serve a critical role in controlling propagation of nociceptive signals. A classic theory of pain physiology posits that transmission of nociceptive signals to the brain is gated at the level of the dorsal horn by inhibitory interneurons (Melzack & Wall, 1965). Competitive modulation of inhibitory interneurons by differing types of primary afferents is hypothesized to modulate pain by a variety of mechanisms, but has classically been suggested to underlying pain relief by tactile stimulation (e.g. rubbing a sore spot), which is supported by evidence of intrasegmental but not intersegmental tactile analgesia (Mancini, Nash, Iannetti, & Haggard, 2014). The role of local inhibitory interneurons in gating pain is demonstrated by studies showing allodynia, or pain response to normally innocuous stimuli (e.g. hair brush), following $GABA_A$ or glycine receptor inhibition (Yaksh, 1989) in otherwise intact rats, and by lowered mechanical threshold for withdrawal flexion in decerebrated rats (Sivilotti & Woolf, 1994). Allodynia and hyperalgesia (sensitization to painful stimuli) brought about by spinal interneuron

synaptic changes occurs without pharmacological intervention following various nociceptive insults (e.g. cutaneous nerve injury, heat injury, etc.; for review see (Woolf, 2011)).

The gating of nociceptive input at the level of the dorsal horn serves a critical role in endogenous analgesic mechanisms. Decades ago it was shown that electrical stimulation of the periaqueductal gray (PAG) could substitute for exogenous analgesics during surgery in rats (Reynolds, 1969), an analgesic phenomenon which has since been widely documented in humans (Bittar et al., 2005; Rasche, Rinaldi, Young, & Tronnier, 2006). This endogenous analgesic mechanism is implicated in cannabinoid (Meng, Manning, Martin, & Fields, 1998) and opiate induced analgesia and is brought about by interactions between the PAG, rostral ventral medulla (RVM) and spinal afferents and interneurons. (for a review see (Fields, Heinricher, & Mason, 1991)). Other mechanisms capable of evoking analgesia have also been identified, most notably spinal-medullary-spinal negative feedback loops. The dorsal reticular nucleus (also known as *subnucleus reticularis dorsalis*, SRD) of the caudal medulla coordinates gating of afferent nociceptive inputs at the level of the dorsal horn to achieve spatial sharpening through a form of nociception driven inhibition of nociception known as diffuse noxious inhibitory control (DNIC). For instance, immersion of a limb in a cold water bath (a conditioning stimulus) has been reported to reduce pain of a distinct thermal stimulation of a different limb (a testing stimulus) (Niesters, Dahan, et al., 2011), and this mechanism has also been credited for the effectiveness of acupuncture (Murase & Kawakita, 2000). On the other hand, both PAG-RVM and SRD mechanisms have been implicated in descending

pain facilitation (Lima & Almeida, 2002; Neubert, Kincaid, & Heinricher, 2004). At the level of the dorsal horn the balance between inhibition and facilitation of nociceptive neurons either directly or indirectly via spinal interneurons is thought to determine the final outcome of this opposition (for a review see (Fields, 2000)). These mechanisms highlight the importance of dorsal horn circuits and spinal-supraspinal interactions in regulating access of afferent nociceptive signals to the brain.

Although local segmental circuits in the spinal cord are capable of gating pain and initiating some forms of defensive behavior, the example of wounded soldiers, if not daily personal experience, clearly shows that pain response takes into account the context of the perceiving subject in a more nuanced way. The fact that nociception impinges on a phenomenal characteristic of consciousness points to an evolutionarily mandated purpose for such nuanced integration of sensory experience. The identification of appropriate response to at least some noxious stimuli in the face of conflicting risks, rewards, and attentional demands, as well as short and long term behavioral adaptation to recover from past and avoid future tissue injury require the involvement of the brain. Therein the representation of and behavioral responses to pain have been attributed to the interaction of a distributed network that includes sensory, motivational, and associative regions.

Nociceptive and relevant sensory information is relayed to the brain predominantly through two pathways. First, spinothalamic nociceptive afferent from the dorsal horn project to both somatosensory areas (Shi & Apkarian, 1995), which are thought to encode spatial and dynamical properties of pain (Chudler, Anton, Dubner, & Kenshalo, 1990; Kenshalo, Iwata, Sholas, & Thomas, 2000; Mancini, Haggard, Iannetti,

Longo, & Sereno, 2012), as well as regions associated with error detection and conflict monitoring, especially the insular and anterior cingulate cortices which are likely involved in the anticipation, motivated response to and learning from pain (Iwata et al., 2005; Rainville, Duncan, Price, Carrier, & Bushnell, 1997). Several of these regions have been implicated in "top down" modulation of pain such as the anterior cingulate cortex in placebo analgesia (Petrovic, Kalso, Petersson, & Ingvar, 2002) and the dorsolateral prefrontal cortex (Lorenz, Minoshima, & Casey, 2003), and may act by recruiting brainstem nuclei to affect inhibition in the spinal dorsal horn (Eippert, Finsterbusch, Bingel, & Buchel, 2009). An additional pathway that is less prominent but perhaps more important to pain regulation arises via the parabrachial nucleus and directly projects to the PAG, amygdala, mesocorticolimbic brain regions (Gauriau & Bernard, 2002), and the ventral tegmental area (VTA)(Coizet, Dommett, Klop, Redgrave, & Overton, 2010). Interaction between mesocorticolimbic regions, specifically ventral frontal cortical structures, the basal ganglia, amygdala and hippocampus, have been implicated in the development of chronic pain and encoding pain affect (Baliki et al., 2012; Mutso et al., 2014). Interest in the role of this brain circuitry in pain has only recently gained momentum, and the understanding of its role in pain modulation remains primitive, but existing evidence suggests it provides a major cognitive-behavioral supplement to the brainstem and spinal mechanisms of pain modulation. For instance, blood oxygen level dependent (BOLD) signal synchrony in the ventral striatum and prefrontal cortex has been related to deliberate cognitive modulation of pain sensitivity

(Woo, Roy, Buhle, & Wager, 2015), and predicts future pain perception in healthy patients prior to a noxious experimental stimulus (Riedl et al., 2011).

The purpose of this exposition was to establish that pain is not a simply continuous representation of sensory input, that it is rather the product of a complex interaction of sensory modalities which converge and diverge at different levels of the nervous system. Many of these levels contribute to pain modulation and analgesia under differing circumstances. Signal detection and amplification at the level of the periphery allows for transmission of signals warning of potential tissue injury to the spinal dorsal horn. The spinal dorsal horn contains local circuits which help select relevant nociceptive stimuli for transmission to the brain, and can be recruited by descending projections from brainstem nuclei to provide context dependent regulation of nociceptive signal propagation. Finally, the brain serves a role in integrating multimodal sensory input with past experiences and predictions of future environmental demands to determine when and to what extent pain should invade conscious perception and initiate defensive or recuperative behavior. The contrast enhancement mechanism following slight offsets in noxious stimulation might be regulated at any one or any combination of these levels of the nervous system. Recent attempts to identify a regulatory site have largely abrogated the problem by comparing and contrasting known mechanisms of spatiotemporal filtering and pain inhibition with neurophysiological responses to offset analgesia.

Offset analgesia and mechanisms of pain modulation

Spatial and temporal filtering is a fundamental property of nervous tissue. For instance, whether a neuron preferentially responds to slow or fast components of a stimulus can be determined by variations in synaptic response and fiber propagation dynamics (e.g. leaky vs. myelinated fibers). Differing temporal filtering strategies can readily be observed in the response dynamics of Aδ and C fibers, the former encoding fast stimulus changes and the latter slower components. These characteristics would be expected in any nervous tissue of similar biophysical constitution (e.g. myelinated integrate and fire vs. leaky integrate and fire neurons), and indeed such general principles or their central analogues following sequential convolution could be hypothesized to underlie offset analgesia. Filtering can simultaneously be achieved in a looser sense with adaptation of primary afferents over time. Sensitization occurs after injury by increasing response of nociceptors to suprathreshold stimuli (Andrew & Greenspan, 1999), but fatigue can also occur at the level of the primary afferent. A simple fatigue response following neurotransmitter depletion or ion channel adaptation might be sufficient to suppress firing in first-order afferents after an intense conditioning stimulus, and nociceptor fatigue has been documented in primary afferents (Peng, Ringkamp, Meyer, & Campbell, 2003). At the same time, filtering can occur at the level of ensembles of neurons, with examples offered in the spatial domain by on-center off-surround dynamics in secondary sensory afferents such as retinal ganglion cells (the classic example) or wide dynamic range neurons of the spinal cord in nociception (Salter & Henry, 1990), which

emerge from the convergence of primary afferents and inhibitory interneurons on these secondary afferents.

Of particular interest in the study of offset analgesia have been central and nociception specific modes of spatial or temporal filtering. For instance, in “wind-up” repeated stimulation of C-fibers at a frequency greater than 0.3Hz progressively increases the response of central neurons through activation of postsynaptic substance-P sensitive G-protein coupled receptors and n-methyl-d-aspartate (NMDA) receptors (De Koninck & Henry, 1991; Dickenson & Sullivan, 1987; Miller & Woolf, 1996). This form of central sensitization corresponds perceptually to the temporal summation of noxious stimuli into progressively more painful sensations. However, while NMDA receptors are necessary for pain amplification in wind-up, ketamine blockade of NMDA receptors was not found to affect offset analgesia (Niesters, Dahan, et al., 2011). Moreover, the magnitude of offset analgesia was not attenuated by capsaicin induced mechanical allodynia or heat induced thermal hyperalgesia (Martucci, Yelle, et al., 2012), further undermining the possibility of an interaction between offset analgesia and the mechanisms involved in central sensitization. The independence of offset analgesia from known spinal mechanisms of nociceptive spatiotemporal filtering and associated spinal changes in plasticity encourages widening the search for underlying mechanisms to include supraspinal pain modulatory systems.

The SRD and PAG/RVM brainstem-spinal circuits are among the best understood examples of supraspinal inhibitory control over noxious afferent input. Of these, the SRD dependent DNIC provides contrast enhancement in the spatial dimension via an analgesic

effect, and could offer some clues regarding supraspinal mechanisms underlying the similar contrast enhancement in the temporal dimension seen in offset analgesia. Additionally, chronic pain patients have shown abnormalities in both DNIC (King et al., 2009; Lautenbacher & Rollman, 1997) and diminished or absent offset analgesia (Niesters, Hoitsma, Sarton, Aarts, & Dahan, 2011), suggesting a potential mechanistic relationship. However, the same study which showed resilience of offset analgesia to ketamine blockade of NMDA receptors nevertheless did show elimination of DNIC (Niesters, Dahan, et al., 2011), and promotes the view that these act by dissociable mechanisms. This view is supported by a more recent functional magnetic resonance imaging (fMRI) study showing distinct brain BOLD signal patterns during DNIC and offset analgesia (Nahman-Averbuch et al., 2014). On the other hand, two other fMRI studies have implicated the PAG/RVM in offset analgesia (Derbyshire & Osborn, 2009; Yelle, Oshiro, Kraft, & Coghill, 2009). However, the PAG is difficult to resolve using current neuroimaging techniques (Linnman, Moulton, Barmettler, Becerra, & Borsook, 2012), casting doubt on the reliability of these findings, and while opiates are classically associated with PAG dependent descending inhibitory control, offset analgesia was shown to be resilient to naloxone inhibition of opiate receptors (Martucci, Eisenach, et al., 2012), further substantiating this doubt. The role of supraspinal mechanisms thus remains obscure, and requires further investigation.

In spite of these failures to identify central mechanisms underlying offset analgesia, there is some minimal evidence which invokes a role for the central nervous system. Induction of offset analgesia proximal but not distal to a separate coincident

noxious stimulus was shown to decrease pain ratings reported for the distal stimulus (Yelle et al., 2008). Because separate pain fiber bundles encode noxious stimuli from spatially segregated regions the involvement of the central nervous system is necessary to explain the finding of this asymmetric interaction. However, this study cannot establish the sufficiency of central mechanisms in offset analgesia, which is what we aim to do here using psychophysical experiments.

Pain psychophysics

The study of pain, an inherently subjective and multidimensional experience, poses a measurement problem which has confounded both medical, sociological and scientific treatments of the phenomenon (Reardon, 2015). The physician, judge and scientist must evaluate how much pain a person feels in response to an injury, but ultimately have no higher standard to appeal to than the individual's own report, which may or may not be reliable. This raises the question of whether or not an objective measure of pain is attainable. While behavioral responses can be used to infer the efficacy of experimental manipulations to affect pain perception (e.g. tail flick reflex in a mouse), only pain ratings from an awake and alert human being can suffice to provide the kind of temporal information needed to track offset analgesia. Thus the problem of measuring subjective experience becomes unavoidable. For the purposes of experimental scientific treatment in health the problem lies not in the possibility that a subject might deliberately deceive the investigator but rather in the possibility that each study participant has privileged access to their own experience and interpretation of that

experience. If the experimenter can appropriately guide the subject's interpretation and a report of experience can be provided in a way that is consistent across subjects, a trans-subjective measurement of experience becomes available for study. Such trans-subjective measurements have in fact been achieved and lead to the apparent laws underlying perception which are testable and reproducible, and thus amenable to scientific treatment.

The measurement of subjective magnitude is not a problem unique to pain, and the field of psychophysics has engaged it head on across sensory modalities and stimulus conditions. The seminal work of S.S. Stevens has shown that human magnitude perception, whether it be the volume of a tone as a function of power per area, the apparent size of a circle as a function of radius, or utility as a function of currency (e.g. dollars), obeys the law of invariance of ratios (S. S. Stevens & Stevens, 1986). Conceptually this means the proportion of two stimuli appears the same regardless of their absolute intensities. For instance, the contrast between two shades of gray appears the same whether under bright or dim lighting, even though the intensity of the shades (measured in candela for instance) may vary considerably as a function of ambient lighting. This invariance of ratios is trans-subjective, accessible to and agreeable across observers, in other words subjective magnitude is reflective of an underlying objective consensus. Mathematically the invariance of ratios entails that magnitude perception can be expressed as an exponential function where the exponent is a modality specific constant that characterizes sensitivity of perception to changes in stimulus intensity.

When asked to rate intensity of a stimulus there is intersubject variability, but this variability can be treated as measurement error. Under similar experimental designs, this

error will cancel out and yield a consistent exponential function, or in other words a constant ratio change in stimulus will yield a constant ratio change in perceived intensity, thus making measurement of perception possible. Critically, this law of the invariance of ratios imposes no constraints on the units of measurement, and thus in fact entails the possibility for robust cross modality matching. Whether the subject is rating pain intensity on a numerical scale, or matching it to lengths of a line on a piece of paper, the power law will emerge.

Measurement of pain specifically suffers from unique problems. Physicians ask able and cooperative patients to rate their pain on a scale from 0 to 10 to monitor fluctuations from visit to visit and make clinical decisions. The underlying objective consensus in magnitude perception makes this possible and convenient. Ratings are internally consistent within a patient, and that consistency is common across patients. However, it turns out not all pain measurement methods are equal and the most clinically convenient is not the best. The existence of an underlying power law, and possibility of cross modality matching, does not free psychophysical measurements from the possibility of introducing an interpretive bias due to poor task design. For instance, in the case of the doctor's pain scale the patient is forced to fit their pain into categories which are implicitly equally spaced, potentially distorting the stimulus-response curve. Studies have shown the least bias to be achieved by continuous visual analog scales anchored with verbal extremes (e.g. "no pain" and "worst pain imaginable") (Price, McGrath, Rafii, & Buckingham, 1983; J. C. Stevens & Marks, 1980). Moreover, pain is characterized by unique temporal characteristics. Self report specifically suffers from memory recall

problems, and its precision becomes unreliable after a delay of only a few seconds (Rainville, Doucet, Fortin, & Duncan, 2004), but at the same time the dynamics of pain are slow and can be tracked in real-time (Cecchi et al., 2012). Real-time rating along a visual analog scale (VAS) thus presents itself as an effective method for obtaining rich information for study of the temporal dynamics of pain magnitude perception across subjects.

Psychophysics can stand in isolation as a discipline within psychology, characterizing the nature of the relationship between stimuli and perception, without concerning itself with the neurobiological mechanisms underlying that relationship, but for the philosopher, neuroscientist or physician psychophysics is at its best when it allows for insights to be gleaned regarding the relationship between perception and neurobiology. Towards this end the tools of psychophysics have seen extensive use in the study of the mechanisms of pain perception. One such use has already been presented, namely the psychophysical phenomenon of temporal summation reflects the underlying wind-up of secondary afferents in the spinal cord. The engagement of myelinated and unmyelinated primary afferents in pain dynamics have also been elucidated using human psychophysical studies by observing pain rating dynamics in response to distal stimulation. Thus psychophysics has proven itself a useful tool in understanding neurobiology, and here it will continue to serve this purpose.

Aims

The aim of this investigation is to use psychophysical experiments to determine whether the neurophysiology of the central nervous system is sufficient to produce analgesia following stimulus offset. Classically, offset analgesia is studied with a single graded thermal stimulus (figure 1, left), which affects a single receptive field. All the physical features of this stimulus affect the same neurons and might be filtered at any point along the signal propagation pathway from periphery to the brain. Here we will continue to investigate offset analgesia using thermal stimulation because graded heat stimuli can be easily controlled and reproduced, but stimulus features will be separated into two distinct stimuli, each delivered using independent thermodes at different locations. One thermode will deliver a constant testing stimulus, while a second thermode will deliver a brief conditioning stimulus of greater intensity which occurs entirely between onset and offset of the testing stimulus, with subjects continuously rating their overall pain. These stimuli are designed such that their composite resembles the time course of a classic offset analgesia experimental stimulus. The presence of an analgesic effect in the testing stimulus following offset of the conditioning stimulus will demonstrate the sufficiency of the central nervous system in effecting offset analgesia, and conversely its absence would demonstrate the necessity of the periphery.

By transmitting nociceptive features along different primary afferent fibers we ensure the primary afferent mediating the signal of the testing stimulus is not directly affected by offset of the conditioning stimulus feature, in contrast to the classic single thermode stimulus. Although processes of primary afferents project from spinal dorsal

root ganglia as a multitude of well defined fiber bundles, and afferents innervating distinct sensory fields are often in close proximity to one another, they do not coordinate their activity in the periphery. Interaction between distinct afferent fibers is unusual in the peripheral nervous system. Electrical coupling has been observed among distinct C-fibers, occurring in the vicinity of the sensory receptor (Meyer, Raja, & Campbell, 1985), but has not been observed across distinct receptive fields. Any analgesic effect demonstrated using sufficiently segregated stimulus features would evidence a central mechanism capable of producing offset analgesia.

Although the dual thermode paradigm can establish the sufficiency of supraspinal or intraspinal neurophysiology for effecting offset analgesia, it cannot under all circumstances rule out interaction between primary afferent synapses which reside within the spinal dorsal horn itself. Afferents innervating common spinal segments might conceivably have neighboring synapses capable of impacting one another's pre or postsynaptic signaling. In fact, the dual thermode paradigm doesn't intrinsically provide any information on the level at which the analgesic mechanism may act within the central nervous system. Different spatial configurations will therefore be used to supplement the use of dual thermodes and advance this point. In one configuration thermodes will be adjacent to one another on the forearm, in another they will be further apart, but still on the same limb, while in a final configuration they will be on opposite sides of the body. The use of ipsilateral stimuli delivered proximally or distally from one another together with contralateral stimuli will provide insight on whether the underlying mechanism is intrasegmental, extrasegmental or supraspinal.

METHODS

Participants

Data was collected from 10 right handed individuals (age: 26±3 years, mean ± SD; 6 males) recruited from the Northwestern University community. Basic demographic information such as age, gender and racial identification were collected from all subjects. All experiments were approved by the Northwestern University institutional review board.

Equipment

Stimuli were delivered by a Medoc PATHWAY ATS: a computer controlled thermoelectric hotplate (30x30mm) with temperature control supplemented by a liquid cooling system. This apparatus was designed for sensory testing and had built-in controls to constrain stimulus intensity and prevent tissue damage. Additionally, subjects at all times had access to – and were instructed in the use of – an emergency stop button which would discontinue stimulus delivery when pressed. Temperature and event related data was sampled at 5ms (200Hz).

Real-time pain ratings were obtained using a hinged potentiometer-based device mounted to the right index finger and thumb. Voltage ratings were converted to a 0-100 scale, such that following subject-specific calibration a closed finger position would correspond to a 0, while a maximal finger span would correspond to 100. These ratings were rendered as a yellow bar alongside a numerical scale spanning 0-100 on a screen which was presented to participants during thermal stimulation trials. As subjects opened

and closed their right hand index finger and thumb the bar would grow or shrink, and they were instructed to rate “no pain” as 0 (closed fingers) and “the worst pain imaginable” as a 100 (maximally open fingers) along this visual analog scale. Thus patients could rate their pain using both numerical and visual cross modality matching. Finger ratings were sampled every 70ms (14.3 Hz) for data collection purposes and reproduced for participant visualization in real-time at the same refresh rate.

Thermodes and pain ratings were all controlled by different computers, each with subtle fluctuations in their internal clocks, which were found to be insufficiently precise to coordinate stimulus delivery or align pain ratings with stimulus delivery in post processing. Consequently, a reference signal was delivered every 2.5s to each participating device during any given experimental trial. These reference signals could be used to trigger stimulus delivery at precise times and in data preprocessing to align stimuli and rating data in a common temporal reference frame.

Task

Two thermodes were used for sensory testing such that it would be possible to apply a test stimulus by one and deliver a separate conditioning stimulus with the other. Three thermode spatial configurations were investigated. Thermodes were placed on the ventral forearms of subjects, but in the first configuration the two thermodes were placed proximally to one another on the left arm but with one more distal from the torso than the other (proximal paradigm), in the second configuration thermodes were placed distally from one another but still on the same arm (distal paradigm), while in the third

configuration one thermode was placed on the left forearm while the other was placed on the right forearm (Figure 2). The stimulation sequence was the same regardless of thermode configuration and the order in which subjects experienced these configurations was selected randomly. Subjects were instructed to rate their overall pain. Although at times they received noxious stimulation from two different sources simultaneously they were not instructed on how to reconcile these into a single pain rating, but were instructed to rely upon their own best judgment to accomplish the task.

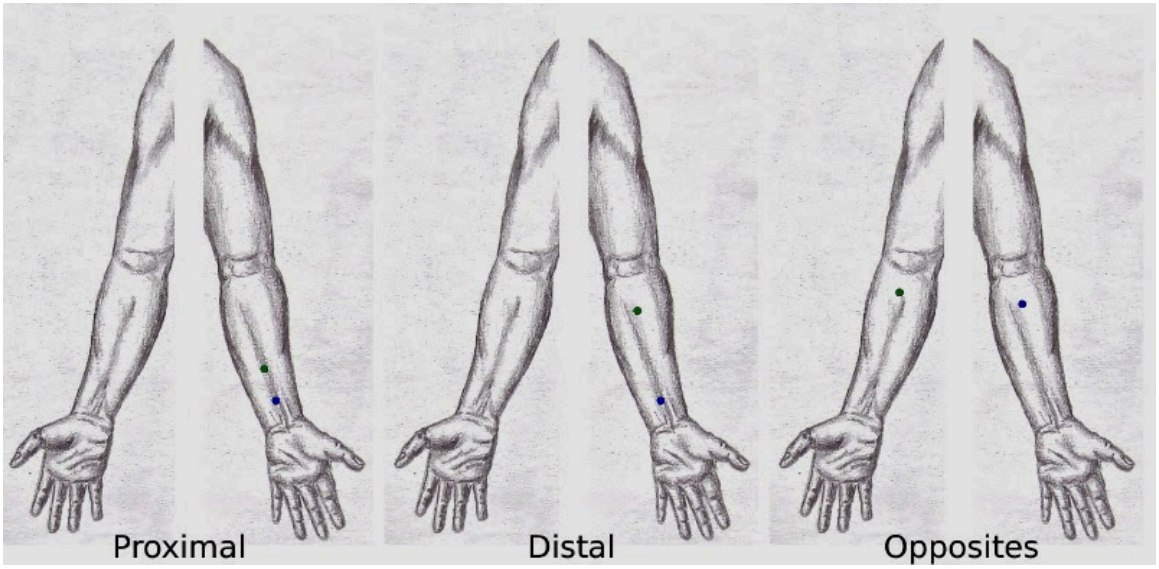

Figure 2 - Three stimulus configurations used for the experimental paradigm are shown. Dots indicate locations of thermodes with different colors indicating different thermodes. Thermodes were always placed over muscle tissue not on the exposed tendons of the wrist. Paradigm names are shown and reflect the orientation of the thermodes with respect to one another.

The stimulus sequence delivered by these thermodes consisted of six stimulation epochs, some involving both thermodes, others involving one alone. The ratings of the first stimulus epoch were discarded because past experience has shown pain ratings for the first stimulation are not robust or reliable within or across subjects, possibly due to

surprise and anticipation effects. The remaining five stimulus epochs are enumerated from 1 to 5, with the discarded initial stimulus designated as epoch 0 when necessary. Absolute stimuli intensities were uniformly increased or decreased by a fixed amount such that stimuli ratings would be of comparable magnitude across subjects after accounting for subject specific differences in thermal pain sensitivity. Three stimuli intensities were used, a baseline temperature, a test noxious stimulus intensity (targeted to evoke a 40/100 VAS rating), and a conditioning noxious stimulus intensity 2C above the test stimulus (Figure 3).

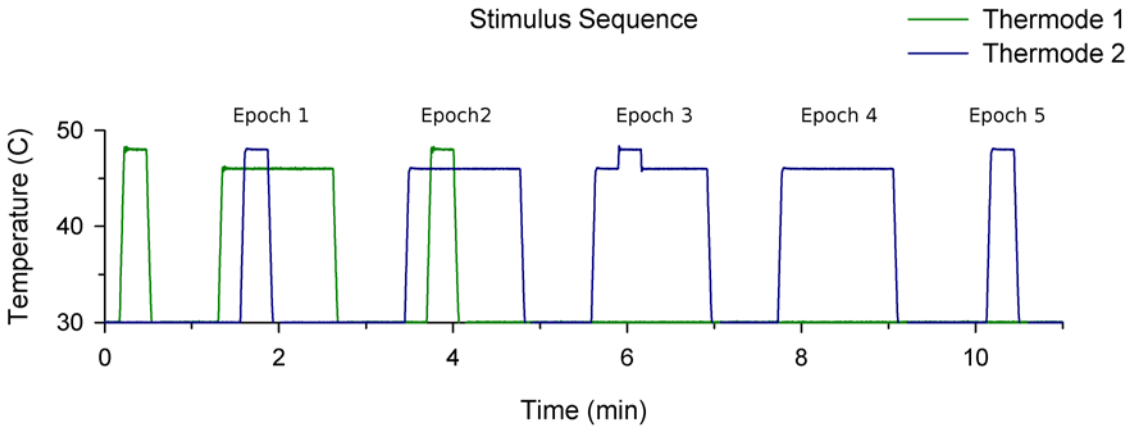

Figure 3 – A stimulation trial involved 6 stimulation epochs. Data from the first was deliberately discarded. Epochs 1 and 2 involved composite stimuli delivered by two thermodes. All stimulation epochs are separated by at least 45s, and each epoch takes 75s to complete (plus an additional 6s needed to achieve thermode temperature changes), although during epoch 5 stimulation only occurs for a portion of this period. The timeseries above were shifted up or down by up to 2C based assessments made during training of individual subject pain sensitivity. Here, a stimulus sequence actually used with a study participant is shown as a representative illustration.

All stimulus epochs had 3 distinct stimulation periods: T1, T2 and T3. All conditioning stimuli were delivered during T2. Epochs 1 and 2 involved the coincident delivery of simultaneous stimuli, one from each thermode. One thermode would deliver a

75s test stimulus before returning to baseline. 15s after the onset of the test stimulus, the second thermode would deliver a more intense conditioning stimulus for 15s before returning to baseline. Cumulatively the two thermodes produce a composite stimulus (e.g. 46-48-46C at 15-15-45s, corresponding to T1, T2 and T3) similar in profile to a classic offset analgesia stimulus, only with distinct stimulus features delivered at distinct sites. For epoch 2 the two thermodes swapped roles. Stimulus epochs 3-5 involved stimulation with a single thermode. Epoch 3 was a classic offset analgesia stimulus sequence (e.g. 46-48-46C at 15-15-45s intervals). Epoch 4 and 5 involved the delivery of individual stimulus features in isolation. Epoch 4 was simply a 75s testing stimulus (e.g. 46C), hereon the "control" stimulus. Although this epoch lacks stimulus features to delineate T1, T2 and T3 periods, they can be defined by time since stimulus onset to coincide with their counterparts in epochs 1-3 and thus provide a reference unconditioned test stimulus for statistical comparisons (Figure 1). Epoch 5 involved delivery of the conditioning stimulus in isolation. All stimulus epochs, including epochs 0 and 1, were separated by at least 45s periods at baseline temperatures (e.g. 30C). Temperature changes were achieved at 6C/s. Stimulus epochs were time-locked to temporal reference signals to ensure the duration between onset of the test and conditioning stimuli would be 15s and to facilitate across-subject alignment of data in data preprocessing. This method is imperfect and occasional failures resulted in data loss, meaning data could not be collected with all 3 thermode configurations from all subjects, but it nevertheless dramatically improved the quality of the data which could be collected. The loss of data quantity in exchange for better quality was deemed to be a favorable exchange.

Before participating in experimental trials all subjects were trained with a stimulation sequence which involved rating 3 pairs of stimuli delivered in random order. Stimulation began with pairs of 44, 46 and 48C stimuli each delivered for 10s and in random order. Based on subject ratings subsequent training sequences had stimuli adjusted up or down until a range was found where maximal stimulus rating was below 100, and the second strongest stimulus was rated between 30 and 50. Subjects repeated this training procedure both until they were consistently rating stimulus pairs of matched intensity (within ±10 VAS units of accuracy), and until an appropriate range of stimuli intensities had been found. Based on performance during this training sequence test and conditioning stimuli were selected for the experimental paradigm. Most subjects were able to achieve these goals within 2 training sequences.

Data analysis

Preprocessing

Data was first preprocessed using custom routines implemented in Matlab. Data was resampled with linear interpolation to 14.3Hz using reference signals to determine appropriate timestamps. Stimulus epochs were analyzed in isolation by aligning data according to the synchronization signal which triggered each epoch. Stimulus-response lag, perhaps attributable to finite tissue energy absorption rates and conduction velocities or time-cost of cognitive evaluative strategies, was assessed using responses to stimulation epochs 4 and 5. These would be expected to show simple linear relationships with stimulus intensity. A cross correlation analysis identified at what time-offset pain

ratings and temperature best correlated with one another, and thus identified each subject's delay in responding to stimulus features. Across subjects, individuals responded to a given stimulus with an approximately 3.36 ± 1.6s lag time (mean ± SD). Finally, onset and offset times for specific features of interest were projected by discounting the temperature rise and fall rates which accounted for up to 6s of each stimulation epoch and incorporating the average stimulus-response lag.

Offset analgesia

Offset analgesia was defined as a response to a conditioning stimulus, and its magnitude is measured in terms of the pain elicited by a test stimulus after conditioning relative to the unconditioned test stimulus. With real-time continuous ratings pain can be defined instantaneously (e.g. maximal or minimal pain) or over some period of time (e.g. average pain). Because offset analgesia has been shown to abide by specific temporal dynamics, with a time to peak analgesia followed by a relaxation period during which the conditioning effect appears to wear off (Yelle et al., 2008), it is best captured by a measure which incorporates both dimensions of magnitude and duration. Mean pain will therefore be used, computed as area under a segment of the pain rating curve divided by the duration of that segment.

The control stimulus of epoch 4 will serve as the unconditioned test stimulus. Because pain perception is adaptive and dynamic even for constant stimuli (Figure 4), equivalent time points during different stimulus epochs must be compared rather than simply comparing preconditioning stimulation periods during the same stimulus epoch

(e.g. period T1 with T3). For evaluating offset analgesia a mean response to the control stimulus will be computed by averaging across all of a subject's epoch 4 ratings, which may be presented up to three times. Using this mean response curve as a reference pain level for evaluating offset analgesia will reduce the effect of rating error for the control stimulus. An exception will be made when investigating sequence effects. No within subject averaging will be performed in that case, and instead stimuli of interest will be compared only to control stimuli from the same stimulation trial.

Statistics

Statistical inferences can be drawn in two ways. First by performing within subject parametric comparisons of absolute pain ratings. Although different subjects may understand different magnitudes of pain ratings to mean different things, training and the law of the invariance of ratios ensures that their pain rating scales are at least internally consistent. Alternatively we can examine pain ratios directly. We can then formally define offset analgesia as $-(P_{us} - P_{cs})/P_{us}$ where P_{cs} and P_{us} are the mean pain ratings for the conditioned and unconditioned test stimuli (respectively) over some period of time. This is equivalent to a ratio of pain ratings minus a constant. Analgesia is designated as a negative value following the convention proposed by (Yarnitsky et al., 2014). Both methods are used.

Evaluating Spatial Filtering

Although this experiment is not designed to investigate spatial filtering or summation effects, it is important to look for any such spatial confound since spatial filtering might result in differing perceptions of conditioning stimuli and spatial summation offset might be mistaken for offset analgesia. The size of spatial effects was evaluated both in terms of magnitude and duration of pain during conditioning (T2) by taking the mean pain rating during this period. A 15s period beginning 3.36s after onset of T2 was used to define this period during epochs 1, 2, 3 and 5. The isolated conditioning stimulus delivered during epoch 5 which was used as a reference. Comparisons of ratios of pain during T2 were made to see if dual thermode paradigms differed from the classic single thermode offset analgesia paradigm.

RESULTS

Classic offset analgesia

Although offset analgesia has been investigated in the past, there are some relatively subtle departures from the classic stimulus sequence in our single-thermode variation of this experiment (epoch 3), and the method of quantifying analgesia is distinct in minor ways to accommodate the stimulus features of the experiment. Here the conditioning stimulus feature is three times as long as in the classic design, and the conditioning feature is twice as large. Moreover, differing methods have been used to quantify analgesia (Niesters, Hoitsma, et al., 2011; Yelle et al., 2008). Reinvestigating the analgesic properties of the classic offset stimulus under these design parameters can therefore provide an informative context for evaluating responses to our dual-thermode stimulus epochs.

After accounting for temperature rise and fall rates analogous 45s periods can be identified across stimulus epochs 3 and 4 (T3). Epoch 4 shows adaptation effects over the course of stimulation with initial responses decaying over time, validating the use of an unconditioned stimulus as a control. A statistically significant difference in mean pain ratings exists across subjects during T3 periods of the two epochs (paired t-test, $t = -2.97$, $p = 0.0156$, Figure 4), consistent with prior studies. 35±17% reduction in pain (mean ± SE) is achieved on average across subjects. Surprisingly one subject showed hyperalgesia during epoch 3, and introduced a significant skew in the distribution of the data. The remaining subjects showed 52±5% reductions in pain during T3 of epoch 3 relative to epoch 4.

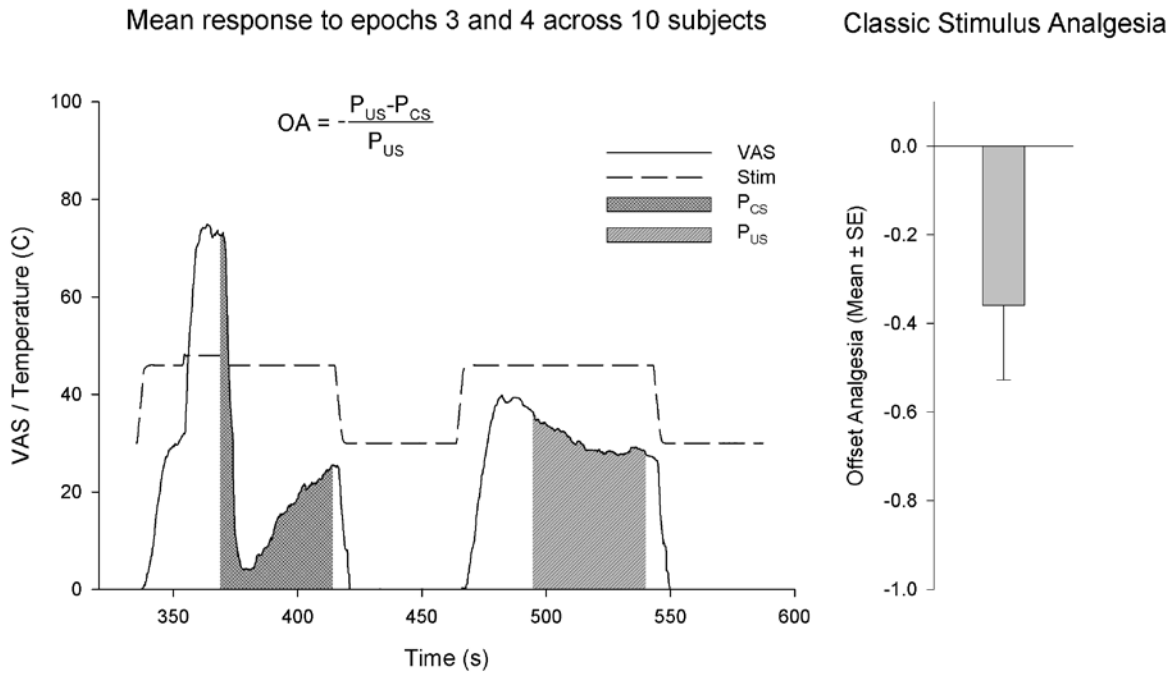

Figure 4 – Stimulus design produces robust offset analgesia, and pain ratings for the classic offset stimulus do not converge with those from an unconditioned stimulus until approximately 45s after offset. Shaded regions indicated areas used to compute magnitude of offset analgesia (OA). Time counted from start of stimulus run. Within subject comparisons show a statistically significant difference in mean pain between those two periods. (paired t-test: t = -2.97, p = 0.0156, N = 10). Mean magnitude of analgesia is illustrated on the right.

Sequence effects

Previous studies have shown a sequence effect for offset analgesia with initial conditioning stimulus features being more effective at inducing analgesia than the same features in subsequent stimulation epochs. Because the novel dual-thermode stimuli delivered in this study are delivered in several configurations, with one configuration following another, a sequence effect might be misinterpreted as a configuration effect and vice versa. Consequently it's important to identify and characterize any sequence effects. The classic offset analgesia stimuli are delivered concomitantly with each dual-thermode stimulus train, so they can be used to study such effects, and lessons learned from the

classic stimulus can then inform results from dual-thermode stimulus epochs. 6 subjects provided ratings for 3 classic stimuli, while 2 more provided ratings for 2, and finally two only rated the classic stimulus once and could not be used in this analysis. No significant sequence effect could be found (paired t-test: trial 1 vs. trial 2 $p > 0.8$, rm-ANOVA, main effect of trial order $p > 0.9$, Figure 5). Although this is inconsistent with previously reported experiments, the thermode delivering the classic stimulus was moved to a new stimulation site between successive ratings of the classic stimulus, and these findings do not rule out the possibility of local adaptation across successive stimulations.

Nevertheless, no local adaption effect can be identified either. The first stimulus run using dual thermode stimuli (whether following the proximal, distal or opposites configuration) can be used to address whether there is any local adaptive effect, and comparing epoch 1 and epoch 2 shows no difference between successive stimulations within the same trial (paired t-test, $p > 0.89$). Although the repositioning of thermodes between successive trials of differing configuration precludes mistaking local adaption for a thermode configuration effect, the absence of local adaption allows us to average responses between epochs 1 and 2. This improves signal-to-noise ratio when analyzing the dual thermode epochs, and simplifies statistical analysis.

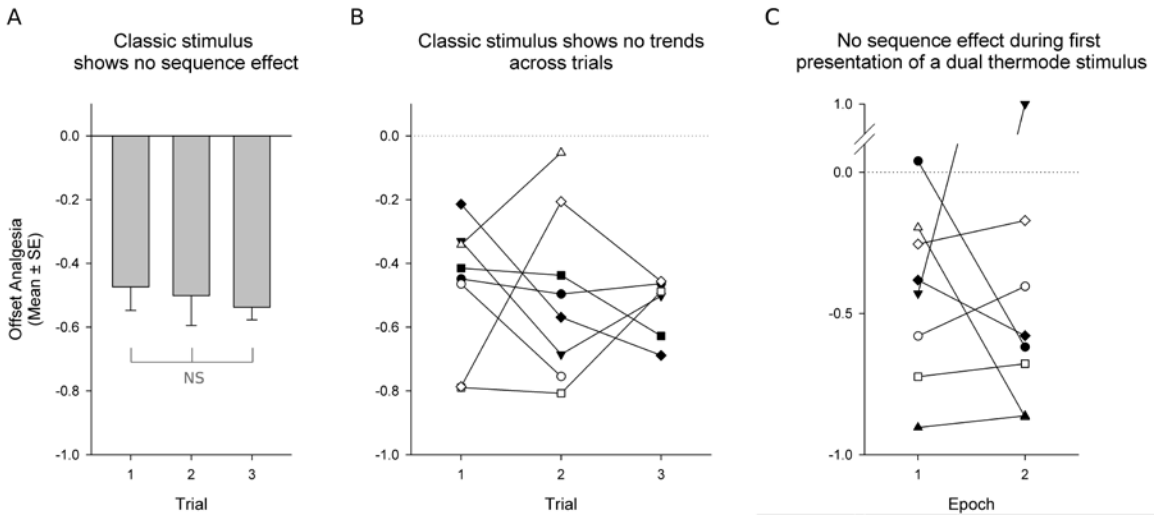

Figure 5 – (A) There is no sequence effect for the classic offset analgesia stimulus using the current design (rm-ANOVA $F_{2,10} = 0.07$, $p > 0.93$). Offset analgesia was measured as illustrated in figure 4. (B) Although considerable intersubject variability exists, there is no trend towards increased or decreased analgesia across successive stimuli. Thermodes were repositioned between repetitions. Each line represents a single subject's trajectory. Higher scores indicate more extensive analgesia. Two subjects did not receive multiple classic stimulations, two more received two instances of the stimulus, while six received three instances. (C) During the first presentation of the dual thermode stimulus, subjects did not show any trend towards more or less pain rated for the first stimulus epoch than for the second. Three subjects rated proximal stimuli first, four rated distal stimuli first and one rated opposite stimuli first. One subject (not shown) showed a massive hyperalgesic response for both stimulus epochs and was discarded as an outlier. Scores above 0 (dotted line) indicate greater pain than the control stimulus (epoch 4). NS, not significant.

Spatial Filtering

The use of dual thermodes introduces the potential for spatial filtering or summation effects in subject perception. Epoch 5 pain ratings can be used as a reference to establish if dual stimuli are rated differently than stimuli delivered in isolation. Spatial filtering might cause mean pain ratings during conditioning (T2) in epochs 1 and 2 to differ from mean pain ratings for the conditioning stimulus feature in the classic single thermode paradigm. Delivery of the conditioning stimulus feature in isolation during

epoch 5 provides a reference point against which to evaluate this potential confound. Examination of the ratio between T2 pain ratings across conditioning paradigms with respect to epoch 5 shows no spatial filtering effects during T2 (Figure 6). The conditioning stimulus is perceived similarly throughout.

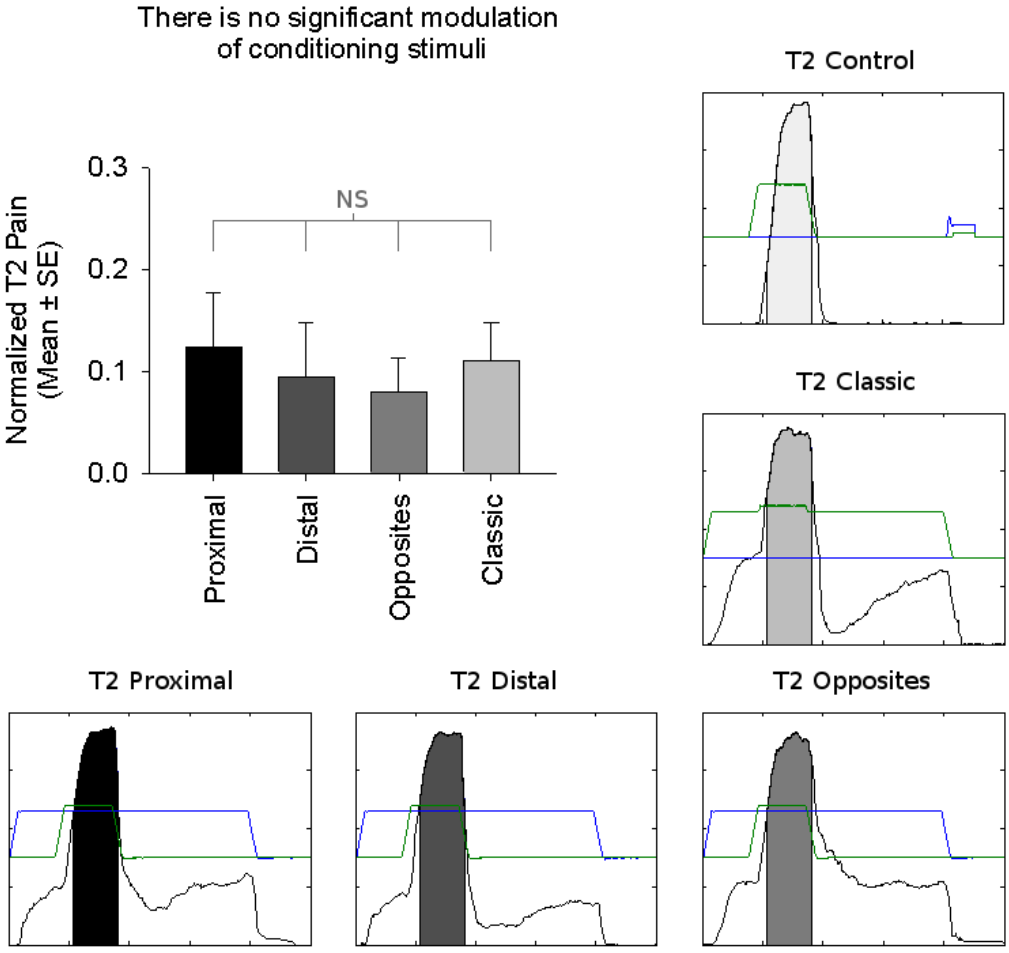

Figure 6 – There is no spatial effect. Conditioning stimuli and stimulus features are perceived similarly across thermode configurations. Stimulus response was calculated as mean pain ratings during T2 period. Conditioning T2 ratings were normalized within subject by taking the ratio of these ratings with respect to epoch 5 T2 ("T2 Control") which was delivered from baseline without any other concurrent experimental stimuli. There was no significant difference in normalized pain across the dual thermode or classic stimuli (rm-ANOVA $F_{3,18} = 0.33$, $p = 0.80$). NS, not significant.

Dual thermode offset analgesia

Period T3 across epochs 1-4 feature identical stimulation, but differ in manner of prior conditioning. In addition, epochs 1 and 2 are delivered with three different configurations, providing additional variety in manner of conditioning. Seven subjects rated all four different manners of conditioning (proximal, distal, opposites and classic) and the unconditioned stimulus, while additional subjects rated some subset of these. In the seven subjects with comprehensive data a significant analgesic effect was found with distal and proximal ipsilateral conditioning but not for contralateral conditioning on the opposite arm (rm-ANOVA, post-hoc Tukey test proximal $p = 0.009$, distal $p = 0.002$, opposites $p = 0.58$, in each case relative to control). The magnitude of analgesia was comparable in overall magnitude to what was found in the response to the classic stimulus (Figure 7). This effect was confirmed by performing additional post-hoc comparisons of proximal and distal thermode configurations using subjects who rated these stimuli and the reference control stimulus but may have been excluded from the prior analysis for failing to provide rating information for other conditions (Table 1).

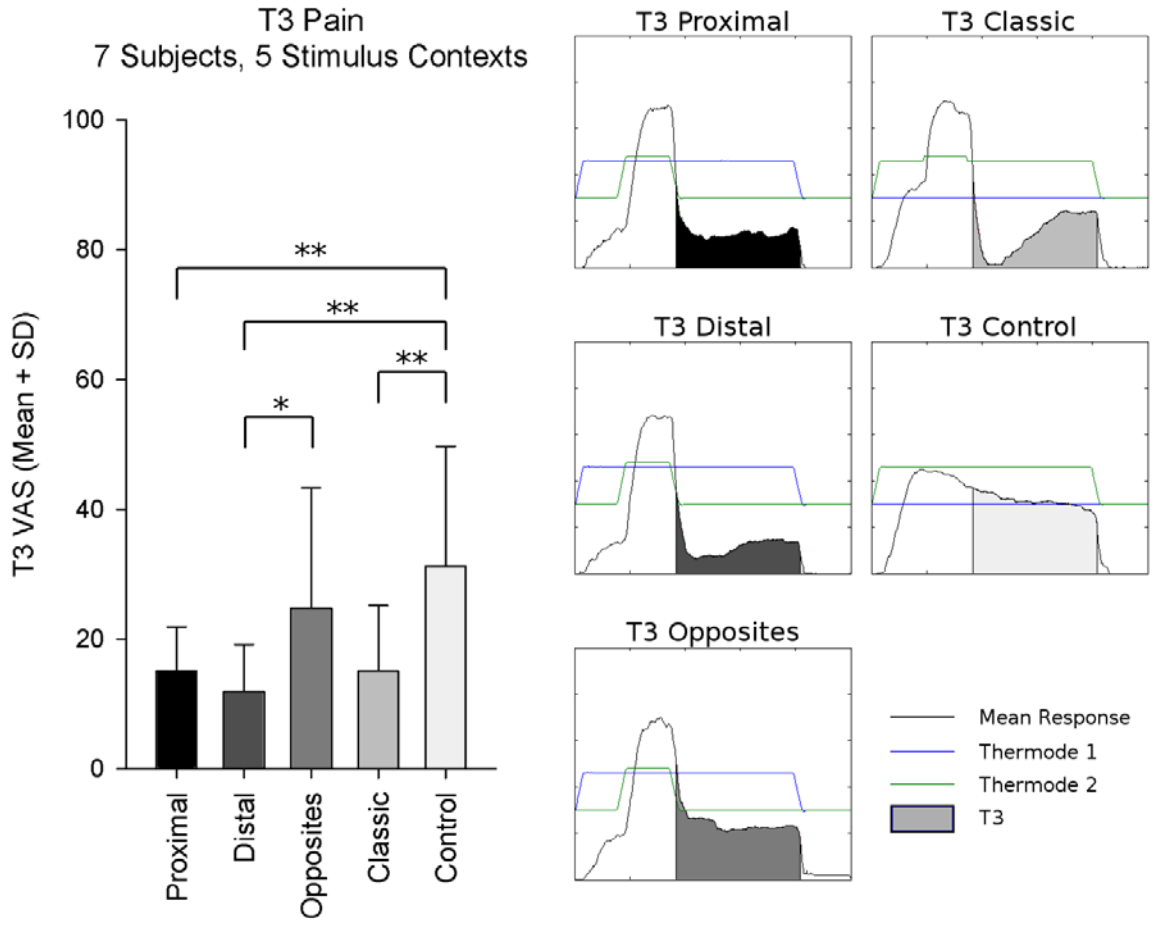

Figure 7 – Offset analgesia is evoked in a test stimulus by offset of a distinct conditioning stimulation of a proximal or distal ipsilateral but not contralateral site on the opposite arm. Overall magnitude of analgesia in the proximal or distal conditioning is comparable to the classic offset analgesia stimulus (rm-ANOVA main effect of conditioning $F_{4,24} = 6.93$, $p < 0.00074$, post-hoc Tukey-test proximal vs. control: $p = 0.009$, distal vs. control: $p = 0.002$, classic vs. control: $p = 0.009$, distal vs. opposites: $p = 0.048$). Mean stimulus-response curves are shown on the right for the control stimulus and for each conditioning configuration. The period during which offset analgesia was evaluated (T3) is shaded. Notably all test stimuli were identical during this period and differed only in the context (conditioning) of that stimulation. * $p < 0.05$, ** $p < 0.01$.

Table 1 Post-hoc paired t-tests confirm analgesia can be evoked by an ipsilateral conditioning stimulus or stimulus feature. This was significantly different from contralateral conditioning.

vs.	control	opposites
proximal	$p = 0.109$ ($n = 9$)	-
distal	$p = 0.005$ ($n = 8$)	$p = 0.039$ ($n = 8$)
classic	$p = 0.016$ ($n = 10$)	-

If similar mechanisms generate offset analgesia in the dual thermode and single thermode paradigm a correlation might be expected between the magnitude of analgesia elicited by each. To test this theory analgesic magnitude between distal, proximal and classic paradigms were compared. No correlations were found in analgesic magnitude across any pairwise comparison of these paradigms.

DISCUSSION

The use of psychophysical measurements and dissociated stimulus features demonstrates the central nervous system is sufficient to produce analgesia following offset of an acute thermal stimulus. When a noxious testing stimulus was conditioned with a second more noxious stimulus, the testing stimulus produced less pain than when the testing stimulus was experienced without conditioning. This effect was comparable in magnitude to analgesia following the T2 to T3 step down in the classic offset analgesia paradigm, and demonstrates antinociceptive adaptation in primary afferents is not necessary to produce the classic offset analgesia response. Moreover, this effect is unlikely to be due to pre or postsynaptic effects at primary afferent synapses in the superficial laminae of the spinal dorsal horn because an analgesic effect was present even with conditioning stimuli delivered at opposite ends of the forearm. Somatotopic mapping of primary afferents to the superficial laminae of the dorsal horn would segregate their synapses spatially and make interaction unlikely (Swett & Woolf, 1985; Yelle et al., 2008). This effect did not show a dependence on whether or not the conditioning stimulus was delivered proximally or distally from the training stimulus relative to the neuraxis, but did appear to show a dependence on lateralization, such that contralateral conditioning was significantly less effective than ipsilateral conditioning.

Direct comparison between the analgesic effects achieved in this study and those of previous studies cannot readily be made due to differences in the method of measuring offset analgesia and difference in stimulus durations. Most have measured analgesia by taking point estimates (e.g. local maxima and minima in VAS ratings). In some cases

these point estimates were compared between T2 and T3 (Grill & Coghill, 2002; Niesters, Dahan, et al., 2011; Niesters, Hoitsma, et al., 2011), while in others they were compared to ratings during unconditioned stimuli (Martucci, Yelle, et al., 2012; Yelle et al., 2008). In at least one case averages over various windows of time were taken rather than point estimates (Derbyshire & Osborn, 2009). Here we use a larger (2C rather than 1C) and longer (15s rather than 5s) stimulus offset during T2 than has previously been reported and examine mean pain during T3 relative to a control (unconditioned) stimulus of equal intensity.

Nevertheless, some cursory observations can be made in favor of the methods used here. Reports of offset analgesia magnitude in the existing literature are never more than 60% and are frequently much less, even with point estimates which would be expected to maximally capture the depth of analgesia. On the other hand, the magnitude of analgesia from our classic stimulus is so robust that within and between subject effects transcend the need for statistical inference and are in every instance discernible by visual inspection. Indeed, visual inspection of the profile of mean response to the classic stimulus train delivered here (Figure 4) suggests analgesia nearly abolishes pain at some timepoint during stimulus delivery with our adjusted stimulus train. Additionally, the control stimulus shows adaptation over time, namely a downward deflection in pain ratings as the stimulus progresses, potentially due to nociceptor fatigue. An adaption effect like fatigue would be expected to be present in both control and conditioned stimuli, emphasizing the importance of abiding by this across-stimulus comparison rather than simply comparing pain during T3 to T2 which might confuse offset analgesia with

independent adaptation effects. This is especially true when T2 has been increased in duration as it has here, providing more time for adaptation.

Although the remarkably complete and sustained analgesic effect seen with this modified stimulus sequence dwarfs receptor fatigue and intersubject variability, it is transient and becomes diluted by averaging over the full duration of T3. Our standard for offset analgesia is thus more conservative than what has been used in the past, but a more robust stimulus response makes up for this loss in power. Furthermore, measuring mean pain during a window rather than at a local minimum reduces the effect of subject rating error over time, and adopting the maximal window allowed by the stimulus eliminates the possibility of any experimenter bias in determining boundary conditions. Additionally, offset analgesia demonstrates intersubject variability, and this variability might be captured in the depth of analgesia, but when an effect is sufficiently potent to produce maximal (100%) analgesia in the majority of subjects then pain ratings become saturated and variability is lost. Most of the variability instead manifests in the rate of return of pain, with some subjects showing faster and others slower return. By taking mean pain ratings over the duration of the T3 epoch rather than a point estimate this variability can be simply albeit indirectly measured. Although the sources of variability remain unknown, using a measurement which reflects it results in more representative statistical analyses and allows for cross paradigm comparisons (e.g. responses to dual thermode vs. single thermode paradigms).

Because we take a conservative approach however, negative findings in general should be interpreted with care. For instance, the absence of a correlation between classic

and dual thermode stimuli, and the absence of spatial or sequence effects may simply reflect a lack of data given this simple measurement technique. Similarly, conditioning stimuli in the dual thermode paradigms were variously delivered proximally or distally with respect to the neuraxis (epochs 1 and 2 feature different stimulus orientations), and the lack of a sequence effect should not be interpreted as the absence of an orientation effect either. With more participants such effects might become discernible, especially in cases where they are confounded by variability in task demands. The task during dual thermode stimulation required evaluation and reconciliation of two stimuli as a single overall pain rating. Pain inherently demands attention, and the rating task becomes uniquely more complex in the dual thermode paradigms where different stimuli compete for this attention. A concomitant decrease in rating precision should be expected. Consistent with this interpretation the dip seen during T3 ratings of the classic single stimulation paradigm is more clearly delineated than in the ipsilateral dual thermode stimulus epochs. Similarly, the absence of analgesia during contralateral conditioning may not be definitive, and may simply reflect a lack of statistical power in the most difficult of this experiment's tasks. Until these phenomena are better understood, conclusions regarding the lateralization of offset analgesia must remain limited. At present the data shows ipsilateral and contralateral conditioning are significantly different in their analgesic effects, but whether contralateral conditioning simply produces no analgesia or produces significantly diminished analgesia is unclear.

On the other hand we are not the first to report absent spatial effects in a stimulation paradigm with clear potential for spatial confounds. In a different study the

progressive decrease but not progressive increase in surface area of stimulation was shown to produce a spatial summation effect. The authors speculated that differential engagement of spatial filtering (e.g. DNIC) might selectively neutralize the effects of spatial summation in the domain of increasing but not decreasing surface area (Marchand & Arsenault, 2002) since spatial filtering is time locked to stimulus onset (Le Bars et al., 1979). If a similar phenomenon were to affect the findings here then it would be possible for a spatial effect to exist during T3 even though there is no discernible effect during T2. With less surface area receiving stimulation, a lower pain rating might be evoked not due to offset analgesia but due to an inverted spatial summation effect. Our experiment was not designed to control for such an asymmetrical spatial effect *a priori*. However, the lack of an analgesic effect during the contralateral conditioning paradigm serendipitously offers just such a control for this possibility. The significant difference in T3 pain between "distal" and "opposite" stimulation paradigms shows that diffuse spatial effects do not underlie the analgesic response seen with ipsilateral conditioning. If there are spatial effects here their magnitude must be on the order of measurement error.

Although the peripheral nervous system is not necessary to produce offset analgesia, our findings do not go so far as to demonstrate the central nervous system is itself necessary. Mechanisms underlying other forms of nociceptive filtering have been observed at multiple levels of the neuraxis, for instance spatial filtering occurs both in the dorsal horn and in brainstem nuclei (Fields et al., 1991). Likewise, offset analgesia may be produced at or receive contributions from different levels of the neuraxis, including primary nociceptive afferents. If antinociceptive adaption exists in primary afferents it

might supplement central mechanisms. Furthermore, even if the peripheral nervous system were not sufficient to produce offset analgesia, it might still interact with central analgesic mechanisms in surprising ways. For instance, the absence of sequence effects across repetitions of the dual thermode paradigms is at odds with previous single thermode studies which showed the first stimulus train to be more effective at producing offset analgesia than subsequent stimuli (Yelle et al., 2008). Additionally, the response profile for the dual thermode stimuli differs from the single thermode stimulus. Such differences highlight the fact that even though offset analgesia is present in our dual thermode stimulation paradigms, the stimulus-response dynamics are not entirely identical to the classic offset analgesia paradigm. There is still room for the periphery to serve a role.

Offset analgesia has been interpreted in the past as a form of edge enhancement (Yelle et al., 2008) for individual stimulus features, but the dual thermode conditioning paradigm induces analgesic effects to manifest in responses to edge-free flat-line stimuli. Here, analgesia rather lends itself to interpretation as a higher order reward phenomenon. Opioid independent dopaminergic projects from the VTA are necessary for striatal reward signals following pain relief, and thus provide an important substrate for motivated learning in response to painful experiences (Navratilova et al., 2012), one which is also consistent with the opioid independence of offset analgesia. Offset analgesia might act to promote escape and avoidance behaviors via the same kind of reward learning as any other form of pain relief. The observation that offset analgesia is absent in chronic pain patients (Niesters, Hoitsma, et al., 2011) supports this argument,

since the striatum in chronic pain patients fails to show an appropriate physiological response to cessation of acute painful stimulation (Baliki et al., 2010). Although this does not imply the mechanism underlying offset analgesia is in the ventral striatum or VTA, it does strongly suggest offset analgesia is interrelated with this reward physiology.

The finding of offset analgesia in the dual thermode paradigm encourages a reevaluation of acute pain in chronicity. Baliki *et al.* hypothesized that acute pain cessation failed to produce an appropriately rewarding physiological response in the striatum because ongoing background pain prevented full realization of relief (Baliki et al., 2010). Here we show an active analgesic response in healthy subjects following offset of one noxious stimulus even in the presence of other ongoing noxious stimulation, strongly suggesting offset of the first acute stimulus is rewarding. If this same principle were followed in chronic pain cessation of acute stimuli would be expected to relieve ongoing pain in patients as well, but the observation that chronic pain patients show reduced or absent offset analgesia under the classic single thermode paradigm strongly suggests such an occurrence would not be observed. This situates offset analgesia well as a model through which to interrogate reward learning circuit abnormalities in chronic pain patients. The issue is especially pertinent considering the critical role served by reward learning circuits in the transition to chronic pain (Baliki et al., 2012) and in establishing the link between chronic pain and its comorbidities (Schwartz et al., 2014) and risk factors (Petre et al., 2015).

The purpose of this study is to definitively and unambiguously demonstrate the sufficiency of central mechanisms for offset analgesia. The burden of proof was to show

at least one spatial configuration of dual thermode stimuli which could produce offset analgesia. Towards that end our method captures the most important stimulus-response features in an unambiguous and simple manner, and even with a coarse quantification technique two of the three thermode configurations are shown to satisfy this burden. Although the central mechanism underlying offset analgesia cannot be specified at this time, these results serve as a guide for future research endeavors.

LIST OF JOURNAL ABBREVIATIONS

Annu Rev Neurosci ..... Annual Review of Neuroscience

Biol Res ..... Biological Research

Brain Res ..... Brain Research

Clin J Pain ..... Clinical Journal of Pain

Curr Biol ..... Current Biology

Eur J Neurosci ..... European Journal of Neuroscience

Eur J Pain ..... European Journal of Pain

Exp Physiol ..... Experimental Physiology

Exp Brain Res ..... Experimental Brain Research

Hum Brain Mapp ..... Human Brain Mapping

J Opt Soc Am A ..... Journal of the Optical Society of America A

J Clin Neurosci ..... Journal of Clinical Neuroscience

J Comp Neurol ..... Journal of Comparative Neurology

J Neurol Neurosurg Psychiatry ..... Journal of Neurology, Neurosurgery and Psychiatry

J Neurophysiol ..... Journal of Neurophysiology

J Opt Soc Am A ..... Journal of the Optical Society of America A

J Physiol ..... Journal of Physiology

Jpn J Physiol ..... Japanese Journal of Physiology

N Engl J Med ..... New England Journal of Medicine

Nat Neurosci ..... Nature Neuroscience

Neurosurg Focus ..... Neurosurgical Focus

Percept Psychophys ..... Perception and Psychophysics

PLoS Biol..... PLoS Biology

PLoS Comput Biol..... PLoS Computational Biology

Proc Natl Acad Sci USA..... Proceedings of the National Academy of Science

Prog Brain Res ..... Progress in Brain Research

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Conference Talks

Petre B, Baliki M, Apkarian AV (2011). Morphological and functional reorganization of the limbic system predicts transition from acute to chronic back pain in humans. *Midwest Pain Interest Group* (PIG) annual meeting, Chicago, IL, July 2011.

Posters

Petre B, Baliki M, Mansour A, Torbey S, Herrmann K, Schnitzer T, Apkarian AV (2013). Back pain intensity differentially engages nucleus accumbens core and shell in humans. *Society for Neuroscience*, San Diego, CA, November 2013.

Baria AT, Petre B, Baliki MN, Huang L, Apkarian AV (2013). Amygdala sub-nuclei differentially encode spontaneous pain and their functional connections are related to pain chronification. *Society for Neuroscience*, San Diego, CA, November 2013.

Mutso A, Petre B, Schnitzer TJ, Apkarian AV (2012). Increased hippocampal functional connectivity in sub-acute and chronic back pain. *Society for Neuroscience*, New Orleans, LA, October 2012.

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