Placebo analgesia: the role of expectation and aversive prediction error

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Emily J Hird

School of Biological Sciences

Contents

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List of abbreviations

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
BOLD	Blood-oxygen level-dependent
CI	Confidence interval
EEG	Electroencephalography
EEP	Electrical-evoked potential
ERP	Event-related potential
FMRI	Functional magnetic resonance imaging
FRN	Feedback-related negativity
GFP	Global Field Power
ICC	Intraclass correlation
LEP	Laser-evoked potential
LMS	Labelled magnitude Scale
ms	Millisecond
NPS	Numerical Pain Scale
OFC	Orbitofrontal cortex
PAG	Periaqueductal gray
PE	Prediction error
PFC	Prefrontal cortex
PRO	Predicted response-outcome model
RVM	Rostral ventromedial medulla
SPM	Statistical parametric mapping
SPN	Stimulus-preceding negativity
TDL	Temporal Difference Learning
TENS	Transcutaneous electrical nerve stimulation

Abstract

Pain perception is remarkably plastic. Expecting low pain decreases perceived pain intensity, as in placebo analgesia. This occurs even when expectations are violated by the actual pain stimulus intensity, when a prediction error is experienced. Prediction error, the difference between an expected outcome and the actual outcome, is signalled in the brain to update expectations to be more accurate in the future. Indeed, according to computational models of learning, neural signalling of prediction error underlies adaptive behaviour. However, in placebo analgesia, pain intensity expectations continue to influence pain perception despite the prediction error associated with high pain stimulus intensity. This thesis examined pain perception in the context of prediction error and expectation.

The structure of the thesis is as follows.

Chapter 1 provides a review of the key concepts present in the thesis. In Chapter 2, the methodology of the thesis is described. The measures used in this thesis were electroencephalography (EEG), chosen for its high temporal resolution, and behavioural pain reports, in response to painful stimulation.

Chapters 3 and 4 explore the EEG correlates of aversive prediction error. Chapter 3 reports an investigation of the best-known EEG correlate of prediction error in response to appetitive and aversive outcomes. In Chapter 4, the EEG correlate of prediction error to pain is investigated, and we show that cue-evoked expectation modulation of the more practical electrical pain stimulation is equal to that of the more commonly used laser pain stimulation. The remaining studies used electrical stimulation to induce pain.

Chapter 5 describes two behavioural studies which explored the effects of cue-evoked expectation and prediction error on pain perception. Here, we show that when prediction error is high, the influence of expectation on pain is reduced, indicating a limit on the influence of expectations on pain. We also test whether the variability of pain modulates the influence of cue-evoked expectation on pain, but do not show a significant modulation. Chapter 6 describes an EEG replication of the placebo analgesia manipulation using a visual cue. This relates the modulation of pain by placebo to that by cue to extend the findings of the thesis to placebo analgesia.

Overall, we report an investigation into the EEG correlates of aversive prediction error, and provide evidence that pain perception is the result of the integration of expectation into pain. Results indicate a role for prediction error in pain perception, and suggest the cued modulation of pain is generalizable across pain sensation types. Our results open new avenues for research into aversive learning using EEG, and have implications for future expectation-based therapies for pain.

Declaration

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The author

Emily Hird completed an undergraduate degree in Biological Sciences and Psychology at the University of Liverpool. She subsequently worked as a research assistant for 18 months at the University of Liverpool, The Walton Centre and the Pain Relief Foundation, before joining the University of Manchester to undertake a PhD. Her PhD research was funded by the Medical Research Council and the President's Doctoral Scholar Award. She is due to commence a study visit at the Karolinska Institute, Stockholm.

Rationale for submitting the thesis in an alternative format

This thesis produced three manuscripts, two of which are published (Chapters 3 and 4), and the third which is in preparation for publication (Chapter 5).

Publications and conferences

Based on Chapter 3:

Hird, E. J., El-Deredy, W., Jones, A., & Talmi, D. (2017). Temporal dissociation of salience and prediction error responses to appetitive and aversive taste. *Psychophysiology*, 1–13. Retrieved from http://doi.wiley.com/10.1111/psyp.12976

Hird E, El-Deredy W, Talmi D (2017). EEG correlates of taste prediction error. Verbal presentation at the *Princeton Neuroscience Institute, Princeton University, Daw lab*

Hird E, El-Deredy W, Talmi D (2016). ERP salience and value representation of primary reinforcers. Verbal presentation at the *PSYCH Associative Learning Symposium*

Hird E, El-Deredy W, Talmi D (2016). ERP salience and value representation of primary reinforcers. Poster presentation at the *Cognitive Neuroscience Society Annual Meeting*

Hird E, Talmi D, El-Deredy W (2015). Neural correlates of emotional response to taste: an EEG study. Poster presentation at the *British festival of neuroscience, British Neuroscience Association*

Hird E, Talmi D, El-Deredy W (2015). Neural correlates of emotional response to taste: an EEG study. Poster presentation at the *The International Society of Research on Emotion*

Based on Chapter 4:

Hird, E. J., Jones, A. K. P., Talmi, D., & El-Deredy, W. (2017). A comparison between the neural correlates of laser and electric pain stimulation and their modulation by expectation. *Journal of Neuroscience Methods*, *293*, 117–127. Retrieved from http://www.sciencedirect.com/science/article/pii/S0165027017303400

Hird E, El-Deredy W, Jones A, Talmi D (2017). Neural correlates of expectation in laser and electrical pain. Poster presentation at the *Cognitive Neuroscience Society Annual Meeting*

Chapter 1: General introduction

Abstract

This chapter reviews the neural expression of prediction error (section 1.1) and mechanisms of pain perception (section 1.2). The modulation of pain signalling by expectation is then discussed (section 1.3). Finally, theoretical models of prediction error, expectation and pain perception are reviewed (section 1.4). The chapter ends with an outline of the thesis aims and hypotheses (section 1.5) and a summary (section 1.6).

1.1. Prediction error (PE)

The world is a dynamic and variable place. As individuals navigating this environment, it is necessary to learn contingencies between events in order to predict the value of future outcomes and build a representative model of the world. One efficient way to maintain up-to-date predictions is to learn through error correction. Here a prediction is made about a future outcome. Upon receipt of the outcome, the error between the expected and the actual outcome is computed. Based on this error, future predictions are updated to more closely reflect the true environment. Prediction error (PE), the discrepancy between the expected value of an outcome and the actual value of an outcome, is central to computational models of learning (Rescorla and Wagner 1972; Sutton and Barto 1990). Updating expectations means that individuals can maintain accurate representations of the environment to maximise rewards such as food, and avoid aversive outcomes such as pain. The neural expression of reward PE has been well-characterised using direct electrophysiological recordings, functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) (Garrison, Erdeniz, & Done, 2013; Sambrook & Goslin, 2015a; Schultz, Dayan, & Montague, 1997). However, the response to aversive PE is not so well-understood, particularly in EEG.

1.1.2. Neural expression of PE

1.1.2.1. Direct measures of PE

The first evidence of the neural expression of PE arose from direct electrophysiological recordings of the phasic activity of monkey midbrain neurons. Before conditioning, these neurons respond positively to the delivery of a taste reward (figure 1.1a). After a conditioning procedure where a cue is repeatedly presented alongside the reward, these neurons respond positively to the presentation of the cue and cease to respond to the reward, because it is now fully predicted by the cue (figure 1.1b). This suggests that the cue gains predictive status, causing reward to be expected, and now the unexpected presentation of the cue itself elicits a PE signal. Neurons signal positive reward PE when the reward is unexpectedly delivered, and this signal is elicited by the taste before learning and the cue after learning. If the reward is unexpectedly omitted the neural firing rate is decreased at the time the reward was expected, which suggests that the neurons signal negative reward PE (figure 1.1c) (Montague, Dayan, and Sejnowski 1996; Schultz, Dayan, and Montague 1997). These results indicate that midbrain dopamine neurons signal reward PE. Reward PE has since been linked to the activity of midbrain dopamine neurons in electrophysiological, lesion, and pharmacological studies (for a review see Niv, 2009).

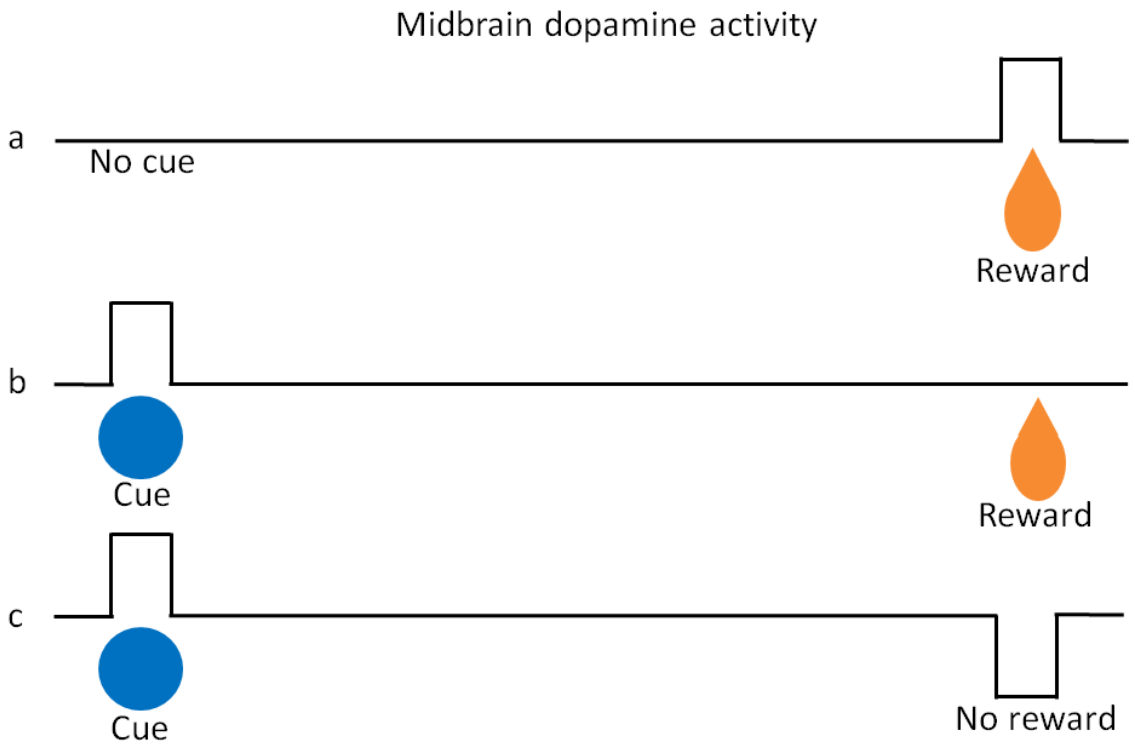


Figure 1.1. *Reward PE in the midbrain dopamine neurons of monkeys; midbrain neural activity is depicted by lines.* *(a) Unexpected delivery of a taste reward elicits phasic dopamine activity. (b) After a visual cue is conditioned to be associated with the taste reward, the cue activates expression of reward PE in phasic dopamine neural activity. Because reward was expected, delivery of the taste does not elicit dopamine activity. (c) If the conditioned visual cue is presented but reward is omitted, dopamine neurons decrease their firing below baseline, expressing negative reward PE at the time that the reward was expected. Adapted from Schultz et al (1997).*

1.1.2.2. Blood oxygen level dependent (BOLD) measures of PE

The results of direct recordings of dopamine neurons have been extended to humans using blood-oxygen level-dependent (BOLD) measures. These measures indicate a signal which correlates positively with reward PE in the ventral striatum, medial prefrontal cortex, amygdala, posterior cingulate cortex and orbitofrontal cortex (Garrison et al., 2013; O'Doherty, Dayan, Friston, Critchley, & Dolan, 2003; Rutledge, Dean, Caplin, & Glimcher, 2010). Reward PE to pleasant taste, which is the rewarding outcome used in Chapter 3 (referred to in the body of this thesis as 'Taste FRN'), correlates positively with activity in value-encoding areas such as the ventral striatum and the orbitofrontal cortex (OFC) (McClure, Berns, & Montague, 2003b; O'Doherty, Dayan, Friston, Critchley, & Dolan,

2003; Pagnoni, Zink, Montague, & Berns, 2002). Reward PE to money correlates positively with activity in value-encoding areas such as the nucleus accumbens, ventral striatum, midbrain, amygdala and cingulate (Abler et al. 2006; Rolls, McCabe, and Redoute 2008; Rutledge et al. 2010). Further, during anticipation of an outcome, the expected value of that outcome is represented. The expected value of appetitive outcomes such as taste and money is represented by areas similar to those expressing PE, such as the midbrain, amygdala, striatum and OFC (O'Doherty et al. 2002; Rolls, McCabe, and Redoute 2008). Overall these results indicate that the expected value of reward and reward PE are represented in the human as well as the animal brain.

There is also evidence that the brain signals aversive PE, the response to the unexpected delivery or omission of aversive outcomes. FMRI studies have captured a correlate of aversive PE to unexpected pain in the midbrain periaqueductal gray (PAG), striatum, anterior insula and amygdala, among other areas (Delgado, Li, Schiller, & Phelps, 2008b; Geuter, Boll, Eippert, & Büchel, 2017a; McHugh et al., 2014; Alexander Ploghaus et al., 2000; Roy et al., 2014b; Seymour et al., 2004; Zhang, Mano, Ganesh, Robbins, & Seymour, 2016). It has been argued that dopamine neurons signal aversive PE, but debate exists as to whether this reflects a salience response, where signal responds positively to the delivery of any important outcome, rather than to the delivery of a positively valued outcome (Bromberg-Martin, Matsumoto, & Hikosaka, 2010; Schultz, 2016). This thesis was particularly interested in aversive PE.

1.1.2.3. Electroencephalography measures of PE

BOLD lacks temporal resolution, with measurement accuracy to the order of seconds, making it difficult to distinguish temporally fine-grained signals which risks the overlapping of responses (Bandettini, 2015; Yael Niv & Montague, 2008). EEG has higher temporal resolution to the order of milliseconds (ms), which means that responses which occur close in time are better differentiated. EEG studies have identified an event-related potential (ERP) component termed the feedback-related negativity (FRN) as the most likely EEG correlate of PE. The literature has historically characterised the FRN as a utility PE signal denoting the value of an outcome from 'good' to 'bad' by measuring its response to reward PE, but the EEG correlate of aversive PE is not so well-understood (Holroyd & Coles, 2002; Sambrook & Goslin, 2015a; Walsh & Anderson, 2012). In Chapter 3 (Taste FRN) we aimed to quantify the FRN to reward and aversive PE.

The FRN peaks frontocentrally 240-340ms after a predictive cue. Source localisation and EEG-fMRI studies have localised the FRN to the anterior cingulate cortex (ACC) and the striatum where it is believed to originate from the phasic firing of midbrain dopamine

neurons (Hauser et al., 2014; Sambrook & Goslin, 2015b; Walsh & Anderson, 2011). The ACC receives signals from the limbic system and prefrontal cortex (PFC) and outputs to motor structures, so it is well-placed to receive PE signals and adjust behavioural outputs (Paus, 2001; Van Hoesen, Morecraft, & Vogt, 1993; Walsh & Anderson, 2012). According to this perspective, the FRN is overlaid on a visual-evoked oddball N200 ERP (Hauser et al., 2014; Heydari & Holroyd, 2016; Holroyd, 2004; Holroyd & Coles, 2002; Walsh & Anderson, 2012b). The FRN has been consistently shown to signal the unexpectedness of outcomes, becoming larger for more unexpected outcomes (Holroyd & Coles, 2002; Sambrook & Goslin, 2015a; Walsh & Anderson, 2011, 2012). A recent meta-analysis showed the FRN is modulated by the unexpectedness and also the magnitude of PE in response to monetary reward (Sambrook & Goslin, 2015a). For example, the difference between a cue signalling a gain of 50 pence and a loss of 50 pence would be larger than the difference between a cue signalling a gain of 25 pence and a loss of 25 pence. These attributes make it a likely EEG correlate for PE. The FRN is calculated by some groups as the difference between ERP amplitude elicited by a cue signalling unexpected delivery and unexpected omission of reward (see figure 1.2 for an example of this) (e.g. Heydari & Holroyd, 2016). To distinguish the FRN as a prediction error signal beyond a general alerting or valence response, other groups calculate it using an axiomatic model of PE, where the difference between delivery and omission of reward must be greater in response to unexpected outcomes than expected outcomes (e.g. Rutledge et al., 2010; Talmi, Atkinson, & El-Deredy, 2013). For completeness, in Chapter 3 (Taste FRN) we calculated the FRN using both of these techniques.

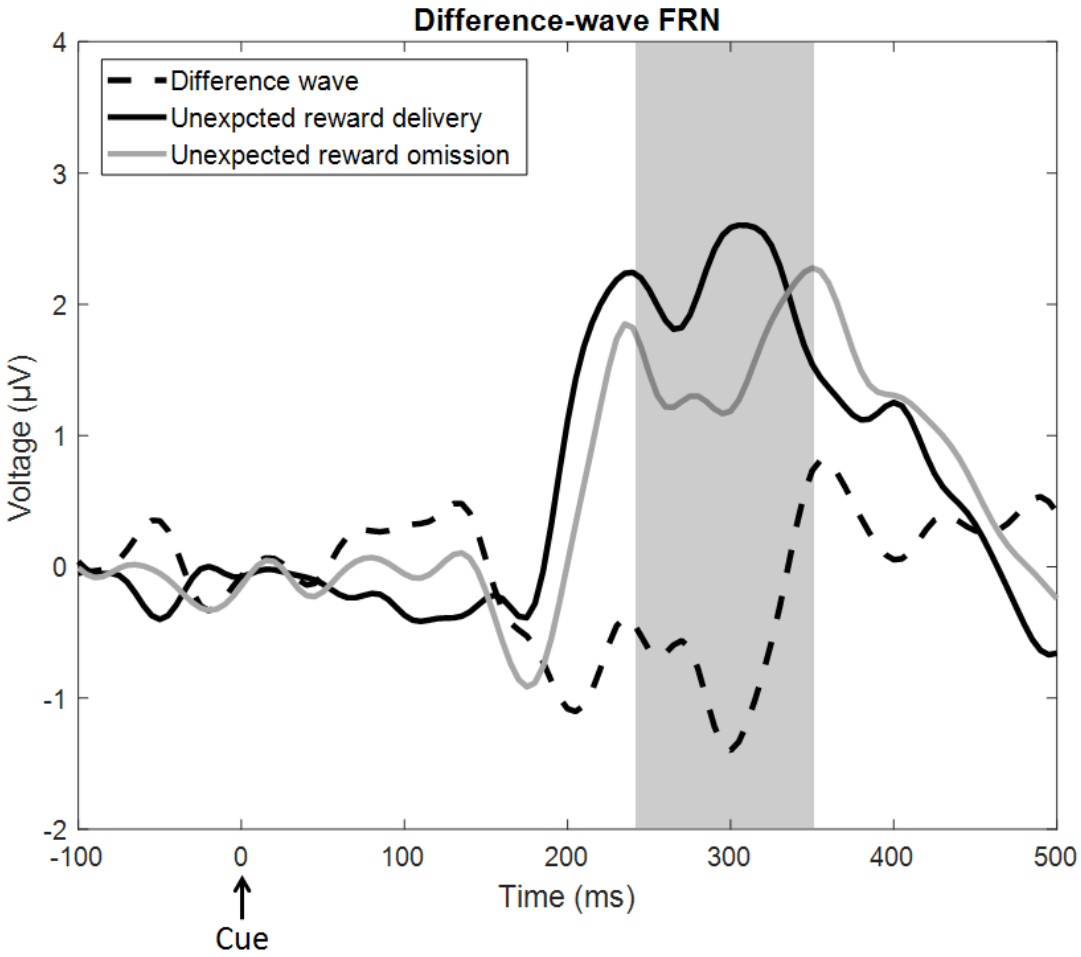


Figure 1.2. *Illustration of the difference-wave reward FRN at electrodes Fz, FCz, and Cz averaged over 37 trials per condition, taken from data collected in Chapter 3 (Taste FRN). The FRN is elicited by a predictive cue presented at 0ms, peaking 240-340ms after feedback (shaded area).*

Recent studies have suggested the FRN signals a general violation in expectation, rather than a utility PE (Ferdinand, Mecklinger, Kray, & Gehring, 2012; Hauser et al., 2014; Huang & Yu, 2014; Oliveira, McDonald, & Goodman, 2007). For example, one study showed that FRN amplitude becomes more negative to the unexpected omission of the expected outcome, be it monetary loss or monetary gain (Huang and Yu 2014). This is operationalised in the predicted response-outcome model, which proposes PE signalling reflects the unexpected omission of both appetitive and aversive outcomes (Alexander & Brown, 2011). This highlights a limitation in the FRN literature. The FRN has chiefly been characterised as a utility PE reflecting the value (better or worse than expected) of monetary reward. However, this is based on the observation that ERPs respond positively to the delivery of money and negatively to the omission of money. If ERPs actually respond positively to the delivery and negatively to the omission of any outcome, the

difference-wave FRN does not reflect utility PE, but it would appear as one in research which used money as reward because the delivery of money has positive utility and the omission of money has negative utility. If ERPs respond positively to the delivery of aversive outcomes, this presents a challenge to the assumption that the FRN signals utility PE specifically. Exploring the FRN response to other modalities of reinforcer would improve understanding of what this ERP represents and indicate whether the FRN also signals aversive PE. This was the aim of Chapter 3 (Taste FRN).

Recent studies using pain as an aversive reinforcer and money as an appetitive reinforcer have provided evidence that the FRN responds in the same direction to aversive PE as reward PE, with ERPs becoming more positive to the delivery and more negative to the omission of aversive outcomes. These studies suggest that the FRN either reflects signalling of aversive PE as well as reward PE, or that it reflects signalling of salience PE, responding positively to motivationally relevant outcomes regardless of their domain (Garofalo, Maier, & di Pellegrino, 2014; Talmi et al., 2013). The comparison between reward and aversive PE in these studies is limited in that pain is a primary reinforcer whereas money is a secondary reinforcer and there are differences between the neural responses to primary and secondary reinforcers, with the amygdala responding to primary but not secondary reinforcers (Delgado, Jou, & Phelps, 2011). Examining the FRN to reward and aversive PE within the same modality of reinforcer would properly reveal whether the FRN reflects a reward PE, or an aversive PE, or utility or salience.

The aim of Chapter 3 (Taste FRN) was to quantify the FRN using a single modality across appetitive and aversive domains, to understand whether the FRN reflects reward or aversive PE, or salience or utility across the domains of reward and aversion.

To summarise, expectations are updated in response to PE. PE has been consistently shown to be expressed in areas such as the midbrain, the basal ganglia and the PAG, and this has been captured on the scalp as the FRN (Eippert & Tracey, 2014; Glimcher, 2011; Holroyd & Coles, 2002; Sambrook & Goslin, 2015a; Schott et al., 2008; Schultz et al., 1997; Walsh & Anderson, 2012).

1.1.3. Placebo: an unusual case of PE?

Maintaining up-to-date expectations is crucial to effectively navigate the environment and understand contingencies between events, and PE is central to this process, as it causes expectations to be updated. However in some contexts expectations influence perception in a way which is not hypothesised by computational models of learning (section 1.4) (Sutton & Barto, 1990; Wagner, 1972). Placebo analgesia is an example of this. In placebo

analgesia, expectations of low pain modulate pain perception despite the PE elicited by the high pain stimulation (Colloca, 2014). Here, expectations of low pain result in modulation of pain perception, evident in the numerous reviews dedicated to placebo analgesia (Atlas & Wager, 2012a; Benedetti, 2014; Fabrizio Benedetti & Frisaldi, 2013; Colloca & Miller, 2011a; Enck, Benedetti, & Schedlowski, 2008; Murray & Stoessl, 2013; Peciña & Zubieta, 2015). This is a powerful effect, reflected in a recent meta-analysis which showed that sham surgery for pain-related conditions achieves a reduction in pain comparable to actual surgery (Jonas et al., 2015). This influence of expectation is particularly puzzling in the context of pain, which represents a potentially damaging stimulus which usually evokes swift withdrawal responses and is risky to ignore (Andersen, Sonnenborg, & Arendt-Nielsen, 1999).

Efforts have been made to image the PE response to pain, which could help to understand the relationship between PE and placebo analgesia. ERP studies have quantified the FRN response to an outcome cue signalling pain (Garofalo et al., 2014; Heydari & Holroyd, 2016; Talmi et al., 2013). In this thesis, when we investigated modulation of pain by expectation, we measured the PE response to the receipt of pain itself rather than to an outcome cue for three reasons. First, the overall aim of the thesis was to explore how the expression of PE to unexpected pain modulates pain perception; an outcome cue may not hold the same level of motivational relevance as delivery of the pain stimulation. Second, presenting an outcome cue signalling pain could inadvertently interfere with the pain expectations elicited by the pain intensity cue. Third, we were interested in the modulation of the brain's response to pain. If we showed modulation of the response to an outcome cue by expectation, rather than to pain, this would only show an effect of expectation on general PE signalling, rather than a specific modulation of the brain's response to pain. The general effect of pain expectations on PE signalling has been previously investigated in a study examining the EEG response to errors on a cognitive task after a placebo analgesia manipulation. This showed some components of task-related error signalling are changed under placebo (Koban, Brass, Lynn, & Pourtois, 2012). However, that study did not test the response to a pain-evoked PE, which makes it difficult to conclude the involvement of pain PE in placebo from these results.

This thesis aimed to explore aversive PE and expectation and their roles in pain perception. After characterising the FRN to aversive taste (Chapter 3, Taste FRN), aim 1 of Chapter 4 (referred to in the body of this thesis as 'Pain FRN and cue modulation of pain') was to extract a pain PE signal directly from the pain-evoked potential. We aimed to avoid the issues linked to measuring the FRN to a cue signalling pain described above and instead to characterise the PE response to pain directly. The remaining studies of the

thesis explored the influence of PE, expectation and pain stimulus intensity on pain ratings and pain-evoked potentials, in response to cued and placebo-elicited expectation. Below, the mechanisms of pain perception and expectation are reviewed.

1.2. Mechanisms of pain perception

See section 2.2 for a review of the technical details of painful stimulation.

1.2.1. Pain-signalling afferent fibres

Pain perception is the result of a complex hierarchy of neural signalling which cumulates in a network of signals reflecting the sensory and affective quality of pain (Bingel et al., 2002; Treede, Kenshalo, Gracely, & Jones, 1999). The response to painful stimulation begins with the activation of pain-signalling Aδ, Aβ and C afferent fibres (Djouhri & Lawson, 2004). Aδ fibres are thinly myelinated and so transmit pain signals faster than C fibres with a conduction velocity of ~11 metres per second. Aδ fibres primarily respond to changes in temperature, pressure, inflammation, and ischemia (Boulais & Misery, 2008). C fibres are unmyelinated and have a conduction velocity of ~1 metre per second (Bromm & Treede, 1983; Brooks & Tracey, 2005; Tillman, Treede, Meyer, & Campbell, 1995; Tran, Lam, Hoshiyama, & Kakigi, 2001; Treede et al., 1998). C fibres also primarily respond to changes in temperature, although they can also respond to inflammation and touch (Boulais & Misery, 2008). There are two methods of phasic pain stimulation. Laser stimulation activates nociceptive Aδ fibres, causing a sensation of heat. Electrical stimulation activates nociceptive Aδ fibres, and also Aβ fibres, which can be nociceptive or somatosensory, and have a faster conduction velocity of ~70 metres per second (Djouhri & Lawson, 2004; Tran et al., 2001). Although electrical stimulation also activates Aβ somatosensory fibres, there is no ERP activity for non-painful stimulation at electrodes where the ERP for pain is recorded, indicating that the ERP response to electrical stimulation chiefly reflects pain signalling (Dowman, 1996; Niddam, Arendt-nielsen, & Chen, 2000).

1.2.2. Central pain signalling

Understanding of the central pain system began with post-mortem human and primate anatomical studies, and later was developed with functional brain imaging (Apkarian & Hodge, 1989; Bowsher, 1957; Jones, 1998; Jones, Kulkarni, & Derbyshire, 2003). These studies showed stimulation of Aδ and C fibres sends a signal into the dorsal horn of the spinal cord (Basbaum and Jessell 2000). Here, the signal passes through a dorsal horn neuron and through the spinothalamic tract to the thalamus. The thalamus is the major

relay site for pain signalling to subcortical and cortical pain processing areas. A meta-analysis of PET, fMRI, EEG and MEG (magnetoencephalography) studies showed the brain areas most commonly activated by painful stimulation included the thalamus, primary and secondary somatosensory cortices, insula, ACC and PFC (Apkarian, Bushnell, Treede, & Zubieta, 2005; Tracey & Mantyh, 2007). The amygdala and the PAG are also major cortical hubs for pain processing and modulation of pain, are densely connected and are involved in aversive learning about pain (Bingel et al., 2002; Eippert & Tracey, 2014; Fields, 2000; McHugh et al., 2014).

1.2.3. The lateral and medial pain processing areas

The categorisation of major pain processing areas into lateral (sensory) and medial (affective) areas, based on the projection sites from the medial or lateral thalamus to the cortex, is arguably a useful way to group regions that have overlapping roles in pain perception (Albe-Fessar, Berkley, Kruger, Ralston, & Willis, 1985). The major pain processing areas are the primary and secondary somatosensory areas, thalamus, insula and the ACC (Brooks & Tracey, 2005). The lateral pain system projects through the lateral thalamic nuclei and includes the primary and secondary somatosensory cortices which signal the sensory aspects of pain including the location and intensity of pain (Bushnell et al., 1999). The medial pain system projects through the medial thalamic nuclei to the ACC, which signals the affective and motivational properties of pain (Mobascher et al., 2009; Vogt, Berger, & Derbyshire, 2003). The mid and posterior insula signals both the intensity and affective aspects of pain and is considered to be part of both the lateral and medial pain system (Brooks & Tracey, 2005; Coghill, Sang, Maisog, & Iadarola, 1999; Craig, Chen, Bandy, & Reiman, 2000; Jones, 1998; Jones et al., 2003). The distinction between the medial and lateral pain system has been highlighted in a study where attending to the location of pain increased activation in lateral pain processing areas such as the somatosensory cortex, whereas attending to the unpleasantness of pain increased responses in medial pain processing areas such as the cingulate, as well as the OFC (Kulkarni et al., 2005).

To summarise, stimulation of peripheral Aδ and C afferent fibres transmits nociceptive signals to the dorsal horn of the spinal cord, the spinothalamic tract and through to the thalamus where it is signalled to higher cortical areas. The brain areas most commonly activated by painful stimulation are the thalamus, primary and secondary somatosensory cortices, insula, ACC and PFC, although the amygdala and the PAG are also central to pain signalling and aversive learning about pain (Apkarian et al., 2005; Bingel et al., 2002; Eippert & Tracey, 2014; Fields, 2000; McHugh et al., 2014; Tracey & Mantyh, 2007).

1.2.4. Pain-evoked potentials

FMRI studies are informative to understand the anatomy of the neural systems underlying pain perception, but they have low temporal resolution compared to EEG so cannot precisely distinguish the temporal sequence of events involved in pain processing. This is a problem, for example, when distinguishing between pain anticipatory and post-stimulus activity; when stimulus intensities are expected, anticipatory activity could be misinterpreted as pain-evoked activity (Brown 2017). EEG components are more temporally sensitive, allowing the distinction between anticipation of pain and responses to the receipt of painful stimulation. However, because EEG potentials reflect a summation of brain activity at a specific point on the scalp and do not have high spatial resolution, pain-evoked EEG potentials do not reflect activity from all regions involved in pain processing. See section 2.1 for a detailed discussion of the limitations of EEG. Below, anticipatory and pain-evoked EEG potentials are reviewed.

1.2.4.1. Pain anticipation

A meta-analysis of fMRI studies of pain anticipation show activation most commonly in areas such as the dorsolateral PFC, mid cingulate and anterior insula, and thalamus. This meta-analysis also showed deactivation in the anterior cingulate (Palermo, Benedetti, Costa, & Amanzio, 2015). The stimulus-preceding negativity (SPN) is an anticipatory slow-wave negative EEG potential which is expressed before pain and which has been localised to the anterior insula and cingulate during anticipation of laser and electrical pain (figure 1.3) (Böcker et al. 2001; Brown, Seymour, Boyle, et al. 2008a). The SPN to electrical pain has also been shown in some studies to be localised to the posterior cingulate and is more pronounced to certain than uncertain pain (Hoflle, Pomper, Hauck, Engel, & Senkowski, 2013; Seidel et al., 2015). The SPN has been shown to track expected pain intensity (Babiloni et al., 2007; Böcker et al., 2001; Brown, 2017; Brown, Seymour, Boyle, et al., 2008b; Jones et al., 2013; Morís, Luque, & Rodríguez-Fornells, 2013; Palermo et al., 2015; Seidel et al., 2015). However, research into the SPN is inconsistent. One study showed no SPN to electrical pain, and another study showed the SPN to laser but not electrical pain (Babiloni et al., 2003, 2007). The SPN has also been shown to signal reward anticipation, which means that its role in pain anticipation is not fully understood, particularly in pain expectation studies where relief of pain could be interpreted as reward (Jones, Brown, & El-Deredy, 2013; Kotani et al., 2003; Leknes, Lee, Berna, Andersson, & Tracey, 2011; Ohgami et al., 2006).

Chapters 4 (Pain FRN and cue modulation of pain) and 6 (Cue and placebo effects on pain) explored the expression of SPN to pain.

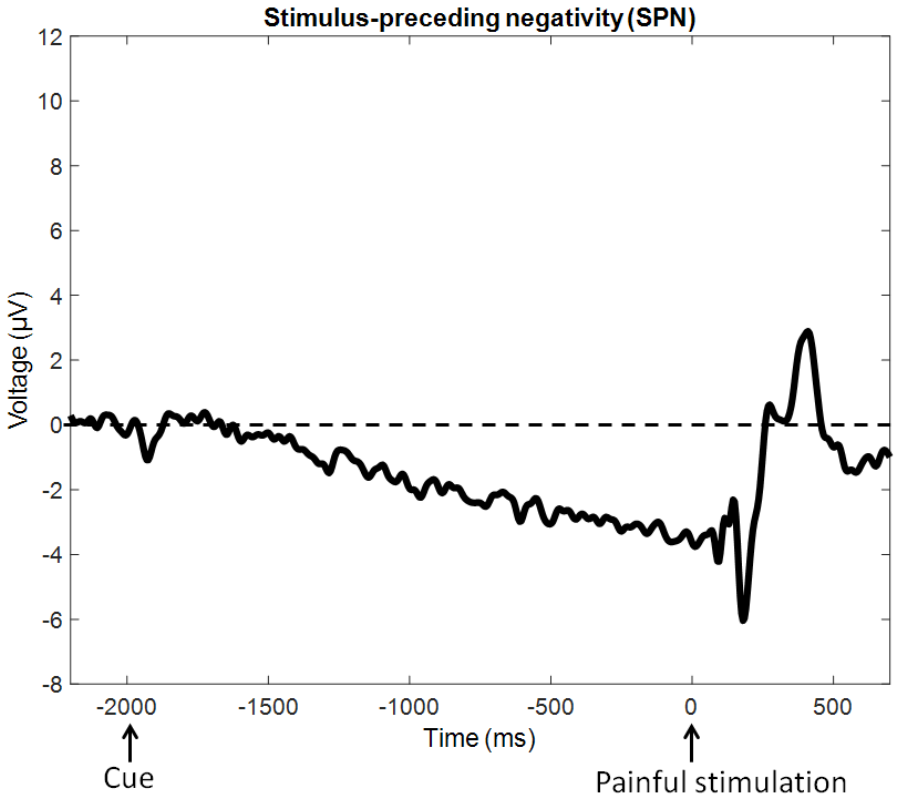


Figure 1.3. *Illustration of a stimulus-preceding anticipatory potential at electrodes CP3 and CP5 averaged over 37 trials, taken from data collected in Chapter 4. At -2000ms, a visual cue is presented informing the participant about the intensity of the pain. The SPN becomes increasingly negative, below 0 μV (dashed line) up to the point of painful stimulation (0ms). Voltage is plotted upwards.*

1.2.4.2. Laser-evoked potentials

Laser stimulation elicits a heat sensation and activity in the somatosensory cortices, hippocampus, amygdala, basal ganglia, cerebellum and insula (Bingel et al., 2002; Bornhövd et al., 2002). Laser-evoked potentials (LEPs) are the ERPs elicited by laser pain, and consist of an N1-P1 and an N2-P2 complex which reflect stimulation of Aδ fibres (figure 1.4). Source localisation studies show N1-P1 and N2 signals originate from the primary and secondary somatosensory cortex and parietal operculum, and the P2 originates from the anterior and posterior cingulate cortex (Bentley, Derbyshire, Youell, & Jones, 2003; Bromm & Chen, 1995; Iannetti, Zambreanu, Cruccu, & Tracey, 2005; Lorenz & Garcia-Larrea, 2003; Tarkka & Treede, 1993; Valeriani, Rambaud, & Mauquiere, 1995).

LEP latencies change as a function of the laser stimulus duration, as temperature peaks towards the end of stimulation. Thulium lasers, as used in Chapter 4 (Pain FRN and cue modulation of pain), elicit LEPs which have an N2 peaking at around 210ms and a P2 at around 330ms after stimulation (Treede, Meyer, & Lesser, 1994). P2 amplitudes are often analysed to capture changes in perceived pain intensity, as in this thesis (Brown, Seymour, Boyle, El-Deredy, & Jones, 2008a; Brown, Seymour, El-Deredy, & Jones, 2008).

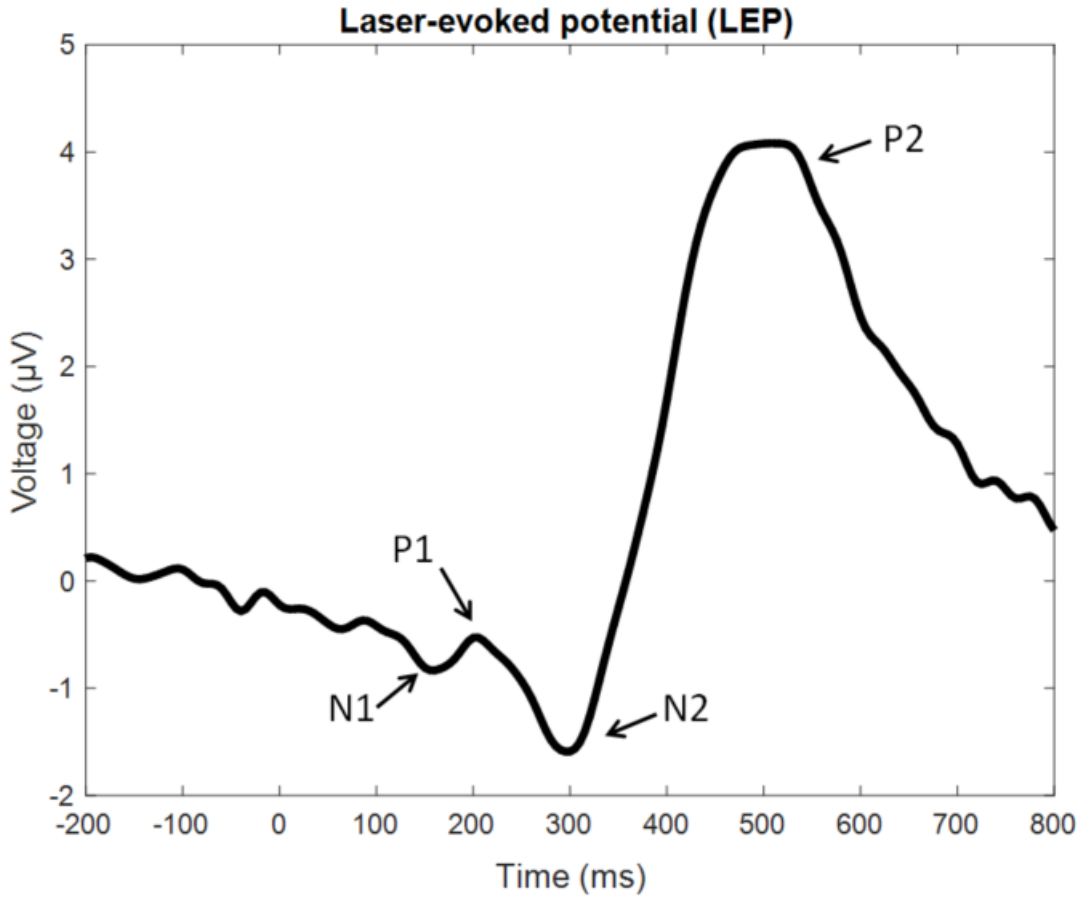


Figure 1.4. *Illustration of a laser-evoked potential at peak electrodes for each participant, averaged over 61 trials, taken from data collected in Chapter 4, with the N1-P1 and N2-P2 complex labelled. Voltage is plotted upwards.*

1.2.4.3. Electrical-evoked potentials

Electrical pain stimulation elicits a shock or a tingling sensation. MEG and MEG-fMRI studies have shown electrical stimulation initially activates the primary somatosensory cortex from 40 to 60ms, and the secondary somatosensory cortex, insula and cingulate from 100 to 300ms (Howland et al. 1995; Joseph et al. 1991; Kitamura et al. 1995, 1997; Lin and Forss 2002). FMRI studies show electrical pain stimulation also activates pain processing areas such as the cerebellum, thalamus, midbrain, PFC and ACC, and this

activation correlates positively with perceived pain intensity (Christmann et al. 2007; Wager 2004a; Yuan et al. 2010). Electrical-evoked potentials (EEPs) have a shorter latency than LEPs (Perchet et al., 2012) and it has been argued that this reflects shorter afferent fibre response times to electrical than laser stimulation because laser stimulation requires heating of the skin (Kunde & Treede, 1993). The EEP expresses a positive peak (P1) around 90ms after stimulation, localised to the primary somatosensory cortex, and a negative-positive complex around 150-250ms after stimulation, localised in somatosensory regions and the cingulate (figure 1.5). The positive peak of this complex is termed the P2 and is commonly used as a measure of perceived pain intensity, as in this thesis (Bromm & Scharein, 1982; Christmann et al., 2007; Joseph et al., 1991; Kitamura et al., 1995) although it does not always correlate with subjective rating of pain intensity (Rütgen, Seidel, Riečanský, & Lamm, 2015).

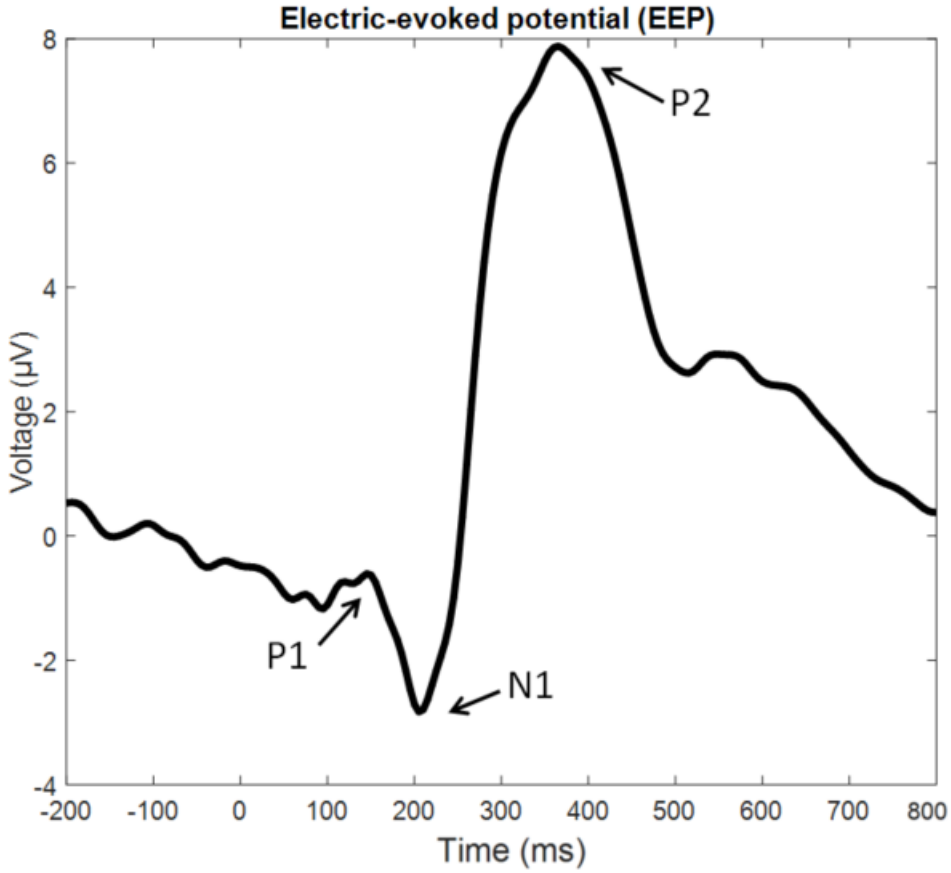


Figure 1.5. *Illustration of an electrical-evoked potential at peak electrodes for each participant, averaged over 68 trials, taken from data collected in Chapter 4, with the P1, N1 and P2 labelled. Voltage is plotted upwards.*

Although laser pain stimulation is more commonly used in placebo analgesia research, electrical pain stimulation has a number of methodological advantages, the greatest one

being that it does not heat the skin and so more trials can be used (see Chapter 4, Pain FRN and cue modulation of pain, for an in-depth discussion of the advantages of electrical pain stimulation).

Aim 2 of Chapter 4 (Pain FRN and cue modulation of pain) was to compare the modulation of electrical and laser pain by expectation, to inform future studies of the optimal method of pain stimulation. Because of the methodological advantages associated with electrical pain (section 4.1) we examined the EEG response to electrical pain anticipation and to the receipt of electrical pain in Chapters 4 and 6 (Cue and placebo effects on pain) of this thesis.

To summarise, both electrical and laser pain stimulation evoke anticipatory and pain-evoked EEG potentials which can be used to study the anticipation and perception of pain (Böcker et al., 2001; Bromm & Scharein, 1982; Brown et al., 2008; Brown et al., 2008; Christmann et al., 2007; Joseph et al., 1991; Kitamura et al., 1995; Scharein & Bromm, 1982). Below, the descending modulation of pain by cognitive factors such as expectation is reviewed.

1.2.5. Descending modulation of pain

The same brain areas that respond to painful stimulation are also involved in descending modulation of pain due to cognitive influences such as expectation. Descending projections from areas such as the rostral ACC and anterior insula to the PAG and the rostral ventromedial medulla (RVM) result in modulation of pain signalling beginning in the cortex and projecting as low as the dorsal horn of the spinal cord (Bee & Dickenson, 2009; Eippert, Finsterbusch, Bingel, & Büchel, 2009; Heinricher & Fields, 2013; Ren & Dubner, 2009). Figure 1.6 gives a summary of the descending and ascending pain processing pathways. The PAG, which is central to descending control of pain, is also involved in the ascending signalling of pain information (Büchel, Geuter, Sprenger, & Eippert, 2014; Johansen, Tarpley, LeDoux, & Blair, 2010; Ritter, Franz, Dietrich, Miltner, & Weiss, 2013). The PAG is thought to be central in descending modulation of pain through the release of endogenous opioids as it contains many opioid signalling neurons, and electrical stimulation of the PAG induces analgesia which is reversed by the opioid antagonist Naloxone (Hosobuchi, Adams, & Linchitz, 1977).

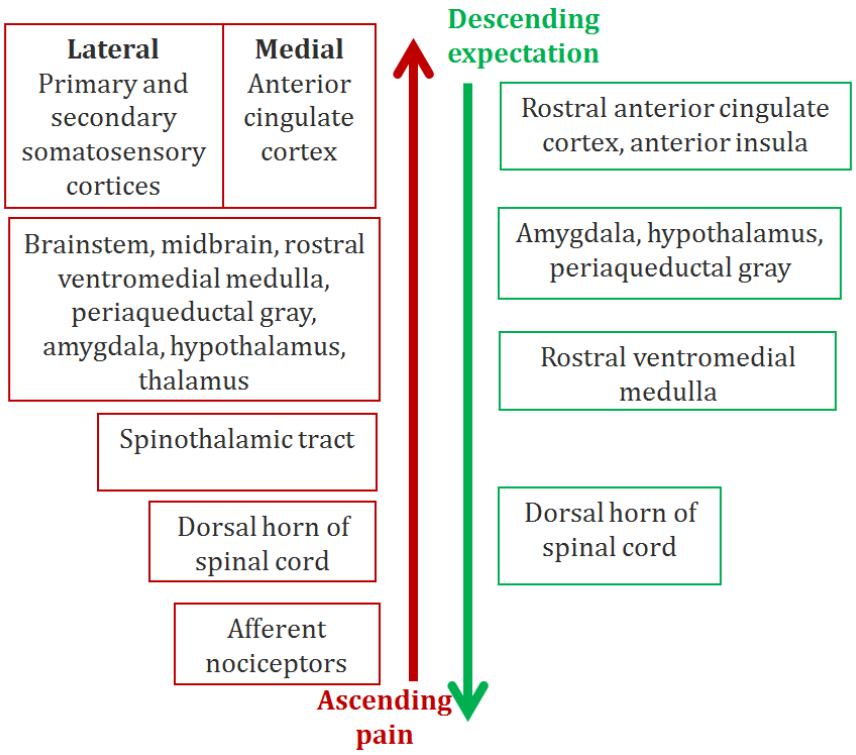


Figure 1.6. *Neural systems involved in ascending (red) and descending (green) modulation of pain. Adapted from Buchel et al. (2014).*

1.2.6. Cognitive influences on pain perception

Pain is modulated by cognitive influences such as attention, emotion and expectation. First, negative emotions influence pain perception. Anxiety increases the perceived intensity of pain and also correlates positively with activity in the entorhinal cortex, and activity here predicts activity in the cingulate, which is involved in affective processing of pain (Ploghaus et al. 2001). Second, distraction increases PAG activity, and this correlates with changes in pain intensity rating between distracted and attended pain (Tracey et al., 2002). Modulation of pain because of distraction is distinct from expectation modulation of pain. Expectation-induced analgesia engages PFC activity during pain anticipation and opioid responses whereas attentional changes are likely to be expressed in a less focused network which is not related to opioid activity (Eippert, Bingel, et al., 2009; Gazzaniga, 2004; Petrovic et al., 2010; Wager, 2004). Further to these cognitive influences, pain perception is also influenced by expectation, as discussed in section 1.3. Expecting pain elicits a sense of anticipation before pain and this anticipation correlates positively with pain intensity ratings, reflected by increased anticipatory activity in areas such as the ventral tegmental area and entorhinal cortex, which correlates positively with increased activity in the insula during pain delivery (Fairhurst, Wiech, Dunckley, & Tracey, 2007). Below, the modulation of pain signalling by expectation is reviewed in detail.

1.3. Modulation of pain signalling by expectation

In this thesis, pain expectations are defined as the beliefs about experienced pain evoked by a pain conditioning procedure or by verbal instructions, alongside the presentation of a pain intensity cue or the administration of a sham treatment.

Placebo analgesia manipulations typically involve both verbal instructions and conditioning, both of which were used in some form in Chapters 4 (Pain FRN and cue modulation of pain) 5 (referred to in the body of this thesis as 'PE size and variability') and 6 (referred to in the body of this thesis as 'Cue and placebo effects on pain') of this thesis. An established placebo analgesia manipulation is the covert reduction of pain stimulation intensity after the administration of an inert cream labelled as analgesic, which leads the participant to believe the cream is an active analgesic (Morton, Watson, El-Deredy, & Jones, 2009; Watson, El-Deredy, Bentley, Vogt, & Jones, 2006; Watson, El-Deredy, Vogt, & Jones, 2007). Here, conscious expectations of analgesia are reinforced by the delivery of low pain stimulation. Verbal instruction alone elicits a weaker placebo response than that elicited by instruction and a conditioning procedure, which suggests that conditioning is important in the placebo response (Colloca and Benedetti 2009). Explicit instructions that the pain stimulation intensity was decreased in the conditioning procedure abolishes placebo responses, which suggests that conscious expectations are also important (Montgomery & Kirsch, 1997). Accordingly, both conscious expectations, which can be manipulated by instruction, and unconscious conditioned responses, which are built by experience, appear to modulate placebo responses (Colloca, Sigaudo, & Benedetti, 2008; Colloca et al., 2008; Colloca, Petrovic, Wager, Ingvar, & Benedetti, 2010; Colloca & Miller, 2011a; Lui et al., 2010; Montgomery & Kirsch, 1997; Yeung, Colagiuri, Lovibond, & Colloca, 2014). Placebo also involves longer term associations between pain relief and clinical cues such as the administration of medication by a clinician (Atlas & Wager, 2012).

Pain expectations can also arise through conditioning between a cue and pain stimulus intensity, without the administration of a sham treatment. Here, a cue is repeatedly paired with low intensity pain stimulation, and if this cue is later presented alongside high intensity pain stimulation, the stimulation is perceived to be lower. This effect is also evident in the opposite direction, where a conditioned high pain cue increases perception of pain stimulation (Almarzouki, Brown, Brown, Leung, & Jones, 2017; Atlas, Bolger, Lindquist, & Wager, 2010; Brown, Seymour, Boyle, et al., 2008a; Webster, Weinman, & Rubin, 2016). Cue conditioning is likely to involve conscious expectations about the cue, alongside a conditioned association between the cue and pain stimulus intensity. In Chapters 4 (Pain FRN and cue modulation of pain), 5 (PE size and variability) and 6 (Cue

and placebo effects on pain) of this thesis, participants were instructed that pain intensity cues predicted the stimulation intensity. Here, we evoked conscious pain expectations using pain intensity cues which were also conditioned by experience.

There are potential differences between the expectations elicited by a sham treatment compared to the expectations elicited by a cue. Whilst placebo analgesia manipulations instruct participants to expect a general pain reduction, which elicits the tonic release of endogenous opioids (see section 1.3.1) (Wager, Scott, and Zubieta 2007), the meaning of cues often changes on a trial-by-trial basis. When the meaning of cues changes trial-by-trial, the mechanism of action must be more phasic (Atlas et al., 2010). Further, placebo analgesia involves expectations about the treatment, whereas cued pain modulation involves expectations about the stimulus itself. Below, the neuroanatomy of placebo and cue effects is reviewed separately for this reason.

Aim 1 of Chapter 6 (Cue and placebo effects on pain) was to formally replicate the sham analgesic procedure using a visual cue, to understand whether the EEG effects of cue modulation of pain are comparable to the EEG effects of placebo analgesia modulation of pain.

1. 3.1. Placebo analgesia modulation of pain processing

Expecting lower pain due to the belief that an analgesic treatment has been administered modulates perceived pain and pain-evoked activity. Both endogenous opioids and dopamine appear to be involved in this process. Placebo analgesia increases the release of endogenous opioids in areas which represent the affective and sensory properties of pain, as well as areas involved in descending pain modulation, and reward processing. This includes the rostral ACC, amygdala, insula, thalamus, OFC and dorsolateral PFC, PAG, and nucleus accumbens. These activations correlate with decreases in pain and unpleasantness ratings (Zubieta et al., 2005; Zubieta, Yau, Scott, & Stohler, 2006).

A meta-analysis of 11 fMRI and PET studies showed that placebo analgesia increases anticipatory activity in areas such as the anterior cingulate, PFC and PAG. The authors suggest this increased anticipatory activity reflects the representation of expectations of low pain (Amanzio et al. 2013; Tracey and Mantyh 2007). Anticipatory activity is negatively correlated with pain-evoked activity in the thalamus, insula and rostral ACC, indicating that expectations decrease pain-evoked activity (Wager 2004a). Disruption of dorsolateral PFC function using transcranial stimulation blocks placebo analgesia, which suggests that the representation of pain expectation is crucial for the modulation of pain perception in placebo (Krummenacher, Candia, Folkers, Schedlowski, & Schonbachler,

2010; Miller & Cohen, 2001). The SPN, which is the best known EEG correlate for pain anticipation, has not been well-characterised in placebo analgesia, although one study showed it was not modulated immediately after placebo conditioning, instead reducing in amplitude in a repeat pain session two weeks later (Morton, Brown, Watson, El-Deredy, & Jones, 2010).

Aim 2 of Chapter 6 (Cue and placebo effects on pain) was to examine the effect of a sham treatment on SPN amplitude, alongside pain-evoked potential amplitude.

FMRI studies show placebo decreases activity in pain-processing areas such as the somatosensory cortex, thalamus, insula and the rostral ACC (Bingel., Lorenz., Schoell., Weiller., & Büchel., 2006; Fardo et al., 2017; Fields, 2000; Petrovic et al., 2010; Petrovic, Kalso, Petersson, & Ingvar, 2002; Wager, 2004). Placebo analgesia even decreases activity in the dorsal horn of the spinal cord, one of the earliest central pain processing regions (Eippert, Finsterbusch, et al., 2009; Goffaux, Redmond, Rainville, & Marchand, 2007; Matre, Casey, & Knardahl, 2006). Placebo analgesia has been consistently shown to decrease LEP amplitude, and less commonly shown to decrease EEP amplitude (Morton, Brown, et al. 2010; Morton, El-Deredy, et al. 2010; Rütgen et al. 2015; Wager, Matre, and Casey 2006; Watson et al. 2007). We explored the effect of a placebo manipulation on perception of electrical pain and EEP amplitude in Chapter 6 (Cue and placebo effects on pain), and compared this with the effect of a cue manipulation.

1.3.2. Cue modulation of pain processing

Cue-evoked expectations modulate pain responses, both behaviourally and at the neural level, but the literature on the modulation of pain by cues is less substantial than that of placebo analgesia (Almarzouki et al., 2017; Atlas et al., 2010; Brown, Seymour, Boyle, et al., 2008a; Brown, Seymour, El-Deredy, & Jones, 2008; Keltner et al., 2006; Kong, Jensen, et al., 2013; Yeung et al., 2014). Cue modulation of the SPN has been explored in two studies. The anticipatory SPN becomes less negative after a low pain expectation cue and correlates with LEP amplitude when the stimulus is predictable, such that the SPN becomes more negative as LEPs become more positive (Brown et al. 2008a). Further, anticipatory activity in the anterior insula predicts the influence of cues on pain perception (Brown et al., 2008). This suggests that before pain stimulation, the anticipation of pain is modulated by cue-evoked expectation. In support of this, research using fMRI shows that functional connectivity between the PFC and the pain-signalling rostral ACC predicts cue effects on pain intensity ratings (Kong, Jensen, et al., 2013). Interestingly, anticipatory activity in value and PE signalling areas such as the OFC and ventral striatum mediates the modulation of pain signalling by cue, implicating value

processing in the cue modulation of pain (Atlas et al., 2010; Hare, O'Doherty, Camerer, Schultz, & Rangel, 2008; Pagnoni et al., 2002). More work is needed to probe the role of value in modulation of the SPN by cue.

Pain intensity cues modulate the perceived intensity of a painful stimulus and activity in pain processing areas such as the secondary somatosensory cortex, insula, thalamus and rostral and caudal ACC (Atlas et al., 2010; Keltner et al., 2006; Kong, Jensen, et al., 2013). LEP amplitude is also modulated by cues (Brown, Seymour, Boyle, et al., 2008a; Lorenz et al., 2005). Chapter 4 (Pain FRN and cue modulation of pain) tested whether EEPs are modulated by pain intensity cues comparably to LEPs. Based on this result, conditioned cues were used to modulate pain intensity expectations in Chapters 5 and 6 of this thesis.

To summarise, expectations about pain can be elicited by verbal instruction and conditioning responses (Colloca, Sigaudo, & Benedetti, 2008; Colloca et al., 2008; Colloca, Petrovic, Wager, Ingvar, & Benedetti, 2010; Colloca & Miller, 2011a; Lui et al., 2010; Montgomery & Kirsch, 1997; Yeung, Colagiuri, Lovibond, & Colloca, 2014). Expectations can either be evoked by the presentation of a visual cue or by the administration of a placebo treatment. Placebo analgesia increases anticipatory BOLD activity in areas such as the anterior cingulate, PFC and PAG, and decreases perceived pain and pain-evoked BOLD activity in pain processing areas (Amanzio et al., 2013; Bingel. et al., 2006; Fardo et al., 2017; Fields, 2000; Petrovic et al., 2010, 2002; Wager, 2004a; Watson et al., 2007). Cues also modulate the perceived intensity of pain, and this is accompanied by increased anticipatory BOLD activity in reward processing areas such as the OFC and ventral striatum, and decreased pain evoked BOLD activity in pain processing areas (Atlas et al., 2010; Brown, Seymour, Boyle, et al., 2008a).

It has been proposed that expectations represented in the PFC signal to the PAG, which modulate pain signals via descending control at the level of the RVM and spinal cord (Büchel et al., 2014; Eippert, Finsterbusch, et al., 2009; Fields, 2004; Johansen et al., 2010; Ritter et al., 2013; Wager et al., 2006). This is in line with a study showing the PFC signals the level of expected pain intensity to the PAG, which computes PE and signals to prefrontal regions, presumably to update pain expectations (Roy et al., 2014). Further, the influence of expectations on perceived pain may be modulated by factors such as the variability of those expectations (Büchel et al., 2014). This is discussed in detail in section 1.4.2. Next, models of PE and pain perception are reviewed to contextualise the involvement of PE in pain perception.

1.4. Theoretical models of expectation and pain perception

The role of PE in reinforcement learning has been summarised using computational models (section 1.4.1). However, these models do not account for the influence of expectations on pain perception and the role of prediction error in this process. A model of perception termed predictive coding better describes the integration of expectations into pain perception. Both reinforcement learning and predictive coding models are reviewed below.

1.4.1. Prediction error in computational models of learning

The Rescorla-Wagner model of reinforcement learning was the first to identify PE as a teaching signal (Rescorla & Wagner, 1972). In this model, PE is computed and expectations are updated at the end of each learning trial. This model accounts for the effects of Pavlovian conditioning where an appetitive or aversive stimulus becomes associated with a cue after a number of learning trials. According to the Rescorla-Wagner model, in Pavlovian conditioning the PE arising from the presentation of an unexpected outcome causes the learner to associate the outcome with the cue, resulting in increased responses to the cue after learning (Buchel & Dolan, 2000; Rescorla, 1988).

One limitation of the Rescorla-Wagner model is that it treats each trial as a discrete event (Niv and Montague 2008). In reality, cues and outcomes can arise at different times, and a learner aims to predict all future outcomes based on past experiences (Niv and Schoenbaum 2008). To account for this, the Rescorla-Wagner model was extended by the temporal difference learning (TDL) model of reinforcement learning. TDL acknowledges that predictions are not made trial-by-trial, but instead extend to all possible future outcomes. This means learning occurs at every time-point rather than at the end of the trial (Sutton & Barto, 1990). In TDL, a prediction consists of the expected immediate outcome and the expected outcome from that time onwards. This prediction is compared against the outcome one time-step later and the prediction of all future outcomes to compute PE (Niv and Montague 2008). In TDL, an association between the outcome and a cue is made based on PE. After learning, if the conditioned cue is presented unexpectedly, this elicits a PE, which represents the expected value associated with that cue. The expected value is represented until the time when the outcome is delivered. There is no PE when the outcome is delivered, because it was fully expected (figure 1.7). The impact of PE is modulated by the learning rate, which defines the degree to which future expectations are updated. It has been demonstrated that the learning rate is modulated by the variability of the environment. When the environment is more variable and predictions

are more uncertain, the learning rate is higher, and PE has a greater impact on future expectations (Behrens, Woolrich, Walton, & Rushworth, 2007; Dayan, Kakade, & Montague, 2000; Pearce & Hall, 1980).

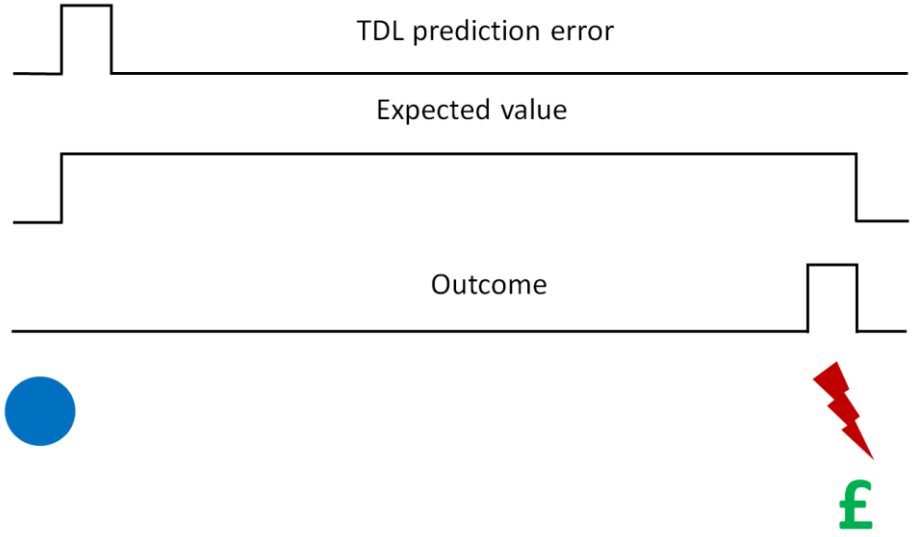


Figure 1.7. *Timeline of temporal difference learning. The cue (blue circle) predicts an aversive or appetitive outcome such as pain or money, at some point in the future. Presentation of the cue elicits a TDL PE which represents the expected value of the outcome predicted by the cue. The expected value signal is signalled until the expected time of the outcome. When the outcome is delivered, no PE occurs because the outcome was fully expected. Adapted from Brown (2017).*

1.4.2. Predictive coding of pain perception

The aforementioned studies in section 1.3 clearly indicate that pain perception is influenced by both placebo and cue-evoked expectations. However, these studies do not explain how expectations of low pain influence pain perception even in light of a high pain stimulus. According to computational models of learning, an error in prediction updates expectations (section 1.4.1). During placebo, expectations modulate pain perception despite the PE elicited by a high pain stimulus. A model of perception termed predictive coding accounts for PE in pain perception. Applied to pain, this model proposes that pain perception is the product of the integration of pain stimulation with prior expectations about the pain, such that errors between expectation and incoming pain stimulus intensity are reduced. This process is thought to be hierarchical, where the perceptual outcome at any level of the neural hierarchy informs prior expectation for the level below (Friston, 2012).

The certainty of expectations and pain stimulus intensity is important in predictive coding of pain. The certainty (the inverse variance) of expectations and pain stimulation dictates their influence on pain perception. If expectations of low pain are more certain (less variable with a tighter distribution), they shift pain perception to be closer to low pain (figure 1.8a) than less certain expectations (more variable with a wider distribution) (figure 1.8b). It has been proposed that the certainty of expectations is signalled by endogenous opioids in the PAG (Büchel et al., 2014).

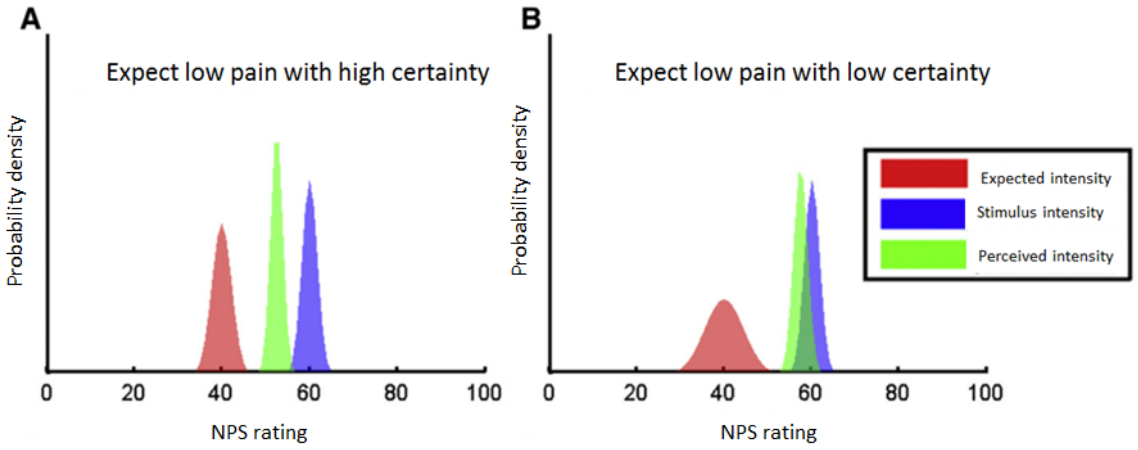


Figure 1.8. *Predictions made by the predictive coding account of placebo analgesia about expectation. Pain perception is shifted by the expected intensity of pain, and this effect is stronger when expectations are more certain. A and B: probability distributions. Expecting low pain (red distribution) shifts pain perception (green distribution) back towards the expectations. This effect is stronger when expectations are certain, with a tighter probability distribution (A) than when they are uncertain (B). Adapted from Buchel et al. (2014).*

There is evidence that increasing the certainty of an expectation increases the influence of that expectation. The influence of expectation on ratings decreases over several days, which suggests a loss of the certainty of expectations over time (Büchel et al., 2014; Colloca & Benedetti, 2006). Certain pain expectations bias perceived pain intensity more strongly than uncertain expectations, and when expectations are certain, anticipatory SPN amplitude correlates positively with LEPs, showing certain expectation predicts pain processing. The authors relate this to the proposition that uncertainty increases attention to stimulus intensity to promote its influence on perceived pain (Brown, Seymour, Boyle, et al. 2008b). More certain placebo instructions induce stronger placebo responses (Pollo et al., 2001; Verne, Robinson, Vase, & Price, 2003). A greater number of conditioning trials leads to a stronger placebo response, and according to predictive coding, a greater

number of conditioning trials would theoretically lead to more certain expectation (Colloca et al. 2010). These studies suggest certainty modulates the influence of expectations on pain perception. One study showed that certain expectations influenced pain perception, but uncertainty about high pain led to a general increase in pain perception which correlated positively with activity in the PAG (Yoshida, Seymour, Koltzenburg, & Dolan, 2013). However, this study used vicarious observation of other people's pain intensity ratings as a manipulator of uncertainty, rather than a probability cue, and social contagion of pain may operate by different mechanisms than expectation modulation of pain.

It has been proposed that more certain (less variable) pain stimulation intensities have a greater influence on pain perception than a less certain stimulation intensity, but this has not been formally tested (Büchel et al., 2014). Here, pain stimulation intensity which is not variable (thus has a tighter distribution, and is highly certain) (figure 1.9a) has a greater influence on perceived pain than pain stimulation intensity which is highly variable (thus has a wider distribution, and is less certain) (figure 1.9b).

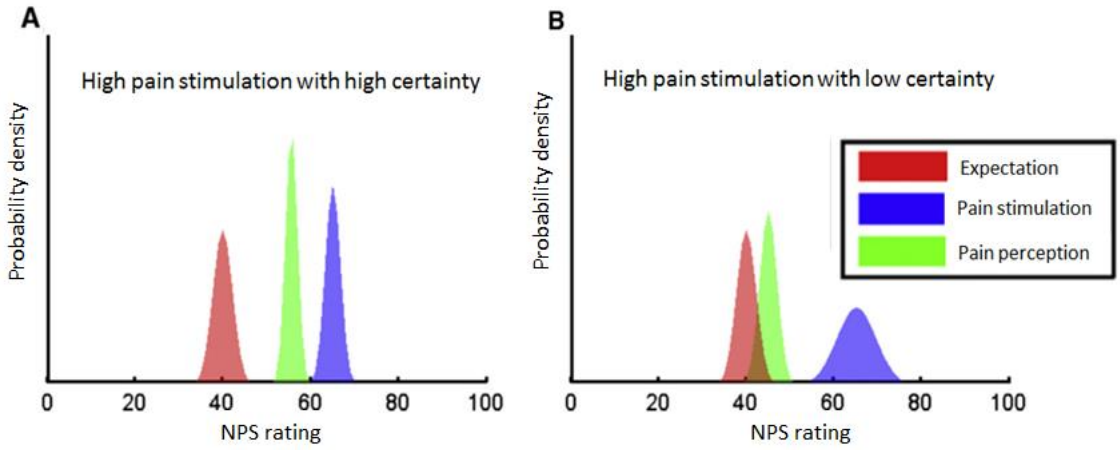


Figure 1.9. *Predictions made by the predictive coding account of placebo analgesia about painful stimulation. A and B: probability distributions. The influence of painful stimulation (blue distribution) compared to expectation (red distribution) on pain perception (green distribution) is modulated by the certainty of the pain. A: a certain high (NPS 7) painful stimulus when expecting low (NPS 4) pain results in perception of NPS 5. B: receiving the same intensity of stimulation with uncertain pain results in perception of NPS 4.2. The influence of the same intensity stimulation had more effect when it was more certain with a tighter distribution (A) than less certain (B). Adapted from Buchel et al. (2014).*

A recent fMRI study shed light on the neural mechanisms which could underlie predictive coding of pain (Geuter et al., 2017). They hypothesised that in line with a predictive coding model, pain processing activity should reflect pain PE and expectation. Indeed, a predictive coding model including these variables performed best at explaining the data. While the posterior insula and parietal operculum represented stimulus intensity, the anterior insula expressed a summation of expectation and PE. In support of this result, anticipatory activity in the anterior insula has been shown to predict pain perception, and aversive PE to pain has been detected in the anterior insula (Ploner et al. 2010; Seymour et al. 2004), indicating that the anterior insula is important for the representation of PE and expectations about pain. Further, confidence in beliefs about pain predicts the impact of anticipatory cues on pain, and this correlates positively with activity in the anterior insula (Brown, Seymour, El-Deredy, et al. 2008; Geuter et al. 2017). The authors note the insula's connections to the PFC and propose that it is in the ideal position to evaluate predictions against sensory information by integrating the sensory signals expressed in the posterior insula with expectations in the PFC to generate pain perception (Chang, Yarkoni, Khaw, & Sanfey, 2013). There is further evidence for this hierarchical modulation of pain perception in the PAG which is involved in both descending modulation and ascending signalling of pain (Ritter et al., 2013). Indeed, predictive coding of pain has been framed within a reciprocal RVM-PAG-spinal cord pain modulatory system (Büchel et al., 2014). Thus, both the anterior insula and the PAG appear to be central to predictive coding of pain.

Finally, a series of recent studies have successfully modelled predictive coding of pain, indicating that pain intensity rating is a function of the influence of both expectation and pain intensity. One model of pain perception in instrumental conditioning used data about expectations from the conditioning session to predict the pain intensity ratings. Stimulus intensity was also modelled. Pain relief was lower when the low certainty analgesia option was selected (Jung, Lee, Wallraven, & Chae, 2017). In another study, cues were associated with high or low pain and participants were instructed that cues were associated with the delivery of transcutaneous electrical nerve stimulation (TENS) analgesia as a placebo manipulation. This study revealed that both prior expectations and cues influenced pain, which suggests that pain perception is influenced by both (Tabor, Thacker, Moseley, & Kording, 2017). In this thesis, as in most previous pain cueing studies, the information provided by a cue is assumed to represent prior expectation, and the stimulus intensity the incoming information (Atlas et al. 2010; Brown, Seymour, Boyle, et al. 2008a; Brown, Seymour, El-Deredy, et al. 2008).

The predictive coding framework of pain perception offers a theoretical framework to make predictions about how expectation modulates pain perception. This framework argues that pain perception is the result of the integration of expectations and stimulus intensity. Aim 1 of Chapter 5 (PE size and variability) was to test whether expected pain intensity and pain stimulus intensity influences perceived pain using a quantitative model.

Although studies show a clear modulation of pain by expectations, it seems likely that there should be some limit to this effect. Even when expectations about pain are certain, if they are strongly violated by stimulus intensity, their influence on expectation should reduce. Otherwise, highly erroneous expectations could lead to inaccurate or even hallucinatory perceptual outcomes. In aim 2 of Chapter 5, we tested for the limits on the influence of expectations on pain. Here we examined whether eliciting a large PE by delivering a pain stimulus intensity which strongly violates expectation reduces the influence of this expectation on pain perception. Indeed, the predictive coding framework argues that that when pain stimulus intensity is similar to expectation, expectations strongly influence perception. However, if the stimulus intensity strongly violates expectation, the influence of expectation on pain is reduced. This is in line with our argument that the influence of expectations on pain should be reduced when pain stimulus intensities strongly violate expectation.

A key prediction of the predictive coding framework is that highly certain expectations have a greater influence on pain perception than less certain expectations, and this has been confirmed by various studies (Brown, Seymour, Boyle, et al. 2008a; Colloca et al. 2010; Colloca and Benedetti 2006; Pollo et al. 2001; Verne et al. 2003). Highly certain pain stimulation should have a greater influence on pain perception than less certain pain stimulation (Büchel et al., 2014). We test this prediction in Aim 3 of Chapter 5 ('PE size and variability').

1.5. Thesis aims and hypotheses

The overall aim of this thesis was to explore the expression of aversive PE and the effect of expectation and aversive PE on pain perception. We aimed to employ the theoretical frameworks of predictive coding and reinforcement learning, and to take behavioural and EEG measures, to examine the interplay between expectation and pain stimulation which produces pain perception.

1.5.1. Hypothesis 1: the FRN is a correlate of pain PE

Chapters 3 (Taste FRN) and 4 (Pain FRN and cue modulation of pain) aimed to quantify the EEG correlate of aversive PE. The aim of Chapter 3 was to characterise EEG correlates of PE using a single modality across appetitive and aversive domains (section 1.1). Chapter 4 extended this to pain: aim 1 of Chapter 4 (Pain FRN and cue modulation of pain) was to capture a pain PE signal directly to pain (section 1.1).

1.5.2. Hypothesis 2: Pain perception reflects the integration of expectation and incoming pain stimulus intensity

We aimed to quantify the influence of expectation and pain stimulus intensity on pain perception, based on measures of pain intensity ratings and pain-evoked potentials. Aim 2 of Chapter 4 (Pain FRN and cue modulation of pain) was to compare how expectation modulates laser and electrical pain, to inform future studies of the best method of pain stimulation (section 1.2). Aim 1 of Chapter 5 (PE size and variability) was to formally test the contribution of cued intensity and pain stimulation intensity on perceived pain using a quantitative model. In aim 2 of Chapter 5, we further evaluated the impact of PE size on pain perception, to test whether the influence of expectation on pain perception is mediated by PE size (section 1.4). The aim of Chapter 6 (Cue and placebo effects on pain) was to replicate the established placebo analgesia conditioning manipulation with a visual cue. Here we aimed to understand whether the conclusions we made about cue modulation of pain in Chapters 4 and 5 of the thesis could be extended to placebo analgesia modulation of pain (section 1.3).

We also aimed to characterise the EEG marker for pain anticipation as a potential measure of pain expectation. Chapters 4 (Pain FRN and cue modulation of pain) and 6 (Cue and placebo effects on pain) probed the SPN and EEP responses to different expectation modulations (section 1.2).

1.5.3. Hypothesis 3: the integration of expectation into pain is modulated by certainty

The influence of certainty on pain perception has previously been investigated from the perspective of the certainty of expectation, but the influence of the certainty of pain stimulation is also important in the predictive coding model, and has not been examined. Aim 3 of Chapter 5 (PE size and variability) was to test whether increasing the variability

of pain stimulation decreased the influence of this stimulation on pain intensity ratings, as predicted by the predictive coding model of perception (Büchel et al., 2014) (section 1.4).

1.6. Summary

There is substantial evidence that PE updates expectations. Neural signalling of reward PE is well-understood but the response to aversive PE, as in response to unexpected pain, is not so well-characterised. Maintaining up-to-date expectations is important to effectively navigate the environment but in placebo analgesia, expectations of low pain continue to influence pain perception despite the PE elicited by the high pain stimulation. This seems to contradict computational models of learning which assume PE updates expectations, but fits with the predictive coding interpretation of pain perception, where expectations influence pain perception, and this process is modulated by the mean and uncertainty of expectation. However, whether pain perception can be explained by predictive coding is relatively unexplored. The purpose of this thesis is to investigate the role of expectation and PE in pain perception and placebo analgesia. We probe this through a series of studies which aim to characterise the EEG correlate of aversive PE, and its expression to pain, and to examine the ERP and behavioural response to unexpected pain, when it is more or less expected, and more or less variable. Finally, the modulation of pain by cue-evoked expectation is empirically linked to placebo-evoked expectation, to place the results of previous chapters in the context of placebo analgesia.

Chapter 2: General methodology

Abstract

This Chapter details the methodology used in this thesis. It begins with a discussion of the neuroimaging methods used. The second part of this chapter gives an overview of the methods of experimental pain stimulation. Following this is the justification for the sample size of each experiment. Finally, three pilot studies reflect the development of paradigms in the thesis.

2.1. Electroencephalography and event-related potentials

EEG is a brain imaging technique which provides a scalp measure of the electrocortical summation of the postsynaptic neural activity of pyramidal cells. Pyramidal cells make up 80% of the human brain and are heavily interconnected and oriented in the same direction. This means when they fire they generate a coherent and measurable scalp EEG signal (see Cohen, 2017 for a recent review on the nature of EEG signals). Post-synaptic potentials are generated when neurotransmitters bind to receptors on the membrane of postsynaptic cells, causing a change in membrane potential for large groups of neurons. These potentials last hundreds of milliseconds and occur simultaneously, which means that the activity summates at the scalp.

EEG provides a measure of ERPs, the electrical scalp potentials elicited by a particular external or internal event. ERP components are the specific scalp-recorded neural potentials which are generated by a specific neural region when a specific computation is performed (Luck, 2005). ERP measures were used as the neuroimaging measure in this thesis. ERP has high temporal resolution, to the order of milliseconds. This temporal resolution means the resulting signal reveals the instantaneous neural response to events such as pain, and how this may be altered by cognitive or emotional processes. The signal reveals the temporal sequence of brain processes, for example a painful stimulus elicits anticipation (reflected in the EEG SPN) early sensory components (N1 component of the pain-evoked potential) to later responses which are more cognitively modulated (P2 component of the pain-evoked potential). For this reason, ERPs are commonly used to study pain-evoked and PE signalling and so were employed in this thesis (Sambrook & Goslin, 2015a; Treede, Lorenz, & Baumgärtner, 2003).

This temporal resolution is not available in haemodynamic measures, which consequently were not used in this thesis. Haemodynamic measures represent a secondary consequence of neural signalling, the blood oxygen level dependent (BOLD) response, and have low temporal resolution to the degree of seconds (Kwong et al., 1992) but provide high spatial resolution. EEG is limited by low spatial resolution, because any given scalp signal could be summated from various different areas of the brain. Brain activity occurs more than a centimetre away from the EEG electrodes which record activity on the scalp, which means the signal crosses cerebrospinal fluid, bone and the scalp itself, and the signal could be distorted by the time it reaches the electrode, leading to decreased spatial resolution. Source localisation techniques may be employed to localise EEG signals to a specific brain region. However, it is well known that these techniques may suffer from the inverse problem (Sarvas, 1987). The inverse problem refers to the observation that various neural

sources could equally feasibly explain the same scalp voltage distribution. This increases error in identifying the correct neural generator of scalp activity through source localisation.

2.1.1. Quantification of event-related potential components

There are a variety of techniques to define ERP components and link them to a specific neural process. One commonly used technique is to quantify components based on qualities such as their pre-defined latency, topography or polarity (Luck, 2005). For example, the FRN is typically defined as a frontocentral component peaking 240-340ms after outcome (Sambrook & Goslin, 2015a). However, this does not take into account the potential for these qualities to shift between participants or studies. This shifting could occur due to variation in EEG cap placement; even a small shift in cap placement would alter the electrode where the ERP peaked. Variations in stimulus characteristics such as the pulse width of a painful stimulus could further alter the latency of an ERP. If a pre-defined latency or electrode location is used to extract amplitudes, and the ERP peak has shifted, amplitudes may not fully represent the component of interest. An alternative to quantifying ERP components based on pre-defined latencies is to measure the peak-to-peak amplitude of the ERP, which is also used in FRN research (Janssen, Poljac, & Bekkering, 2016). Here, the difference between the most negative and the most positive peak within a pre-specified time-window is compared between conditions. However, this still requires the specification of an electrode of interest. A third technique available is the difference-wave technique which involves subtracting the ERP amplitude of one condition of interest from another. This is the most common way to calculate the FRN, and was used in Chapter 3 (Taste FRN). This has the advantage of removing any variance which is not associated with the effect of interest because it reflects the difference between two conditions. Again, this requires the specification of a latency and electrode of interest (Luck, 2005).

Data-driven techniques offer alternative methods of component identification. These techniques do not rely on a rigid definition of a component at a single electrode or time-window, and so circumvent the risk of losing data due to signal variability between participants or experiments. Data-driven techniques were used in Chapter 3 (Taste FRN). Here, SPM (Statistical Parametric Mapping) was used to analyse the data. The SPM analysis program shows differences in ERP amplitude between conditions across all available time-points and electrodes. Data from all electrodes are interpolated to produce a scalp map and responses are tested over the scalp. Testing for effects has to correct for the number of statistical tests carried out, by correcting for multiple comparisons. The

more statistical tests are carried out, the greater the chance of obtaining a false positive. The standard way of correcting for multiple comparisons in statistics is to apply a Bonferroni correction, but this assumes observations are independent, whereas in EEG, observations from neighbouring samples are highly positively correlated, and the data represent a continuous image rather than a series of independent tests. This problem applies both to the number of time-points and the number of voxels from the scalp map. Significance values are instead adjusted by calculating the probability of the topological features of the statistical map exceeding a certain threshold by chance (Kilner & Friston, 2010). The other known issue in SPM is that it does not specifically identify ERP components, instead identifying any changes in signal amplitude, which could be any number of milliseconds, from very short to very long, which may not represent an ERP component.

One way to increase the specificity of SPM analysis is to constrain the analysis to particular electrodes or time-windows. Constraints can be identified in advance, based on the literature. Alternatively, Global Field Power (GFP) offers a data-driven method to constrain SPM analyses, and was used for this purpose in Chapter 3 (Taste FRN). GFP provides a measure of the variance across participants, electrodes and conditions to identify latencies and topographies of high variance, which are likely to reflect a component of interest. This facilitates the identification of optimal time-windows and electrodes where amplitudes are extracted from. In Chapter 3 we used GFP to constrain the latency and topography of analysis for a data-driven approach with SPM to find the FRN. Tellingly, this technique yielded a different result to the difference-wave method described above. This illustrates how the method used to identify ERP components can fundamentally influence conclusions about data. Another way to increase the specificity of SPM analysis is to apply a mask to statistical tests. For example, inclusive masking involves specifying the search volume for a statistical test to electrodes where a previous statistical test showed an effect. Exclusive masking involves excluding a search volume where a previous statistical test showed an effect. Masking is useful to unpack statistical interactions.

Another data-driven technique, which is useful when latencies and topographies are variable between individuals, is to identify the latency of 50% of the maximum amplitude of a pre-specified ERP peak averaged across conditions and participants (Luck, 2005). This technique was used in Chapters 4 (Pain FRN and cue modulation of pain) and 6 (cue and placebo effects on pain), because the P2 peak of the pain-evoked potential was variable between individuals. Each participant's maximum peak across conditions within this latency is identified, and ±40ms time-window is extracted around this, to include the

peak of the maximum. This captures approximately 50% of the component's peak height, which means that it represents the ERP component peak. This allows computation of a mean for each condition of interest at the latency and electrode of interest.

These EEG techniques all share a limitation inherent to EEG. An experimental manipulation could elicit neural processes which are separate to the component of interest but which are expressed at the same topography and latency as the component of interest. These components could be generated from the same neural source, or different neural sources which are expressed at the same point on the scalp. If this occurs, it is not possible to decompose these processes from the component of interest using standard ERP techniques. This is a limitation because small differences in experimental conditions between studies could inadvertently modulate neural processes outside of the component of interest, and overlapping components may alter the characteristics of the component of interest, producing spurious results. One example for this is in the FRN literature. Here, the response to unexpected outcomes could represent a general alerting response rather than a PE response. There are numerous accepted ways to identify the FRN (summarised above). This poses a problem for the literature because it makes it difficult to compare results between studies. The most accepted way is to calculate a difference wave, which we did in both Chapters 3 (Taste FRN) and 4 (Pain FRN and cue modulation of pain), but the difference wave approach also has limitations because it requires the pre-selection of an electrode and latency of interest. In Chapter 3 we also employed an axiomatic model, which defined the FRN as an interaction between whether an outcome was expected or unexpected, and whether an outcome was omitted or delivered. The signal difference between unexpected delivery and omission should be greater than the signal difference between expected delivery and omission. Because each of the conditions is of interest in the unpacking of this interaction, the difference wave approach, which computes the difference between two conditions, is not useful as a test for this model. The axiomatic approach ensures that the effect is not observed because of a main effect of expectation or outcome, either of which could represent a general alerting response rather than a PE response.

2.2. Painful stimulation

Short phasic painful stimulation where pain is perceived immediately upon stimulation is the most appropriate technique for ERP analysis measuring the instantaneous neural responses to pain. The two established methods of phasic pain stimulation are laser and electrical stimulation, both of which were used in this thesis. In this section we review the

technical details of painful stimulation. The neural responses to pain stimulation and cognitive modulation of pain are discussed in sections 1.2 and 1.3.

2.2.1. Laser stimulation

Laser stimulation is a well-validated method of experimental pain induction (Garcia-larrea et al., 2003; Iannetti et al., 2004; Lorenz & Garcia-Larrea, 2003; Treede et al., 2003). The laser delivers brief radiant heat which activates peripheral nociceptive Aδ and c fibres (see section 1.2.1, introduction) (Iannetti et al., 2004; Tran et al., 2001) and elicits a heat pain sensation, which can be manipulated according to the intensity of the laser stimulation.

A thulium laser was used in this thesis. CO2 lasers are more commonly used for pain induction but have a number of disadvantages. CO2 lasers emit infrared radiation which has an penetration depth of 10μm, which is less than the depth range of pain afferents in the skin (20-570 μm) (Tillman et al., 1995; Treede et al., 1998). This means pain afferents are only stimulated by heating the skin beyond 60°C, which can damage the skin (Bromm & Treede, 1983; Haimi-Cohen, Cohen, & Carmon, 1983; Spiegel, Hansen, & Treede, 2000). The time it takes for nociceptive afferent fibres to be activated is dependent on how long the skin takes to heat, usually 20-40ms, which means that CO2 lasers take longer to activate afferent fibres than thulium lasers do (Bromm, Jahnke, & Treede, 1984; Treede & Kunde, 1995). Thulium lasers, on the other hand, have a penetration depth of 360 μm because they deliver near-infrared radiation. This means that they stimulate nociceptive afferents directly, and stimulation time can be decreased to 1ms (Spiegel et al., 2000). Hence, they are less likely to cause cumulative heating of the skin, which means that more pulses can be delivered in a session. They also show good signal-to-noise ratio and highly consistent LEPs between trials (Kazarians, Scharein, & Bromm, 1995; Spiegel & Hansen, 1996; Weiss, Kumpf, Ehrhardt, Ingmar, & Miltner, 1997).

A key limitation of both C02 and thulium laser stimulation is that it leads to cumulative heating of the skin and consequent sensitisation, which could confound the pain stimulation intensity. Despite the directness of the stimulation in thulium lasers, these lasers can still heat the skin because of absorption of heat by the skin. This provided one motivation for the comparison between expectation modulation of laser and electrical pain in Chapter 4 (Pain FRN and cue modulation of pain), as electrical pain does not heat the skin. If electrical pain stimulation is modulated by expectation similarly to laser stimulation, electrical stimulation would be a preferable method of painful stimulation because it is safer to use. We aimed to minimise heating in Chapter 4 by marking the stimulation site, the dorsum of the arm, with a 3cm x 4cm stimulation grid, where each square was 1cm^2 in size, and delivering stimulation to the site sequentially between grid

squares. This meant the same site was not repeatedly stimulated in a short space of time. See table 2.1 for the energy output values for the thulium laser, and figure 2.1 for plots of the thulium laser energy output values.

Table 2.1. *Details and stimulation parameters of the laser stimulator*

Chapter	Stimulator type	Stimulation area	Pulse width	Energy delivered at 50%	Wavelength
4 (Pain FRN and cue modulation of pain)	30-watt IPG Photonics Corporation class 4 thulium laser, US/ TLR-30-2050	Dorsum of arm	150ms	2.25 joules	2050 nanometres

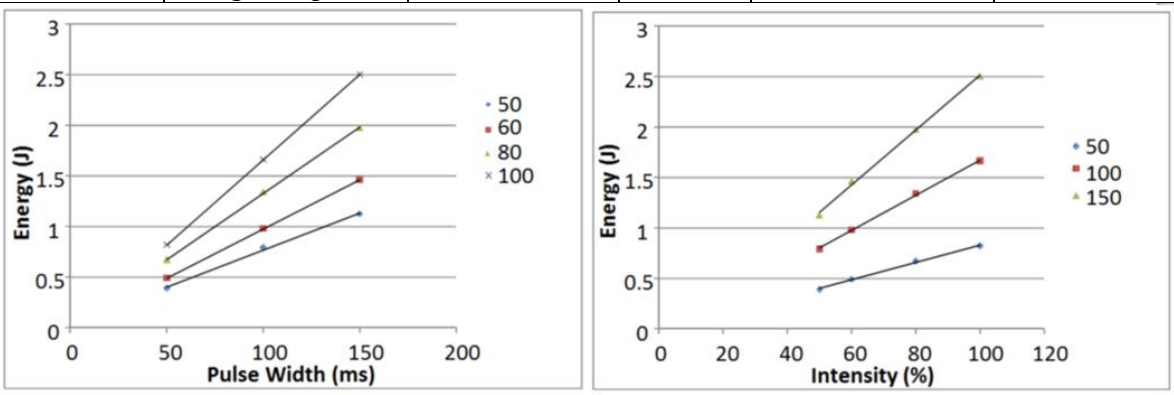


Figure 2.1. *Energy output values for the thulium laser. Left: Joules output as a function of pulse width, at varying levels of stimulation intensity (%, depicted in the key, where on 0% is minimum intensity, 100% is maximum intensity, relative to the output range of the stimulator). Right: Joules output as a function of intensity, at varying pulse widths (ms, depicted in the key) (Watson, Jones & Rainey, 2017)*

2.2.2. Electrical stimulation

Transcutaneous electrical nerve stimulation also activates peripheral afferent fibres, causing a pain sensation. This has been used previously as an aversive stimulus in conditioning experiments, and in placebo and cueing experiments (e.g. Atlas, Bolger, Lindquist, & Wager, 2010; Garofalo, Maier, & di Pellegrino, 2014; Talmi, Atkinson, & El-Deredy, 2013; Wager et al., 2004). Electrical stimulation activates myelinated somatosensory Aβ fibres and, above threshold, nociceptive Aδ fibres, eliciting a pain sensation. Electrical stimulation does not cause cumulative skin heating, so there is no limit on the number of trials that can be used in this respect. However, because electrical stimulation elicits somatosensory Aβ alongside nociceptive fibres the resulting EEP does not reflect purely nociceptive activity. The somatosensory activity could add signal noise to

the subtle effects of expectation in the EEP. Chapter 4 (Pain FRN and cue modulation of pain) of this thesis describes a study designed to compare the expectation modulation and corresponding EEG correlates of laser and electrical pain, to ascertain whether the EEP is appropriately modulated by expectation. We showed equal modulation of LEP and EEP by expectation despite the potential for extra somatosensory noise in the EEP. On balance, electrical stimulation was deemed more appropriate for the remaining chapters of this thesis, due to the constraints on trial numbers and the ethical implications of the risk of skin lesions in laser stimulation.

The electrical stimulator used in Chapter 4 (Pain FRN and cue modulation of pain) was a TENS machine built by the University Medical Physics team (Salford Royal NHS Foundation Trust) which delivered stimulation via two silver/silver chloride cup electrodes which were taped to the middle phalanx of the right index finger. The electrical stimulator used in Chapters 5 (PE size and variability) and 6 (Cue and placebo effects on pain) was a DS5 Isolated Bipolar Constant Current Stimulator (Digitimer DS5 2000, Digitimer Ltd., Welwyn Garden City, UK) and was operated via an electrode strapped to the dorsum of the hand. See table 2.2 for details of the stimulators used in Chapters 4, 5 and 6, and see figure 2.2 for the energy output values of the TENS machine used in Chapter 4, provided by the Medical Physics team (Salford Royal NHS Foundation Trust). There was no equivalent plot available for the DS5 Isolated Bipolar Constant Current Stimulator, but the figure would look similar, with a lower 120 volts limit. The maximum voltage for the TENS machine was higher than for the DS5 Isolated Bipolar Constant Current Stimulator. We moved to use the DS5 to decrease the risk of skin breakdown to participants which was present because of the higher voltages as in the TENS stimulator. The lower maximum voltage meant the range of painful stimuli was smaller for the DS5 (table 2.2). However, pain intensity ratings of DS5 stimulation in Chapters 5 and 6 reached 'highest tolerable' levels in all participants, so this limited voltage range did not cause an issue.

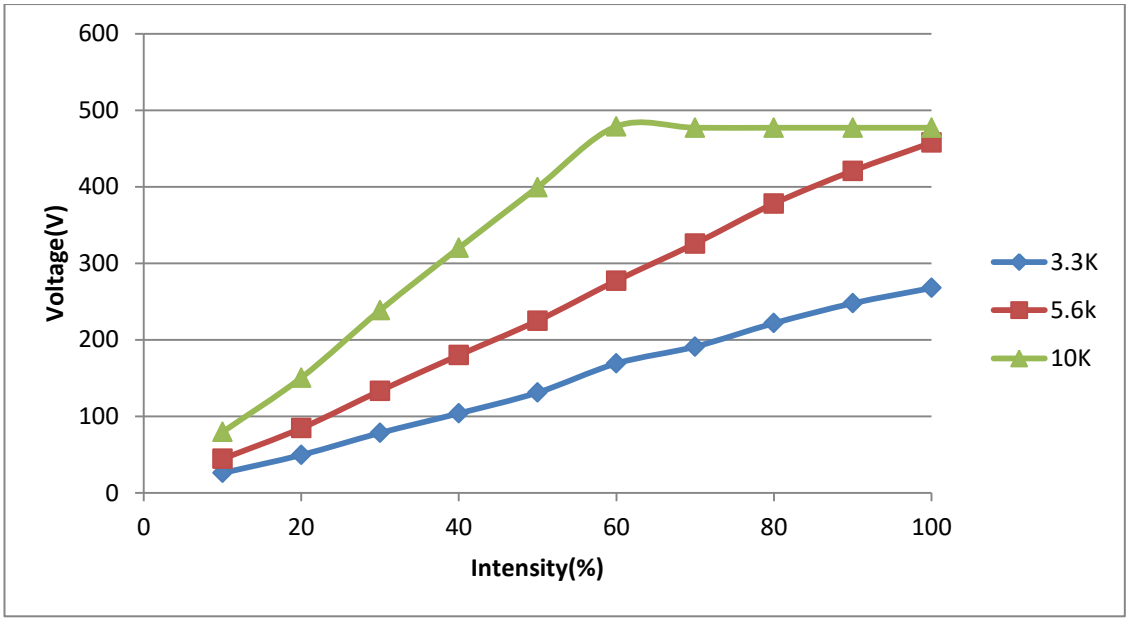


Figure 2.2. *Energy output values for the TENS stimulator. Voltage output as a function of the stimulation intensity of the TENS machine: on the x axis, 0% is minimum intensity, 100% is maximum intensity, relative to the output range of the stimulator. The different colours correspond to the test-load used to simulate skin impedance (depicted in the key). As the TENS machine is a constant current source it adjusts its output voltage according to impedance to deliver the requested intensity. The machine was tested with 3.3, 5.6 and 10 KiloOhm (KΩ) resistors (Watson, Rainey, & Jones, 2017a).*

Table 2.2. *Details of the TENS stimulators. Note that the maximum voltage was higher for the TENS machine built by Medical Physics, and this stimulation was unipolar, whereas stimulation for the DS5 was bipolar. A bipolar pulse means that the applied current can be reversed in direction through the skin without physically switching the electrodes across, and is used to avoid electrolysis which could increase the risk of skin ulceration.*

Chapter	Stimulator type	Stimulation area	Pulse width	Maximum voltage	Maximum current	Maximum output power
4 (Pain FRN and cue modulation of pain)	TENS machine built by Medical Physics	Middle phalanx of the right index finger	230-300ms	477 volts	81 milliamps	37 watt
5 (PE size and variability) and 6 (Cue and placebo effects on pain)	DS5 Isolated Bipolar Constant Current Stimulator	Dorsum of right hand	5ms	± 120 volts	±50 milliamps	37 watt

2.2.3. Habituation

Habituation can add noise to pain responses by causing a decreased response to stimulation over time which is not accounted for in the analysis. During habituation the responses to painful stimulation decrease with repetition of stimulation. Both subjective ratings and neural responses to laser pain habituate (Mobascher et al., 2010). Subjective ratings of electrical pain have been shown to habituate in one study (Colloca, Benedetti, & Pollo, 2006). However, most studies show that the amplitudes of EEPs habituate but the subjective ratings of pain do not (Babiloni et al., 2004; Christmann et al., 2007; Hoflle et al., 2013; Miltner, Larbig, & Braun, 1987; Rütgen, Seidel, Riečanský, et al., 2015). A difference in habituation rate may explain why EEP amplitude does not always correlate with pain intensity ratings, for example in Chapter 4 (Pain FRN and cue modulation of pain). Habituation can be both central and peripheral (Greffrath, Baumgärtner, & Treede, 2007; Milne, Kay, & Irwin, 1991). Habituation leads to decreased activity in pain processing areas such as the thalamus, insula and somatosensory cortex, suggesting habituation modulates the central processing of pain (Bingel, Schoell, Herken, Büchel, & May, 2007; Handwerker & Kobal, 1993). We varied the inter-stimulus interval of all pain trials in the thesis to minimise central habituation to a stimulus by maximising attention to the stimulus, as has been previously recommended (Treede et al., 2003). Moving the laser stimulation site by a small amount on each trial is also often employed to decrease

nociceptor fatigue, which we employed (Andreatta, Michelmann, Pauli, & Hewig, 2017; Babiloni et al., 2007; Brown & Jones, 2008; Brown, El-Deredy, & Jones, 2014; Treede et al., 2003). This is not possible in electrical stimulation because the electrode is fixed to the stimulation site. Despite this difference between laser and electrical stimulation, similar habituation responses have been shown between laser and electrical pain stimulation (Babiloni et al., 2007).

2.2.4. Calibration of stimulation intensity

Sensitivity to painful stimulation varies according to the site of stimulation and between individuals, influenced by factors such as gender and skin colour (Arendt-Nielsen & Bjerring, 1988). Calibrating the stimulation intensity at the beginning of an experimental session to each individual's sensitivity ensures the same subjective intensity of pain is felt by all participants. During the calibration procedure participants receive pulses of increasing intensity and give verbal feedback using a NPS (Numerical Pain Scale) from 0 (no pain) to 10 (maximum possible pain) (figure 2.3). The stimulation begins with a low intensity which may be below the threshold for detection, and is increased to an intensity participants call 'high but tolerable pain'. Other important verbal anchors are 'just painful' and 'medium pain'.

2.2.5. Numerical Pain Scale

It is important to measure the subjective experience of pain stimulation intensity and unpleasantness when investigating neural pain responses. This is because pain-evoked potentials do not always correlate with pain report, indicating they may not consistently reflect the pain experience (e.g. Chapter 4, Pain FRN and cue modulation of pain). Pain is difficult to quantify, but the NPS provides a series of verbal anchors to quantify an individual's pain experience as objectively as possible, and allows comparison across participants and across studies (Johnson, 2005). The NPS has been recommended for measurement of subjective pain reports (Hjermstad et al., 2011). The pain ERP literature typically uses a NPS rating where 3 is "just painful" and 7 is "highest tolerable pain" (e.g. Brown, Seymour, El-Deredy, & Jones, 2008; Morton, Brown, Watson, El-Deredy, & Jones, 2010b; Watson et al., 2006). To maximise comparability with this literature, in Chapters 4 and 7 we used the same NPS. In Chapter 5 (PE size and variability), the lowest pain was defined as level 2 on the NPS, and the maximum tolerable pain level 8 on the NPS, which has also been used previously (Atlas et al., 2010). The wider NPS in Chapter 5 was designed to give participants a broader verbal range of pain intensity ratings to encourage

finer differentiation of pain levels (figure 2.3). This was a key requirement for Chapter 5 which measured the effect of small variations in pain intensity (section 2.5.2).

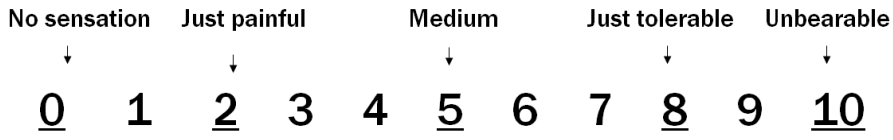


Figure 2.3. *Numeric Pain Scale. Participants rated painful stimulation from level 0 (no sensation) to level 8 (just tolerable). This NRS was used in Chapter 5 (PE size and variability). In Chapters 4 (Pain FRN and cue modulation of pain) and 6 (Cue and placebo effects on pain), the verbal anchors differed: a score of 3 indicated "just painful", and a score of 7 indicated "just tolerable".*

2.3. Labelled Magnitude Scale

In Chapter 3 (Taste FRN) the Labelled Magnitude Scale (LMS) was used to measure hedonic responses to taste. The LMS is a quasi-logarithmic scale with verbal anchors describing hedonic intensity from "strongest imaginable" sensation to "no sensation", which has been validated for research into intensity responses and is commonly used in taste research (Cardello, Schultz, Lesher, & Merrill, 2005; Dinehart, Hayes, Bartoshuk, Lanier, & Duffy, 2006; Duffy & Bartoshuk, 2000; Green et al., 1996; Hayes, Allen, & Bennett, 2013; Schutz & Cardello, 2001). The LMS was developed based on research into responses to taste, which showed responses to different intensities are non-linearly spaced (Green, Shaffer, & Gilmore, 1993) (figure 2.4).

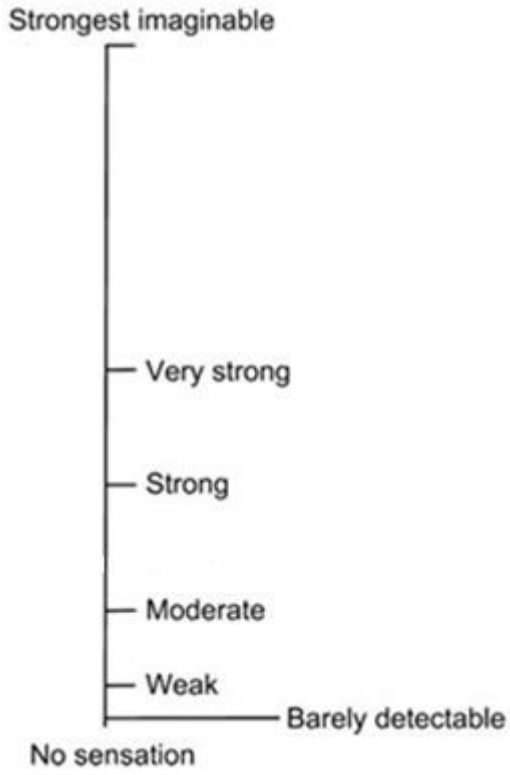


Figure 2.4. *Labelled magnitude scale. Participants rated the hedonic intensity of taste from no sensation to strongest imaginable. This LMS was used in Chapter 3 (Taste FRN).*

2.4. Sample size

In Chapter 3 (Taste FRN) and 4 (Pain FRN and cue modulation of pain) of this thesis, the standard convention of acquiring 16 datasets for EEG analysis was employed. To account for noisy data or problematic files, 20 participants were collected (Luck, 2005).

Chapter 5 (PE size and variability) was a behavioural study. The effect size was not known in advance, so the sample size instead matched previous studies investigating the effect of expectation on electrical pain and more subtle effects such as the effect of certainty or subliminally presented cue on pain intensity rating which typically recruit 15-30 participants (e.g. Brown, Seymour, Boyle, El-Deredy, & Jones, 2008b; Colloca & Benedetti, 2006; Jensen et al., 2012). We hence elected a sample size of 30 participants.

Chapter 6 (Cue and placebo effects on pain) investigated the effect of cued expectations (study 1) and a sham treatment (study 2) on EEPs. We aimed to collect 30 participants per group following previous placebo analgesia studies (Morton et al., 2010, 2009). Midway through data collection, a manipulation check revealed a procedural problem in study 2. We continued collecting data for study 1, and restarted study 2 with new participants after conducting additional research to improve the procedure. Consequently, a sample of

N=30 participants per group was collected for study 1 (33 females, mean age 22 years) and N=22 per group for study 2 (29 females, mean age 22 years).

2.5. Paradigm development

2.5.1. Hedonic taste concentrations

2.5.1.1. Purpose and objectives

For Chapter 3 (Taste FRN), a key feature of the design was the presentation of appetitive and aversive tastes which were hedonically equidistant. This was a novel paradigm which required concentrations of appetitive sweet and aversive bitter taste which were rated as equally intense in terms of their respective pleasantness and unpleasantness in the majority of participants. For this we asked participants to rate the hedonic intensity of a variety of taste concentrations.

2.5.1.2. Method

This pilot employed a within-participants design, where each participant (N=11) received a variety of taste samples. Participants were instructed to avoid food and water for two hours beforehand, to ensure they had the same levels of satiety in the pilot as in the experiment as this could influence the hedonic intensity of the taste. The participant's head was secured with a chin rest with rubber tubes (bore 2 mm, wall 0.5 mm, Altec Products) attached to the chin rest and to the participant's face with medical tape. The tubes were attached to 50ml syringes (Plastipak syringe, 50 ml Luerlok, Fisher Scientific) operated by pumps (Harvard Apparatus, pump 33) which were controlled by software (MATLAB, MathWorks).

Participants received four concentrations of bitter taste and four concentrations of sweet taste, which were each delivered five times in a random order. Bitter and sweet tastes were administered in separate blocks and block order was randomized. The tastes consisted of 1, 0.6, 0.2 and 0.1 mole of sucrose, and 1.0, 0.75, 0.25 and 0.05 millimole of quinine. Each solution also included 10 millilitres of lemon taste per litre of liquid to counteract the sweetness of the sucrose to make the taste pleasant whilst maintaining symmetry between sweet and bitter tastes. Participants rated the hedonic intensity of each taste from 'strongest imaginable taste' to 'no sensation' on the LMS. Participants placed a mark on the line where their perceived intensity of pleasantness for sweet, or unpleasantness for bitter, lay. Data were entered into a 2 (taste) x 4 (magnitude) repeated-measures ANOVA.

2.5.1.3. Results

The ANOVA revealed a main effect of magnitude ($F(3,27)=34.83$, $p<.001$, $\eta_P^2=.8$), but no significant effect of taste ($F(1,9)=4.1$, $p=0.07$, $\eta_P^2=.31$) or a magnitude x taste interaction ($F(3,27)=0.84$, $p=0.49$, $\eta_P^2=.09$). This suggested that increases in magnitude changed the hedonic intensity rating, but there was no difference between bitter and sweet tastes. See figure 2.5 for a plot of these data.

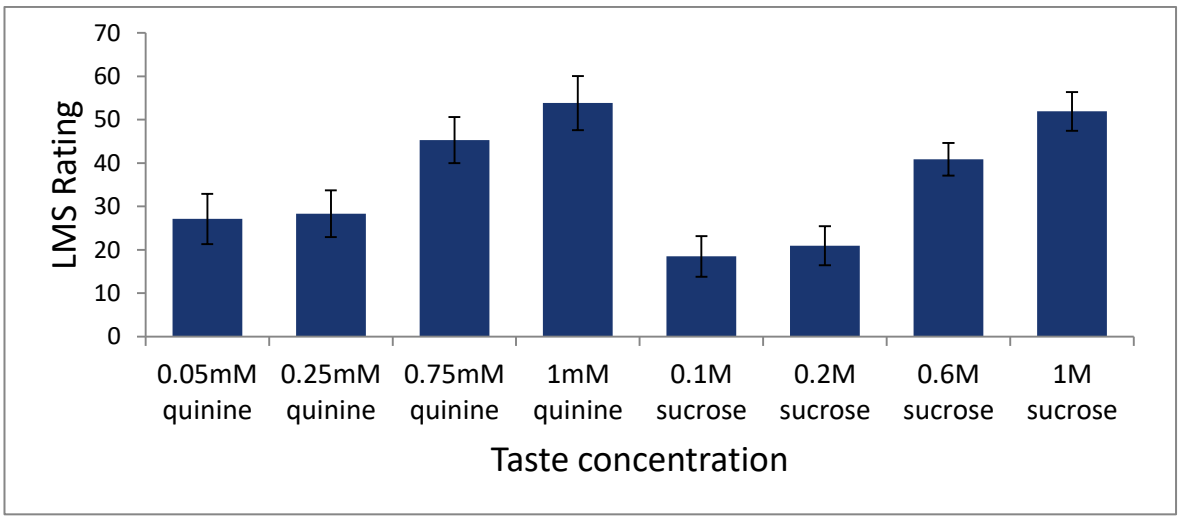


Figure 2.5. *Hedonic taste ratings. Participants rated the hedonic intensity of increasing concentrations of bitter quinine and sweet sucrose taste. The data show a range of responses to the various intensities which suggested these concentrations would be appropriate to elicit hedonic intensity ratings which were equivalent between the bitter and sweet tastes. Error bars represent the standard error of the mean.*

2.5.1.4. Discussion

We identified four concentrations of bitter and four concentrations of sweet taste which elicited hedonic intensity ratings which were widely ranged within sweet and within bitter, but comparable between bitter and sweet. These concentrations allowed for selection of a high magnitude bitter and a high magnitude sweet concentration whose hedonic ratings were within 10% of one another, and a low magnitude sweet and a low magnitude bitter concentration whose hedonic ratings were within 10% of one another for a given participant. With these concentrations we could also identify low magnitude concentrations whose hedonic ratings were at least 20% less than the high magnitude concentrations for that participant. We used these eight concentrations in the main experiment (Chapter 3, Taste FRN).

2.5.2. Differentiation between electrical pain at multiple intensity levels

2.5.2.1. Purpose and objectives

Chapter 5 (PE size and variability) employed a design which required the delivery of a range of pain intensities, and it was important that participants could differentiate the different intensities for the manipulation to be effective. For this reason, we conducted a pilot study which tested participant's ability to differentiate different levels of the electrical stimulation used in Chapter 5.

2.5.2.2. Methods

6 participants underwent a pain calibration procedure where they verbally rated the pain intensity of increasing intensities of stimulation using a NPS. We presented participants with a NPS scale where a level 2 pain was labelled as "just painful" and a level 8 pain was labelled as "highest tolerable" pain. Painful stimuli were electrical pulses delivered to the dorsum of the right hand via a concentric electrode by a constant current stimulator (Digitimer DS5 2000, Digitimer Ltd., Welwyn Garden City, UK). The pulse width of the electrical stimulation was 5 milliseconds. All stimuli were delivered on a Matlab platform (Mathworks) which interfaced with the pain stimulator via a digital-to-analogue convertor (Multifunction I/O device, National instruments, Measurement House, Berkshire, UK).

Once stimulation intensities for their levels 1 to 8 had been established, participants took part in two studies designed to assess how well they could differentiate the different intensities.

In study 1 a series of pulses identified as that participant's NPS 2, 4, 6 or 8 in the staircase procedure were delivered to participants pseudorandomly. Participants were asked to identify the NPS intensity of each pulse. Each pulse was delivered 10 times.

In study 2 participants were presented with a pulse and asked to identify what NPS intensity they believed the stimulus intensity was. On each trial, participants were instructed that the pulse could be one of two NPS intensities. This was either level 1 versus 6, or level 2 versus 6, or level 3 versus 6, or level 4 versus 6, or level 5 versus 6. The number of correct responses was plotted as a percentage for each condition, to test the hypothesis that as the discrepancy between the two options increased, accuracy increased. For example, we predicted participants would be more accurate at detecting whether a

stimulus was level 1 versus 6, compared to if the option was level 5 versus 6. Participants received 10 pulses per condition, five at each level.

2.5.2.3. Results

Because of the low sample size, we did not carry out statistical analysis on these data. The results of study 1 showed that on average, participants rated the pulses as similar to how they had rated them in the pain calibration procedure, although they rated level 2 and 4 as slightly higher than they had in the pain calibration procedure, and level 6 and 8 as slightly lower than they had in the pain calibration procedure (figure 2.6).

Study 2 showed an overall trend which supported our hypothesis: as the discrepancy between potential outcomes decreased, percentage accuracy across participants and trials on identifying the stimulus decreased.

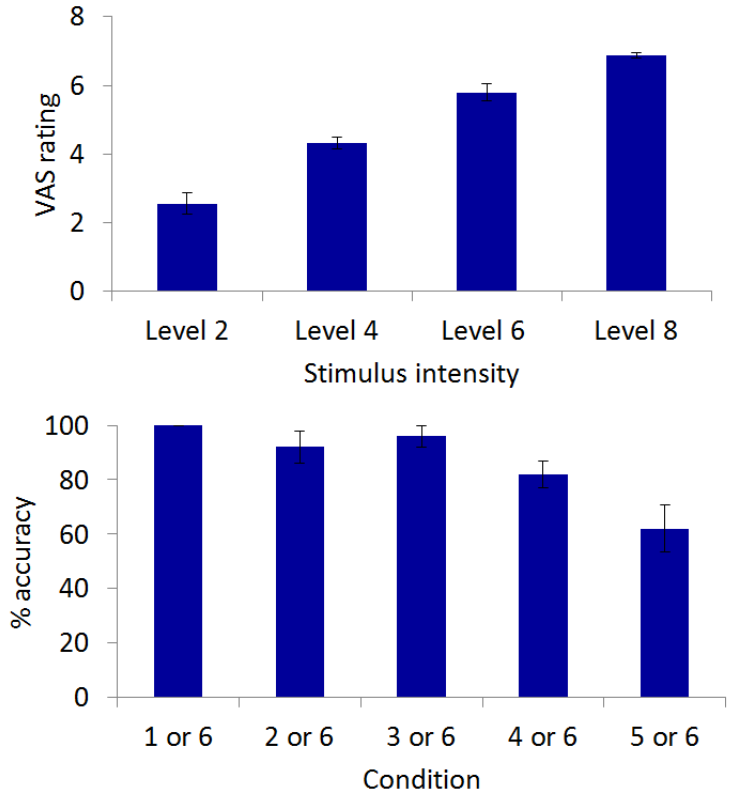


Figure 2.6. *Pain intensity rating and accuracy. Upper plot: Study 1: Participants rated the intensity of stimulation corresponding to their level 2, 4, 6 and 8. On average, participants rated the pulses as similar to how they had rated them in the pain calibration procedure, other than level 8, which was rated as slightly lower than in the pain calibration procedure. Lower plot: Study 2: participants were presented with a pulse and asked to identify what they believed the stimulus NPS was, between two potential NPS intensities. Accuracy decreased as the discrepancy between the two potential NPSs decreased.*

2.5.2.4. Discussion

Results from study 1 showed that participants successfully differentiated between level 2, level 4, level 6 and level 8 NPS stimulation intensities. This indicated that electrical stimulation was an appropriate tool to use for the PE manipulation in Chapter 5 (PE size and variability) where participants needed to differentiate between small differences in stimulation intensity for the manipulation to be successful. The fact that pain intensity ratings were slightly decreased at levels 6 and 8 suggests sensitivity to the high stimulation intensities decreased between the pain calibration procedure and the pilot procedure. This could be the result of habituation to the stimulation or that the stimulation was perceived as higher in the pain calibration procedure when it was accompanied by the

knowledge that the intensity was increasing pulse by pulse. The results of study 2 indicated that participants were able to identify a difference between two pain stimulation intensities. Participants performed at an above chance level even when pain stimulation intensities were very similar (level 5 versus level 6). This indicated that participants could detect a discrepancy between pain intensities, which meant that for the certainty manipulation in Chapter 5, we could deliver pain intensities which were discrepant to cued intensities and test the effect of the size of discrepancy on pain intensity ratings.

2.5.3. Placebo analgesia to electrical pain stimulation

2.5.3.1. Purpose and objectives

While placebo analgesia modulation of laser pain is established (e.g. Brown et al., 2011; Morton, Brown, et al., 2010a; Morton, El-Deredy, Watson, & Jones, 2010; Morton et al., 2009; Watson et al., 2009b, 2006, 2007), the effects on electrically induced pain perception are not so well-established, particularly using placebo cream and EEG. This may be because the application of placebo cream could increase the sensitivity of Aδ fibres to electrical stimulation which could confound placebo responses (see Chapter 6 for a full discussion).

An interim analysis of data collected in Chapter 6 showed that NPS ratings in the experimental and the control groups increased after application of the placebo cream. In other words, ratings of the same stimulus were higher in block 3 (after cream application) than in block 1 (before cream application). We conducted a series of 3 pilots to investigate the reason for this increase. For completeness, we present the initial results collected before the interim analysis, followed by the series of 3 pilots.

2.5.3.2. Methods

In the initial data collected before the interim analysis, 42 participants (22 control and 19 experimental participants) underwent the placebo conditioning procedure described in section 6.4. In all the three pilots described below we replicated the procedure of the placebo experimental group (i.e. we carried out a placebo analgesia manipulation) because we were interested in eliciting a placebo response. Because the overall sample size here was 41, we carried out statistical analysis on these data. Average NPS pain intensity ratings for each participant and condition were entered into a 2 (group: experimental vs. control) x 2 (block: 1 vs. 3) mixed-model ANOVA.

In pilot 1, the placebo cream was immediately before block 2 instead of immediately after block 1, in order to decrease any peripheral effects of the cream being on the skin for a

sustained amount of time during the 10 minute break between block 1 and 2. 11 participants took part in this pilot. In pilot 2, we removed the influence of the placebo cream altogether by instead using a 'transdermal analgesic patch' as the placebo manipulation. Topical pain relief patches such as fentanyl patches are available commercially so should be reasonably familiar to participants as an analgesic treatment. Five participants took part in this pilot. In pilot 3, we made three final adjustments to the procedure. We delivered a minimal and controlled amount of cream to the participant. We also delivered five test pulses immediately before block 2 to test whether participant sensitivity to the stimulation had changed, and if it had, we adjusted the intensity of block two stimuli to account for this. To increase expectation of pain relief, we instructed participants that the analgesic was fully effective for one hour after administration. Eight participants took part in the final pilot. Because the sample size was low in all pilots, we did not carry out statistical analyses.

2.5.3.3. Results

In the initial data collected before the interim analysis, both the experimental group and the control group showed an increase in pain intensity rating in block 3 compared to block 1 (see table 2.3). The mixed-model ANOVA revealed a main effect of block ($F(1,34)=10.65$, $p=0.003$, $\eta_P^2=.24$) (block 3>block 1), but no interaction with group ($F(1,34)=.24$, $p=0.63$, $\eta_P^2=.01$), suggesting that pain ratings increased in block 3 in both the experimental and control group.

In Pilot 1, block 2 pain intensity ratings were decreased, suggesting applying the placebo cream at the beginning of block 2 had minimised any increased sensitivity to the stimulation. However, pain intensity ratings still increased between block 1 and block 3. Introduction of the analgesic patch in pilot 2 resulted in similar results to Pilot 1, with block 2 ratings decreased, but block 1 lower than block 3 ratings (table 2.3). In pilot 3, participants showed a strong reduction in pain intensity ratings in block 2, suggesting that stimulation in block 2 was being felt as adequately low to achieve placebo conditioning. Pain intensity ratings did not increase between block 1 and block 3 (table 2.3). See figure 2.7 for a summary plot of all pilot data.

Table 2.3. *Sample sizes and pain intensity ratings for block 1, 2 and 3 of the pre-interim data check and the three pilots*

Session	Group	N	Block 1 average pain intensity rating (*sd*)	Block 2 average pain intensity rating (*sd*)	Block 3 average pain intensity rating (*sd*)
Pre-interim check	Experimental	19	6.1 (0.8)	5.2 (1.33)	6.6 (1.31)
	Control	22	5.9 (1.03)	3.7 (1.27)	6.5 (0.89)
Pilot 1	Experimental	11	5.6 (0.87)	4.2 (1.05)	6.2 (1.05)
Pilot 2	Experimental	5	5.6 (0.93)	3.9 (1.11)	6.1 (1.74)
Pilot 3	Experimental	8	6.1 (0.81)	3 (0.9)	6 (1.18)

2.5.3.4. Discussion

Analysis of the data collected before the interim analysed revealed that pain intensity ratings were increased in block 3 compared to block 1. Inspection of the group means revealed that pain intensity ratings of block 2 (the conditioning block) in the experimental group were too high to achieve a placebo conditioning effect (table 2.3). We deduced that the placebo cream which was left on the arm for the ten minute break between block 1 and 2 may have increased sensitivity to electrical stimulation.

In pilot 1 the placebo cream was applied immediately before block 2 instead of immediately after block 1, so it was not on the arm for the ten minute period. In pilot 2 we used a placebo transdermal analgesic patch instead of a placebo cream. Both of these pilots resulted in decreased pain intensity ratings of block 2. Despite resolving this potential issue in the conditioning block, pain intensity ratings still increased in block 3 compared to block 1.

We did not use a placebo transdermal analgesic patch in the main study because the aim was to replicate previous placebo cream studies using electrical pain. In pilot 3, to address any potential peripheral effects of the cream, we delivered a minimal, controlled amount of cream to the participant. We also delivered five test pulses immediately before block 2 to test whether participant sensitivity to the stimulation had changed, and if it had, we adjusted the intensity of block two stimuli to account for this. Finally, to address any psychological difficulties in the manipulation, to increase participant's expectation of pain relief, we instructed participants that the analgesic was fully effective for one hour after administration. The results showed that pain intensity ratings of block 2 stimuli were not increased by the administration of the placebo cream. Also, the pain intensity ratings did not increase in block 3 compared to block 1. These ratings did not exhibit a placebo effect (a reduction in block 3 compared to block 1), but we reasoned that the placebo effect would be unlikely to be observed with a sample size of 8. With a greater sample size, the

placebo effect may be observed. Hence, we collected a sample size of 40 participants, having made these adjustments.

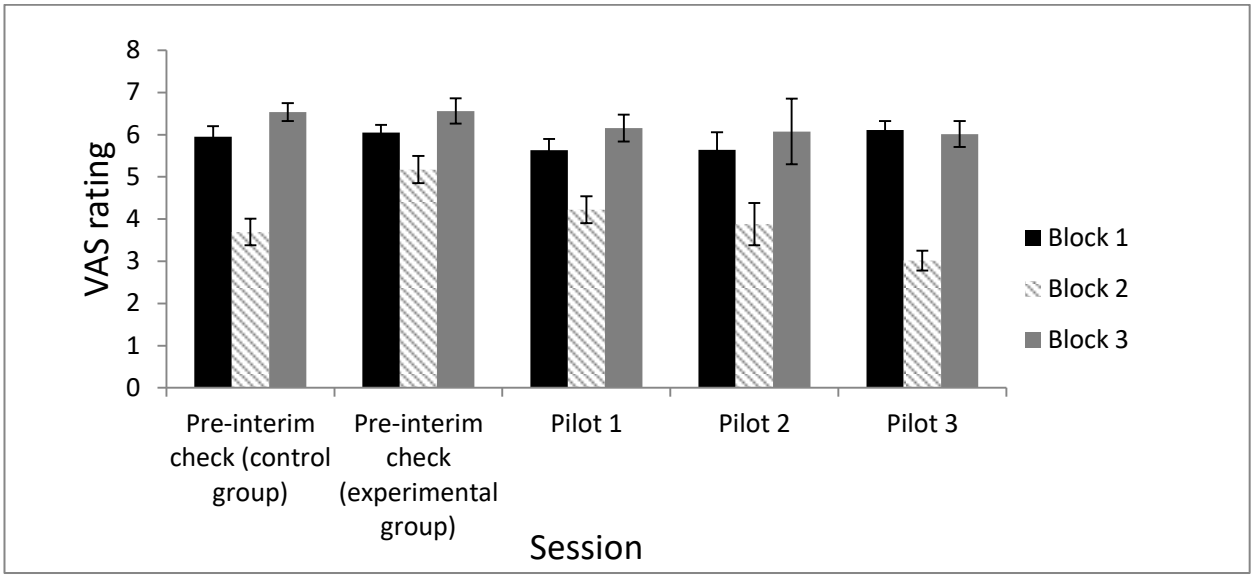


Figure 2.7. *Placebo analgesia to electrical pain stimulation pilot data. Pain intensity ratings of painful stimulation in block 1, 2 and 3 for the four datasets collected. Ratings of block 2 and 3 were decreased by the adjustments made by the manipulation.*

Chapter 3: Temporal dissociation of salience and prediction error responses to appetitive and aversive taste

A version of this Chapter is published in *Psychophysiology*.

Hird EJ, El-Deredy W, Jones A, Talmi D. Temporal dissociation of salience and prediction error responses to appetitive and aversive taste. *Psychophysiology*. 2017;1–13. https://doi.org/10. 1111/psyp.12976

The results were verbally presented at the PSYCH Associative Learning Symposium (2016) and the Princeton Neuroscience Institute, Princeton University, Daw lab (2017). The results were also presented in a poster format at the British festival of neuroscience, British Neuroscience Association (2015), The International Society of Research on Emotion (2015) and The Cognitive Neuroscience Society Annual Meeting (2016).

Emily Hird, Wael El-Deredy, Anthony Jones and Deborah Talmi designed the study. Emily Hird collected the data, analysed the data, and wrote the paper, with input at all stages from Wael El-Deredy, Anthony Jones and Deborah Talmi.

Abstract

The FRN, a frontocentral ERP occurring 200-350 ms after emotionally-valued outcomes, has been posited as the EEG correlate of reward prediction error, a key component of associative learning. Recent evidence challenged this interpretation and has led to the suggestion that this ERP expresses salience, instead. Here we distinguish between utility prediction error and salience by delivering or withholding hedonistically matched appetitive and aversive tastes, and measure ERPs to cues signalling each taste. We observed a typical FRN (computed as the loss-minus-gain difference wave) to appetitive taste, but a reverse-FRN to aversive taste. When tested axiomatically, frontocentral ERPs showed a salience response across tastes, with a particularly early response to outcome delivery, supporting recent propositions of a fast, unsigned and unspecific response to salient stimuli. ERPs also expressed aversive prediction error peaking at 285ms, which conformed to the logic of an axiomatic model of prediction error. With stimuli that most resemble those used in animal models we did not detect any frontocentral ERP signal for utility prediction error, in contrast with dominant views of the functional role of the feedback-related negativity ERP. We link the animal and human literature and present a challenge for current perspectives on associative learning research using ERPs.

3.1. Introduction

The reward prediction error hypothesis of associative learning provides a foundational understanding of adaptive behaviour and is used widely to explain the neuroimaging correlates of associative learning in humans (Holroyd & Coles, 2002; O'Doherty, Hampton, & Kim, 2007). The seminal finding that sparked much of this research is that midbrain dopamine neurons express reward prediction error, increasing their firing to unexpected reward and reducing their firing to unexpected omission of reward (Schultz et al., 1997), with an impressive homogeneity in the firing of individual dopamine neurons (Eshel, Tian, Bukwich, & Uchida, 2016). The term 'reward' is inherently related to the term 'utility', an economic term that denotes the subjective value of an outcome, from 'good' to 'bad' (Friedman & Savage, 1952). Reward prediction error specifically refers to the signal the brain is thought to compute when it encounters an unexpected delivery or omission of an appetitive outcome, but the literature often assumes that reward prediction error signals actually reflects the utility of either appetitive or aversive outcomes (Schultz, 2016). This assumption means that unexpected delivery of appetitive outcomes should be signalled similarly to the unexpected omission of aversive outcomes.

This interpretation of the midbrain dopamine signal has been challenged by evidence that some dopamine neurons respond with phasic bursts to the delivery of *both* appetitive and aversive outcomes, suggesting that they code salience, instead of utility (Brischoux, Chakraborty, Brierley, & Ungless, 2009; Bromberg-Martin et al., 2010; Joshua, Adler, Mitelman, Vaadia, & Bergman, 2008). Schultz (2016) proposed that the challenge salience presents to the interpretation of midbrain dopamine firing as a reward prediction error can be addressed by distinguishing between two temporally separate signals within 500 ms of a predictive cue: an initial non-discriminative response to various forms of salience, between 100-200ms from the cue, followed by a utility prediction error signal from 150-350ms (Schultz, 2016). Schultz (2016) listed three reasons for this initial salience response. Physical salience refers to physical attributes such as size and colour, motivational salience refers to the ability of an outcome to elicit attention due to its high motivational relevance, while surprise salience refers to the unexpectedness or novelty of an outcome. Clearly, both appetitive and aversive outcomes can have high motivational and surprise salience (Sambrook & Goslin, 2014). Higher salience of stimuli increases their ability to capture attention. For example, an unpleasant taste is low in utility and so could elicit a negative reward prediction error, but is also high in motivational salience, because it represents a potentially harmful substance which would attract attention so that it is avoided in the future (Esber & Haselgrove, 2011). A negative reward prediction error

would be expressed by a reduction in neural activity, whereas a salience response would be expressed by an increase.

Distinguishing between utility and salience is fundamental in the burgeoning literature in neuroeconomics and affective neuroscience. This is evident in recent meta-analyses of human neuroimaging (Bartra, McGuire, & Kable, 2013; Lindquist, Satpute, Wager, Weber, & Barrett, 2015; Sambrook & Goslin, 2015a). The doubt regarding what dopamine neurons compute has triggered a re-examination of the functional role of a key ERP that is thought to be dopaminergically mediated. The FRN is thought to originate from dopaminergic projections to the ACC, evident by the finding that it is modulated by dopamine agonists (Holroyd & Coles, 2002; Santesso et al., 2009; Walsh & Anderson, 2012) and combined EEG-fMRI work (Hauser et al., 2014) . The dominant theory contends that the FRN expresses utility prediction error, but recent studies provided evidence that it expresses salience (Garofalo et al., 2014; Hauser et al., 2014; Huang & Yu, 2014; Pfabigan et al., 2015; Sambrook & Goslin, 2015b; Talmi et al., 2013). Those studies showed that the FRN difference-wave reflects a negative deflection when any outcome – appetitive or aversive – is unexpectedly omitted, as proposed by the predicted response-outcome (PRO) model (Alexander & Brown, 2011) and in line with an interpretation of the FRN as expressing motivational salience rather than utility prediction error signal.

A criticism of those studies is that most appetitive and aversive outcomes were not well-matched. For example, in two studies that used money and physical pain as reinforcers (Heydari & Holroyd, 2016; Talmi et al., 2013) the response latency of the frontocentral EEG signal differed (Heydari & Holroyd, 2016). While disparate spatiotemporal dynamics are unsurprising when appetitive and aversive outcomes have different hedonic values and are drawn from different modalities, as in both of these studies, such spatiotemporal differences make it difficult to argue that a signal expresses the psychological variable of salience in both appetitive and aversive conditions. Importantly, in traditional FRN studies that use financial outcomes, ostensibly matching modality and outcome value, monetary gain may represent a different level of motivational salience than monetary loss, so that the dimension of value could be confounded by that of salience (e.g. Bai, Katahira, & Ohira, 2015; Hauser et al., 2014; Weismueller & Bellebaum, 2016). Studies that employ financial outcomes rarely match the utility of reinforcement (e.g. Bai et al., 2015; Hauser et al., 2014) so that an FRN could stem from greater emotional and ensuing cognitive impact of losses over gains, as described in prospect theory (Sambrook, Roser, & Goslin, 2012). Indeed, there is evidence that midbrain neurons signal to negative more than positive prediction errors, suggesting negative prediction errors have greater salience (Rodriguez, Aron, & Poldrack, 2006). Because electrophysiological recordings demonstrate that different

temporal ranges of the signal express different psychological variables, it is important to match outcome modality and utility.

An fMRI study that addressed the issue of salience and utility with well-matched outcomes, using taste reinforcers, observed a salience but not a utility prediction error response (Metereau & Dreher, 2013), in accordance with other fMRI studies that examined this distinction with other outcomes (Gu et al., 2016; Hauser et al., 2014). However, the recent realisation that differences between the dopaminergic signature of utility prediction error and salience can be discerned in different temporal ranges of the signal (Schultz, 2016) means that unique utility responses in those studies were perhaps lost to temporal smearing in fMRI work. Here we used EEG, which has greater temporal resolution, to test whether any ERP could be detected which expresses utility prediction error rather than salience across well-matched appetitive and aversive outcomes.

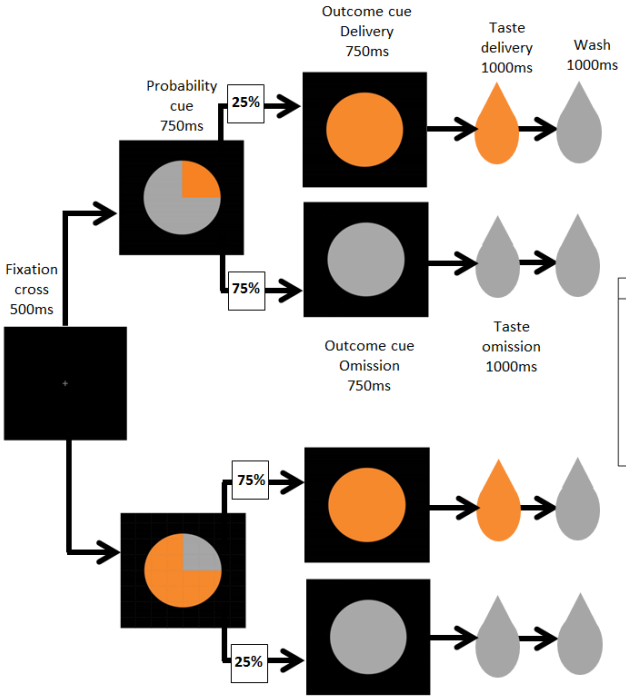
We used taste reinforcers, which closely resemble reinforcers used in the animal models that the associative learning literature uses as its key interpretative framework. Indeed, while previous research has demonstrated the feasibility of using taste in appetitive conditioning in EEG (Franken, Huijding, Nijs, & van Strien, 2011), ours is the first ERP study of taste prediction error. Importantly, we used hedonically equidistant sweet and bitter tastes, which circumvents the confound of utility and motivational salience present in traditional FRN studies and allows a direct comparison of the signal in the appetitive and aversive conditions, to distinguish between utility and salience response. We observed an FRN for appetitive outcomes, but a reverse-FRN for aversive outcomes. Further analysis exploited the logic of the axiomatic model of prediction error (described under 'Design' below) to assess whether signal codes salience or utility prediction error (Roy et al., 2014; Rutledge et al., 2010). We found ERPs that expressed salience and an aversive prediction error; we did not observe an ERP expression of utility prediction error. These results are important because they contradict the dominant theory of the FRN and reveal the limits in what we can observe on the human scalp.

3.2. Methods

3.2.1. Design

Axiomatically, a neurobiological signal of utility prediction error should be expressed as a specific form of interaction between outcome and expectancy (Rutledge et al., 2010). When good outcomes (here, delivered sweet and omitted bitter taste) and bad outcomes (omitted sweet and delivered bitter taste) are expected, the signal should not differentiate between them, so expected outcomes form a baseline for comparison, in agreement with

direct recordings of dopaminergic neurons (Schultz et al., 1997). The difference between good and bad outcomes should be pronounced when outcomes are unexpected. This logic of the original model requires a manipulation of valence and expectancy but not of outcome domain (appetitive/aversive). It would be enough, for example, to cross expectancy with delivered and omitted sweet taste, or with delivered sweet and delivered bitter taste. A manipulation of valence and expectancy is not enough, however, to convincingly show that a signal expresses utility prediction error. This is because a utility prediction error signal must express both appetitive and aversive prediction errors, namely, it must exhibit the interaction described above separately in the appetitive (here, sweet) and aversive (here, bitter) domain. Moreover, although it is not known whether *more* dopamine corresponds to *more* or *less* positive amplitude in any particular instance, if the funnel plot for the appetitive domain shows that unexpectedly delivered positive outcomes are more positive than unexpectedly omitted positive outcomes, the opposite should then hold in the aversive domain, where unexpectedly delivered negative outcomes should be *less* positive than unexpectedly omitted negative outcomes. Therefore, to investigate whether the ERP signal expresses utility prediction error or salience we manipulated taste: (bitter/sweet), expectancy (expected/unexpected) and outcome (whether taste is delivered/omitted) within subjects (Figure 3.1). This factorial design is appropriate to investigate whether any neurobiological signal conforms to the axiomatic model (Rutledge et al., 2010). A difference wave approach cannot tell us, for example, whether unexpected good outcomes diverge away from expected outcomes in the opposite direction than unexpected bad outcomes.



Taste	Magnitude	Expectancy	Outcome	Number of trials
Sweet	High	Expected	Delivered	135
			Omitted	135
		Unexpected	Delivered	45
			Omitted	45
Bitter		Expected	Delivered	135
			Omitted	135
		Unexpected	Delivered	45
			Omitted	45

Figure 3.1. *Experimental paradigm. Left: The timeline of each trial. After a fixation cross, a veridical probability cue set the expectation of the taste delivery/omission chance of the trial. An outcome cue followed, declaring the impending true outcome of the trial, deliver/omit, which triggered a theoretical prediction error. The taste was delivered, or omitted, based on the outcome cue. A rinse solution of artificial saliva prepared the participant for the next trial. Right: Number of trials in each condition. 'Expected' outcomes were presented 75% of the time, and 'unexpected' outcomes 25% of the time, to match the veridical probability cue.*

Motivational salience is operationalised as a main effect of outcome, differentiating cues that predict delivered taste from those that predict omitted taste. Surprise salience is operationalised as a main effect of expectancy, differentiating cues that are expected from those that are unexpected. These main effects could be expressed regardless of whether the taste is appetitive or aversive. To maintain the perceived salience of experimental taste delivery, a subset of additional trials in the experiment delivered low magnitude tastes (slightly sweet, slightly bitter), which were administered in keeping with the same experimental design, but not analysed. Bitter and sweet tastes were delivered in separate blocks, to prevent reward generalisation (Schultz, 2016), namely a response to the bitter taste because participants were expecting the sweet taste.

3.2.2. Participants

Twenty participants aged 18-35 (12 females, mean age 20 years) received £20 compensation or course credit points for participation in the study. Participants had normal or corrected-to-normal vision. They had no history of neurological or psychiatric conditions, no metabolic disorder and were not taking any centrally-acting medication or any medication which could make it difficult to fast for 2 hours. Ethical approval was granted by the University of Manchester, where the study took place.

3.2.3. Materials

Pilot studies identified four concentrations of sucrose and water (sweet) and four concentrations of quinine and water (bitter), which elicited a range of hedonic ratings of pleasantness and unpleasantness, respectively. For sweet those were 1.0, 0.6, 0.2 and 0.1 mole of sucrose, and for bitter those were 1.0, 0.75, 0.25 and 0.05 millimole of quinine. Each solution also included 10 millilitres of lemon taste per litre of liquid to balance the sweetness of the sucrose whilst maintaining symmetry between sweet and bitter taste.

For each participant, two concentrations from each taste were selected, corresponding to equivalent high and low taste magnitude pairs, whose hedonic ratings were within 10% of one another (see procedure). The low magnitude hedonic ratings were at least 20% less than the high magnitude concentrations for that participant.

A neutral solution was designed to mimic the ionic balance of saliva to minimise reward associated with the liquid, and comprised of distilled water, 0.012 moles sodium bicarbonate and 0.012 moles potassium chloride.

Taste magnitude and likelihood were communicated to participants by visual cues, represented in figure 3.1, which were matched for luminance. The experiment was conducted on a Matlab platform (Mathworks).

3.2.4. Apparatus

Participants' heads were stabilized using a chin rest. Four rubber tubes (bore 2mm, wall 0.5mm, Altec Product LTD) were attached to the chin rest and to the participant's face with medical tape. The tubes were attached to 50ml syringes (Plastipak syringe 50ml Luerlok, Fisher Scientific) fitted into pumps (Harvard Apparatus, pump 33) in the neighbouring room. Pump activity was controlled by software (Matlab, Mathworks).

3.2.5. Procedure

Participants made two visits to the lab, and were instructed to avoid food and water for two hours prior to each visit.

On the first visit, in order to establish equivalent behavioural responses in bitter and sweet, participants rated four concentrations of quinine and four concentrations of sucrose using the LMS, a validated scale for collection of intensity ratings (Hayes et al., 2013). Participants were instructed to place a mark on the line where their perceived intensity of pleasantness for sweet, or unpleasantness for bitter, lay. Bitter and sweet were administered in separate blocks. Block order was randomised. The taste stimulus was delivered for 1000ms. Participants then rated the taste on the LMS and had a sip of water before moving on to the next taste. Participants rated each stimulus five times in each block. Trial order was randomised. On the basis of those ratings, four concentrations were selected for each participant (see Materials).

On the second visit, participants were sat in a quiet, dimly lit room. A total of 720 trials were administered in the high magnitude condition. Each trial lasted 10 seconds, and the entire session lasted 3 hours. Bitter and sweet were administered in separate, alternating and counterbalanced blocks. The different magnitude and probability conditions were presented pseudorandomly within each block.

Participants were advised at the beginning of a block whether it was a 'sweet' block, or a 'bitter' block. Figure 3.1 depicts a schematic of the design and a timeline of each trial. A fixation cross was presented for 500ms followed by a veridical probability cue presented for 750ms which set up expectations: either 75% probability of taste delivery and 25% taste omission, or 75% probability of taste omission and 25% taste delivery. Next, an outcome cue was presented for 750ms. The outcome cue signalled the actual outcome (with 100% probability) of either delivery (orange) or omission (grey) of the taste. The probability of the deliver/omit outcome cues followed the statistics of the expectation cues: On trials with 25% probability of taste delivery, the taste delivery outcome cue was presented 25% of the time, and on trials with 75% probability of taste delivery, the taste delivery outcome cue was presented 75% of the time. Next, the taste was delivered, or omitted, based on the outcome cue. On 'omit' trials a neutral solution was delivered. Both taste solutions and neutral solutions were administered for 1000ms. Finally, a wash of neutral solution for 1250ms prepared the participant for the next trial. There was a pause of 2500ms during which the screen was black before the next trial began (see figure 3.1).

Breaks were provided every 40 trials. Every 80 trials a randomly selected cue was presented to participants and they were asked to identify whether they had seen this in the last 40 trials. This was designed as a check that participants were paying attention to the images on the screen.

3.2.6. EEG recording

Continuous EEG recording was acquired at a sampling rate of 512 Hertz using a 64 electrode Active-Two amplifier system (Biosemi, Amsterdam, Netherlands) with Actiview acquisition software (Biosemi, Netherlands). Here, an active and passive electrode replaces the ground electrode to create a feedback loop that drives the average potential of the subject (the common mode voltage) as close as possible to the analogue-to-digital converter reference voltage in the analogue-to-digital box. Vertical and horizontal electro-oculograms (EOG) was measured for detection of eye-movement and blink artefacts. Impedances were kept at 20 KΩ or less. The experiment was conducted in a dimmed, quiet room.

3.2.7. EEG data analyses

3.2.7.1. Preprocessing

The ERP time-locked to the outcome cue was preprocessed using SPM12 (Ashburner et al., 2013; Litvak et al., 2011). The signal was re-referenced to the mean of all scalp electrodes, downsampled to 200 Hertz (Hz), and filtered with a Butterworth filter between 0.1 and 30 Hz. Epochs were extracted 200ms before the outcome cue to 600ms after, importantly avoiding the actual delivery of any fluid, which occurred 750ms after the outcome cue. Artefact rejection was achieved by following two steps. Firstly, eyeblinks were modelled and underwent artefact rejection at a lenient threshold of 150uV. The resulting eyeblinks model was used to correct for eyeblinks, using the singular value decomposition (SVD) technique implemented in SPM12. Any remaining trials in which the signal in any of the electrodes exceeded 80μV were rejected. On average, 17% of trials were removed across participants and conditions. One participant was removed from the analysis due to high noise levels in the ERP signal. Single-trial data were averaged separately for the eight conditions using the "robust averaging" method in SPM12b (Litvak et al., 2011). Robust averaging takes into account distribution of data for every channel and trial by down-weighting outlier trials. Weights were determine for each condition separately so as not to unduly distort signal in unexpected trials which, by definition, had fewer trials than the expected condition. Averages were then filtered with a low-pass filter with a cut-off of 30Hz to remove high frequencies introduced by the robust averaging method. These preprocessed signals were

used in all of the analyses reported later on in the manuscript. They were only additionally manipulated in the SPM analysis, as reported below.

3.2.7.2. Analysis of habitation

We obtained ERPs time-locked to taste delivery to test for ERP habituation to the actual delivery of taste over time. For the purpose of the habituation analysis we analysed response to the taste itself (rather than to the cue). Although there is a risk that signal-to-noise ratio may be decreased in taste ERP trials due to muscle artefacts associated with the stimulus, previous research has confirmed an ERP response to taste at electrodes Fz, F3, F4, Cz, C3 and C4 (Franken et al., 2011; Kobal, 1985). We averaged across these electrodes within the window of taste delivery (0-1000ms after taste onset) for the first and second half of the experiments for the four conditions where taste was delivered, regardless of how expected the taste was. These data were entered into a 2 (taste) x 2 (experiment half) repeated-measures ANOVA using a threshold of $p < .05$ in SPSS.

3.2.7.3. Difference wave analysis of the FRN

First, we conducted a difference-wave analysis to facilitate comparison with previous results. We extracted data from vertex electrodes Fz, FCz and Cz, 240-340ms after the outcome cue, following the recommendations of a meta-analysis which identified this spatiotemporal window as the most likely latency and location of the FRN signal of prediction error (Sambrook & Goslin, 2015a). Difference waves representing unexpected outcomes were computed using the conventional loss-minus-gain technique. In sweet we computed the omission-delivery difference wave, and in bitter we computed the delivery-omission difference wave. We conducted a one-sample t-test on this signal, following the analysis protocol of a recent study of the FRN to appetitive and aversive outcomes using a threshold of $p < .05$ (Heydari & Holroyd, 2016).

3.2.7.4. SPM analysis of frontocentral electrodes in the 200-380ms time window

The FRN literature lacks consistency in measuring the FRN. The meta-analysis we relied on to compute the difference waves above (Sambrook & Goslin, 2015a) acknowledged that variability in methods may complicate a blanket application of that time window. Though the FRN is linked to activity in the rostral and dorsal ACC (Hauser et al., 2014; Nieuwenhuis, Slagter, Von Geusau, Heslenfeld, & Holroyd, 2005; Walsh & Anderson, 2012) and well-defined as being expressed at frontocentral electrodes after 200ms, the specific electrode and latency varies between studies, meaning the peak signal may be overlooked.

This is a particular issue in more complex experimental designs or where novel modalities, such as pain, could change the morphology of the signal (Garofalo et al., 2014; Talmi et al., 2013). Because we used a novel feedback modality here (taste), and because the difference-wave approach does not allow a test of the predictions of the axiomatic model (Talmi, Fuentemilla, Litvak, Duzel, & Dolan, 2012), we employed an additional data-driven approach for data analysis.

GFP is a technique that measures variance across all electrodes, conditions, and participants, and has traditionally been used to select spatiotemporal analysis windows (Lehmann & Skrandies, 1980; Skrandies, 1990). Using GFP we observed two peaks in frontocentral ERP activity, where the FRN is normally expressed (Walsh & Anderson, 2012), 200-380ms from the outcome cue (figure 3.2). This window was used for statistical analysis with SPM, an established technique (Litvak et al., 2011) which employs the General Linear Model to estimate parameters over electrodes and time, and which has been successfully used to study the FRN (Hauser et al., 2014; Litvak et al., 2011; Talmi et al., 2013). For this analysis the preprocessed data were converted to a single three-dimensional space by time image for each subject and condition. This conversion is achieved by generating a scalp map for each condition and stacking these maps over peristimulus time. The resulting images were smoothed using a Gaussian kernel full-width at half-maximum of 8 mm/ms. Individual smoothed images for each condition were entered into two statistical models, one for each taste, and analysed with a 2 (expectancy: expected/unexpected) x 2 (outcome: delivered/omitted) repeated-measures ANOVA. Higher-order effects were analysed first, and, used to mask exclusively the analysis of lower-order effects. Following (Talmi et al., 2013), a peak threshold of $p < .005$ and a cluster extent threshold of 100 voxels was used. All key results are reported corrected for multiple comparisons at the cluster level using a strict FWE < .05.

3.3. Results

3.3.1. Behavioural results

We ran a 2 (magnitude: high, low) x 2 (taste: bitter, sweet) factorial ANOVA on the ratings of intensity for bitter and sweet for the concentrations we selected for each participant. Unsurprisingly, there was a significant main effect of magnitude on the ratings, where high magnitude tastes were rated as significantly more intense than low ($F(1,18) = 62.17$, $p<.001$, $\eta_P^2 = .775$). There was no significant effect of taste ($F(1,18) = 3.135$, $p=0.94$, $\eta_P^2 =.148$), and no interaction ($F(1,18) =.835$, $p=.373$, $\eta_P^2 =.044$). Participants were 97% accurate in

identifying whether or not they had seen a cue in the previous 40 trials, which confirmed that they were attending to the cues.

3.3.2. Electrophysiological results

Figure 3.2 shows the detailed ERP responses to taste, with a more positive response to delivery than omission of unexpected bitter and sweet taste.

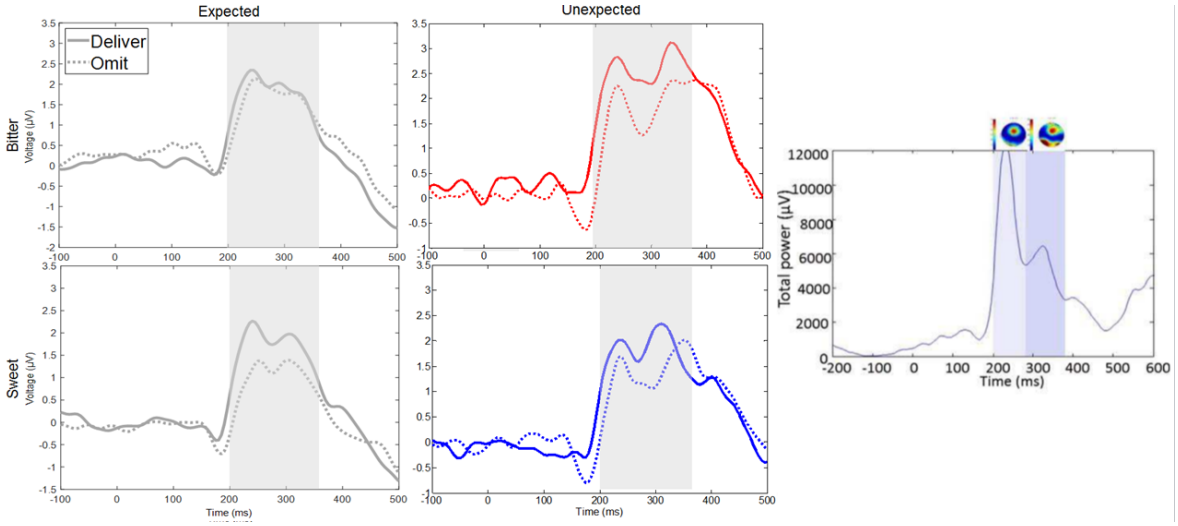


Figure 3.2. *Detailed results. Left: group average ERPs for all conditions. ERPs at frontocentral electrodes over an average of 112 trials in the expected conditions and 37 trials in the unexpected conditions and time-locked to the outcome cue presented at oms. Right: Global field power. Global field power identified two peaks in time windows 200 to 380ms post outcome cue. Topographic plots at the top of this panel show the topography of the global field power for the two time-windows.*

3.3.2.1. Habituation

Taste-elicited amplitudes showed no significant effect of taste ($F(1,18)=1.309$, $p=.268$, $\eta_P^2=.068$), experiment half ($F(1,18)=2.204$, $p=.155$, $\eta_P^2=.109$), or interaction ($F(1,18)=.016$, $p=.902$, $\eta_P^2=.001$), suggesting participants did not habituate to the taste.

3.3.2.2. Difference-wave analysis of the FRN

Figure 3.3 depicts a significant FRN in sweet ($t(18)=-2.152$, $p=.045$), replicating previous work. Crucially, in bitter we observed a significant 'reverse' FRN ($t(18)=2.78$, $p=.012$), replicating our previous findings with pain (Talmi, Anderson & El-Deredy, 2013) (figure 3.3).

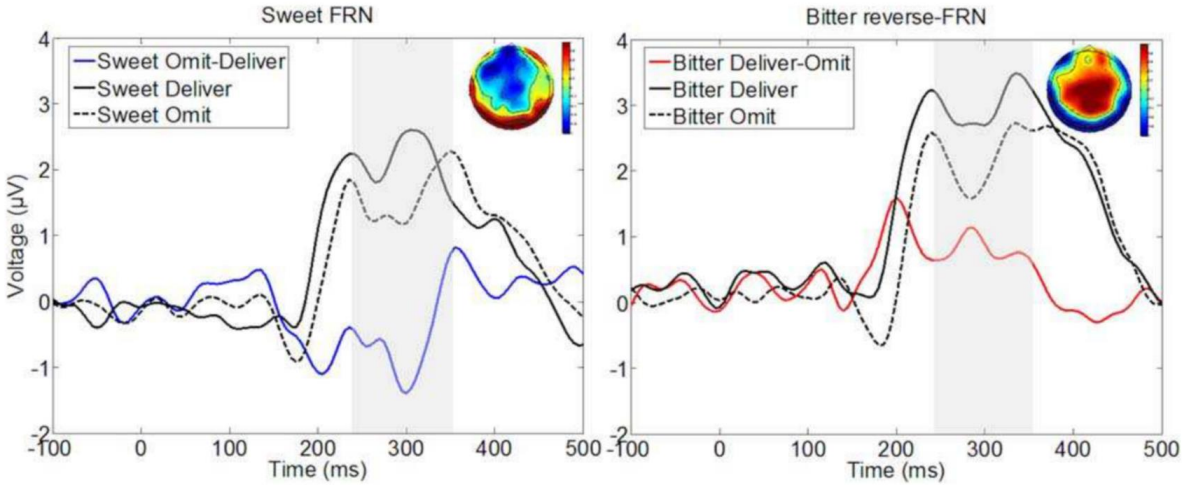


Figure 3.3. *Difference-wave FRN analysis. Difference waves subtracting unexpected loss from unexpected gain at Fz, FCz and Cz over an average of 112 trials in the expected conditions and 37 trials in the unexpected conditions and time-locked to the outcome cue presented at oms. The search volume (240-340ms), based on a meta-analysis, is shaded. Topographic plots show the unexpected loss-gain difference wave topographies for the shaded time-window.*

3.3.2.3. SPM analysis of frontocentral electrodes in the 200-380ms time window

3.3.2.3.1. Aversive and reward prediction error.

The outcome by expectancy interaction was analysed for each taste. Figure 3.4 shows that the pattern of this interaction was similar in both tastes, but more pronounced in bitter. In bitter, the interaction was expressed in a significant cluster peaking at 285ms, which extended 251-317ms after the outcome cue (peak at C2, x=17.00, y=-3.25, cluster p(FWE) =.032, cluster size 618 voxels). We conducted further analyses to unpack this interaction. Masked inclusively by the interaction of outcome and expectancy, unexpected delivered outcomes were significantly more positive than omitted (peak at C2, x=12.75, y=-8.63, extending 200-368ms after the outcome cue, cluster p(FWE)<.001, cluster size 2855 voxels). There was no significant difference between expected delivered and omitted outcomes. Unexpected delivered outcomes were significantly more positive than expected (peak at Cz, x=0.00, y=-8.63, extending 200-380ms after the outcome cue, cluster p(FWE) =.003), and unexpected omitted outcomes were significantly more negative than expected (peak at FC4, x=34.00, y=7.50, extending 269-295ms after the outcome cue, peak p (FWE)<.001). As shown in figure 3.4, this funnel-shaped signal adhered to the criteria of an axiomatic model of prediction error in the aversive domain. In sweet the outcome by

expectancy interaction did not survive our significance threshold. It can be readily appreciated that the numerical pattern of the nonsignificant interaction in sweet contradicts an interpretation of the frontocentral signal as a utility prediction error, because in both tastes unexpected delivered outcomes – good in sweet, bad in bitter - yield more positive amplitudes than unexpected omitted outcomes.

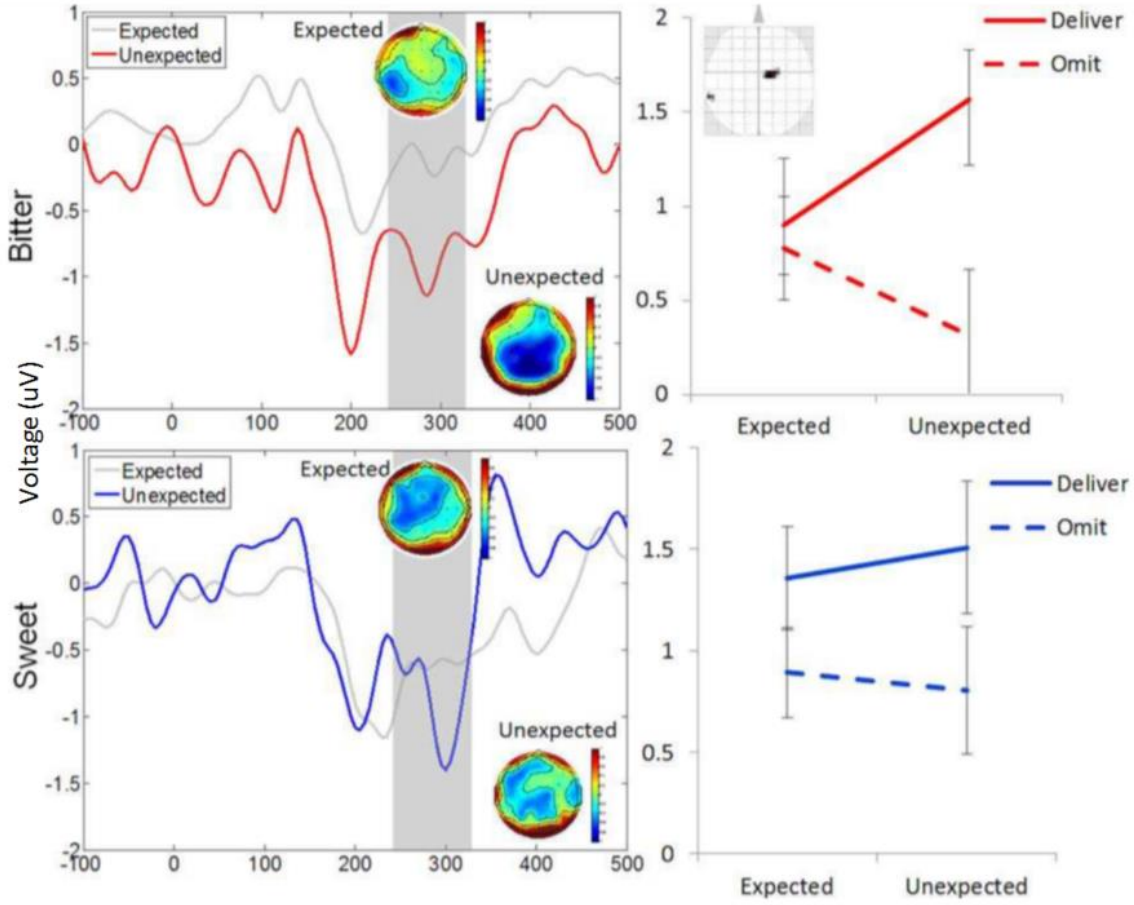


Figure 3.4. *Aversive prediction error but no reward prediction error. Left: Difference waves subtracting delivered from omitted outcomes. The difference waves were averaged over frontocentral electrodes across participants over an average of 112 trials in the expected conditions and 37 trials in the unexpected conditions and time-locked to the outcome cue presented at 0ms. The temporal boundaries of the significant interaction are shaded in grey. In the sweet condition (lower left) the interaction was not significant. Right: Voltages at the peak voxel of the cluster (inset, plotted on the SPM glass brain) corresponding to the significant outcome by expectancy interaction cluster in the bitter condition (upper right) and the non-significant outcome by expectancy interaction in the sweet condition (lower right). Error bars represent the standard error of the mean.*

3.3.2.3.2. Motivational salience

The analysis of the main effect of outcome in each taste was masked exclusively by the interaction of outcome and expectancy. In bitter, the effect of outcome yielded a frontocentral cluster where amplitude for delivered outcomes was more positive than for omitted outcomes, peaking at 215ms and extending 200-287ms after the outcome cue (peak at FC1, x=-21.25, y=2.13, cluster p(FWE)<.001, cluster size 3582 voxels). A similar result was obtained in sweet, where a frontocentral cluster peaked at 220ms and extended 200-265ms after the outcome cue (peak at C1, x=-17.00, y =-3.25, cluster p(FWE) =.001, cluster size 2181 voxels).

3.3.2.3.3. Surprise salience

The analysis of the main effect of expectancy in each taste was again masked exclusively by the interaction of outcome and expectancy. In bitter, the effect of expectancy was expressed in a frontocentral cluster peaking at 370ms and extending 306-380ms, where amplitude for unexpected outcomes was more positive than amplitude for expected outcomes (peak at FCz , x=-8.50, y=7.50, cluster p(FWE)<.001, cluster size 2861 voxels). Again, a similar result was obtained in sweet, where the effect of expectancy yielded a frontocentral cluster peaking at 375ms (peak at C1, x =-8.5, y=-3.25, 309-380ms, cluster p(FWE)<.001, cluster size 2525 voxels).

3.3.2.3.4. Analysis across tastes

The analyses above yielded a number of similarities across tastes which are explored here with a more comprehensive model, including taste as an additional within-subject factor. Although the outcome by expectancy interaction was of different magnitudes in bitter and sweet, the three-way interaction between outcome, expectancy, and taste was not significant. The two-way outcome by expectancy interaction (across bitter and sweet tastes) yielded a frontocentral cluster peaking at 285ms which extended 273-317ms after the outcome cue (peak at C2, p<.001, x=21.25, y=-3.25). While significant, this activation did not survive our conservative correction for multiple comparisons; this is clearly because the weak interaction in sweet attenuated the highly significant interaction in bitter. The main effect of outcome, masked exclusively by the interaction of outcome and expectancy, yielded a frontocentral cluster peaking at 220ms and extending 200-342ms after the outcome cue (peak at C1, x=-21.25, y=-3.25, cluster p (FWE)<.001, cluster size 4168 voxels). The main effect of expectancy masked exclusively by the interaction of outcome and expectancy yielded a frontocentral cluster peaking at 370ms and extending 203-380ms

after the outcome cue (peak at FCz, x=-4.25, y=2.13, cluster p (FWE)<.001, cluster size 3902 voxels).

3.4. Discussion

This is the first study to characterise ERP expression of prediction error using taste, thus bridging a gap between human EEG studies on reinforcement learning and those carried out in animal models (Holroyd & Coles, 2002; Schultz, Dayan, & Montague, 1997). We delivered and omitted expected and unexpected hedonically matched appetitive and aversive tastes. Our goal was to distinguish between utility prediction error signals, where amplitude should be most positive (or negative) for delivered appetitive and omitted aversive tastes and most negative (or positive) for omitted appetitive and delivered aversive tastes, and a salience response, where amplitude should be most positive (or negative) for the delivery of both appetitive and aversive tastes compared to their omission. Following the logic of the axiomatic model (Caplin & Dean, 2008; Rutledge et al., 2010) we were particularly interested in signal that differentiated aversive and appetitive taste and taste omission cues more strongly when they were unexpected. For both appetitive and aversive taste, we observed continued expression of salience (Sambrook & Goslin, 2015b) across the entire 200-380ms time window. There was also an expression of aversive but not reward prediction error at the latency most characteristic of the FRN, peaking at 285ms (Holroyd, 2004; Sambrook & Goslin, 2015a).

The latency of the FRN, considered the EEG correlate of prediction error, is variable between studies and is usually identified between 200-350ms after the outcome (Hauser et al., 2014; Heydari & Holroyd, 2016; Holroyd, Hajcak, & Larsen, 2006; Holroyd, 2004). The lack of consistency in selection of latency windows makes it difficult to conclude whether the same signal is being studied across laboratories. A meta-analysis suggested that frontocentral vertex electrodes are most likely to express reward prediction error, mainly related to monetary reward, around 240-340ms, although the latency could be influenced by the modality of the outcome and other features of the experimental set-up (Sambrook & Goslin, 2015a). Here we examined the ERPs from 200ms onwards, firstly employing the traditional difference-wave approach within the electrodes and time-window recommended in the meta-analysis, and secondly in through a data-driven analysis in the spatiotemporal window of 200-380ms identified through coarse GFP analysis.

The commonly-used loss-gain difference-wave analysis (Heydari & Holroyd, 2016) was conducted to facilitate comparison between this study and previous FRN studies. We observed significant expression of appetitive FRN and aversive reverse-FRN. This result was driven by an increased positivity in response to delivered over omitted tastes in both

appetitive and aversive domains and directly replicates our previous work, where we observed increased positivity to the delivery of pain and money (Talmi et al., 2013), with a reverse-FRN for pain. The implication of these results is that the FRN may express response to more and less salient stimuli, rather than differentiate 'bad' from 'good'.

In the data-driven analysis ERPs had a more positive amplitude for tastes that were about to be delivered compared to those that were about to be omitted, and were also more positive for tastes that were unexpected compared to those that were expected. Using the terminology of Schultz (2016), this expression of motivational salience and surprise salience, respectively, was observed for both appetitive and aversive outcomes (i.e. sweet and bitter tastes). The response to motivational salience peaked particularly early, around 220ms, in agreement with recent proposals of an initial unsigned response that represents various forms of salience rather than utility (Schultz, 2016). This finding replicates our previous work with pain and money outcomes, where ERPs differentiated between pain and money that were about to be delivered and those about to be omitted around 200-290ms after the cue (Talmi et al., 2013). It is important to note that the main effect of outcome here drove the results reported above for the traditional difference-wave analysis. As per previous suggestions, the early response to motivational salience is likely related to the oddball N200 potential, which is associated with response conflict (Holroyd, 2004; Sambrook & Goslin, 2015b).

Frontocentral ERPs at 285ms in the aversive domain, in experimental blocks where only delivery or omission of bitter taste was possible, conformed to the logic of an axiomatic model of prediction error. The key pattern specified by the Axiomatic Model of prediction error is an interaction between outcome and expectancy (Rutledge et al., 2010). At that time window in the bitter condition ERPs were more positive for outcome delivery relative to omission, a difference that was more pronounced when outcomes were unexpected. The signal was also significantly more positive for delivered unexpected than delivered expected outcomes, and more negative for omitted unexpected than expected outcomes, in line with the predictions of the axiomatic model. On its own, as discussed earlier, the signal observed in the bitter condition adheres to criteria for an aversive prediction error, but cannot be interpreted unambiguously as a utility prediction error signal.

In the appetitive domain at that same latency, in blocks where only delivery or omission of sweet taste was possible, the signal followed a similar pattern to that found in the aversive domain, but while the main effects of outcome and expectancy were significant, the interaction between them was not. Two aspects of this result are important. First, because this outcome by expectancy interaction is a hallmark of a neurobiological reward prediction

error signal we conclude that we could only observe an aversive prediction error here, but not a reward prediction error. Clearly, the finding that frontocentral ERPs did not express prediction error in the appetitive domain already means that they also did not express utility prediction error, a quantity which should be equally well observed in both appetitive and aversive domain; yet the null interaction between outcome and expectancy in sweet could be just due to low power. Crucially, a utility prediction error signal should present with an *opposite* sign in each domain, but the direction of averages in all four conditions was the same. Clearly, a neurobiological signal of utility prediction error would not be expressed as greater positivity for both unexpectedly good and bad outcomes. Taken together, the frontocentral signal observed in this experiment did not express a utility prediction error. Other aspects of the data suggest that ERPs also did not track utility *per se*. For example, ERPs did not differentiate expected omission and delivery of a bitter taste (which have different utility), but did distinguish between expected and unexpected sweet taste (which have the same utility).

We did not observe a reward prediction error pattern in the appetitive domain, although such a pattern has been readily observed in previous work (Sambrook & Goslin, 2015a; Walsh & Anderson, 2012) including in our own previous study (Talmi et al., 2013). Instead ERPs in the appetitive domain expressed motivational and surprise salience. This may be due to the reinforcement modality we selected. Very few ERP studies of FRN used primary reinforcers. Although some previous ERP studies of aversive prediction error used pain, which is a primary reinforcer (Garofalo et al., 2014; Heydari & Holroyd, 2016; Talmi et al., 2013), all previous ERP studies of reward prediction error, including those that used pain in the aversive domain, used money, a secondary reinforcer, in the appetitive domain. We used primary taste reinforcers because appetitive taste is known to elicit a dopamine response. Second-by-second dopamine release in response to food cues signals future appetitive outcomes (Hamid et al., 2015), and anticipation of appetitive taste activates the dopaminergic system (O'Doherty et al., 2002). In agreement with previous research (Nitschke et al., 2006), we did not see neural habituation to appetitive taste. Moreover, we followed routine practice in animal models and ensured that the hedonic value of our taste reinforcers was titrated so that it was positive and high for each individual participant, and we deprived participants of food and water beforehand, which enhanced the incentive salience (Berridge, 2012; McClure, Daw, & Montague, 2003) of the sweet taste. We propose, therefore, that the sweet taste is more salient than the small amounts of money participants gained in previous work, and so ERPs to sweet taste prioritised expression of salience, rather than prediction error. This hypothesis can be tested in future work where monetary and taste reinforcers are directly compared. We also acknowledge that it is possible, as the use of sweet taste as an appetitive reinforcer in humans is fairly novel, that

the sweet taste was salient, but not appetitive, despite titration of the hedonic intensity of the sweet taste, and so elicited a salience response. However, previous research has successfully used sweet appetitive taste reinforcers (Franken et al., 2011; Kim, Shimojo, & O'Doherty, 2011; McClure, Berns, & Montague, 2003), so this hypothesis remains questionable. Future studies could acquire trial-by-trial ratings of hedonic intensity and appetitive value of sweet taste to test this.

We employed a Pavlovian task because this task established the fundamental reward prediction error result in animal models, which we aimed to be closest to. However, the fact that we employed a passive Pavlovian task, in contrast to the majority of FRN experiments which tend to employ instrumental learning tasks, could also contribute to the lack of reward prediction error signal. This is in keeping with previous work showing a smaller effect of reward prediction error in passive tasks (Sambrook & Goslin, 2015a). Therefore, it is possible that the FRN signals an instrumental, rather than a Pavlovian, reward prediction error. The fact that the ERPs signalled prediction errors in the aversive domain clarifies that domain cannot be ignored in the pursuit of an ERP signature of prediction error.

As we did not use source localisation analysis, we cannot make claims about where the signal originated from. A number of different regions expressed salience in previous fMRI work, summarised in a recent meta-analysis (Lindquist et al., 2015). The most likely sources of the salience response are the ACC, which receives prediction error signals from the midbrain, and the insula, which together with the dorsal ACC form the salience network (Seeley et al., 2007). The dorsal ACC was the source of the frontocentral salience response to money in a previous ERP-fMRI study, and this signal was shown to be signalled directly from dopaminergic sources to the dorsal ACC (Hauser et al., 2014). Furthermore, the ACC is known to be the source of the FRN ERP (Miltner, Braun, & Coles, 1997; Walsh & Anderson, 2012). The anterior insula and striatum have also been shown to express salience to appetitive and aversive tastes (Metereau & Dreher, 2013). The aversive prediction error signal we recorded on the scalp may have originated from the dopaminergic midbrain, involved in previous studies of aversive prediction error (Brischoux et al., 2009; Seymour et al., 2004), but its pathway to influencing scalp ERPs awaits further work.

Our findings go beyond existing fMRI work to exploit the temporal resolution of EEG and contradicts the dominant perspective on the FRN ERP as a signal of reward prediction error (Abler, Walter, Erk, Kammerer, & Spitzer, 2006; Carlson, Foti, Mujica-Parodi, Harmon-Jones, & Hajcak, 2011; Hauser et al., 2014; Holroyd, 2004; Holroyd & Coles, 2002; Walsh & Anderson, 2012). They agree better with recent work that proposes this signal expresses salience (Garofalo et al., 2014; Hauser et al., 2014; Huang & Yu, 2014;

Pfabigan et al., 2015; Sambrook & Goslin, 2015b; Talmi et al., 2013). Our findings also agree with the PRO (Predicted Response-Outcome) model, which asserts that ACC activity reflects negative surprise, responding to the unexpected omission of both appetitive and aversive outcomes (Alexander & Brown, 2011), in that ERPs were more negative to omissions across the board.

Our data show conclusively that frontocentral ERPs at the time-window of the FRN do not express outcome valence, contradicting the interpretation of the FRN as a utility prediction error signal (Holroyd, 2004). Across the time window of interest and across tastes ERPs were more positive for cues that predicted salient outcomes, namely, delivered or unexpected outcomes. The spatiotemporal evolution of the signal was differentially sensitive to the feature that rendered the predicted outcome salient, with a particular time window where the response in the aversive domain appeared to go beyond salience to resemble an aversive prediction error.

Chapter 4: the FRN to pain and expectation modulation of laser and electrical pain: an ERP study

A version of this Chapter is published in *The Journal of Neuroscience Methods*.

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The results were presented in a poster format at The Cognitive Neuroscience Society Annual Meeting (2017).

Emily Hird designed the study, collected the data, analysed the data, and wrote the paper, with input at all stages from Anthony Jones, Deborah Talmi and Wael El-Deredy.

Abstract

Pain perception and associated evoked potentials are modulated by expectation, but the PE response directly to pain has not been quantified. The first aim of this study was to quantify the most likely EEG correlate of PE to pain by violating cue-evoked expectations of high and low pain in a within-participant design. The second aim of this study was to ascertain the best pain stimulation technique for pain expectation studies. ERP studies of the influence of expectation on pain typically utilise laser heat stimulation to provide a controllable nociceptive-specific stimulus. Painful electrical stimulation has a number of practical advantages, but is less nociceptive-specific. We (1) tested for an FRN, defined as the difference-wave between higher and lower-than-expected pain and (2) tested whether perceived pain and pain-evoked potentials induced by electrical stimulation were modulated by expectation, whether this expectation elicited anticipatory EEG correlates, and how these measures compared to those associated with laser stimulation. (1) The difference-wave between higher and lower-than-expected pain did not yield an FRN, suggesting the FRN is not a useful correlate of prediction error to pain. (2) Despite sensory and affective differences between laser and electrical pain, intensity ratings and pain-evoked potentials were modulated equivalently by expectation, though ERPs only correlated with pain intensity ratings in the laser pain condition. Anticipatory correlates differentiated pain intensity expectation to laser but not electrical pain. Though laser-evoked potentials express a stronger relationship with pain perception, both laser and electrical stimulation may be used to study the modulation of pain-evoked potentials by expectation.

4.1. Introduction

Our experience of pain is profoundly influenced by what we expect to feel. For example, expecting a visit to the dentist to be painful is likely to increase the pain experienced during that visit. Experimentally induced pain is also influenced by expectations. Pain expectations can be experimentally manipulated through administration of a sham analgesic (Morton, Brown, Watson, El-Deredy, & Jones, 2010b; Wager, 2004; Watson et al., 2009a) or by eliciting cue-evoked expectations (Atlas, Bolger, Lindquist, & Wager, 2010; Brown, Seymour, Boyle, El-Deredy, & Jones, 2008a). Pain stimulus intensities which are higher or lower than expected result in pain PE which, according to computational models of learning, causes expectations to be updated to be more representative of reality in the future (Rescorla & Wagner, 1972; Sutton & Barto, 1990). FMRI studies have captured a correlate of PE to pain in areas such as the PAG, striatum, anterior insula and amygdala (Delgado et al. 2008a; Geuter et al. 2017; McHugh et al. 2014; Ploghaus et al. 2000; Roy et al. 2014; Zhang et al. 2016) However fMRI lacks temporal resolution, meaning fine differences in pain anticipation and the response to pain may be overlooked when pain is expected, whereas EEG has a greater temporal resolution. The FRN is the most likely EEG correlate of PE (Sambrook & Goslin, 2015a). Whilst some studies show an FRN response to a cue signalling pain, no study has shown the FRN directly to pain (Garofalo et al., 2014; Talmi et al., 2013). The first aim of this study was to capture the FRN response to pain.

ERP studies have focused on the modulation of LEPs by expectation (Colloca et al., 2008; Lorenz et al., 2005; Lyby, Aslaksen, & Flaten, 2011; Martini, Lee, Valentini, & Iannetti, 2015; Morton, Brown, Watson, El-Deredy, & Jones, 2010b; Morton, Watson, El-Deredy, & Jones, 2009; Wager, Matre, & Casey, 2006; Watson et al., 2009b; Watson, El-Deredy, Vogt, & Jones, 2007). LEPs result from laser heat stimulation of nociceptive Aδ and c fibres (Iannetti et al., 2004; Iannetti, Zambreanu, & Tracey, 2006) and therefore have the advantage of being nociceptive-specific. LEPs are a well-validated method for assessing pain perception and its neural basis (Garcia-larrea et al., 2003; Mobascher et al., 2009; Treede et al., 2003).

An alternative method of pain induction is TENS, which activates myelinated Aβ somatosensory fibres as well as Aδ nociceptive fibres and elicits an EEP. Across studies, it has been shown that both EEPs and LEPs express the P2 component which is closely linked to activity in the operculum, SII and the cingulate cortex (Bentley et al., 2003; Christmann et al., 2007; Garcia-larrea et al., 2003). Some studies have shown modulation of electrically induced pain by expectation, chiefly behavioural studies (Colloca, Sigaudo,

& Benedetti, 2008; Colloca & Benedetti, 2006, 2009b; Colloca, Petrovic, Wager, et al., 2010; De Pascalis, Chiaradia, & Carotenuto, 2002; Yeung, Colagiuri, Lovibond, et al., 2014), and fewer fMRI studies, showing modulation within the rACC, insula and thalamus (Wager, 2004b), and one ERP study (Rütgen, Seidel, Riečanský, et al., 2015). Laser and electrical stimulation activate similar areas of the brain, sharing activation across key structures of the lateral and medial pain system. Expecting both types of stimulation modulates activity in areas such as the cingulate, insula, dorsolateral prefrontal cortices and thalamus (Amanzio et al., 2013; Bentley et al., 2003; Christmann et al., 2007; Wager, 2004b). As similar neural areas are activated by the two pain types, and as one would expect expectation to influence pain perception independent of the modality of pain stimulus, it is possible that expectation could modulate the ERP and intensity ratings of the two types of stimulation equivalently. We compare the effect of cue-evoked expectation on the P2 component of EEPs and LEPs.

Awareness of imminent pain elicits an anticipatory slow-wave EEG correlate termed the SPN (Böcker et al., 2001). A marker for anticipation is a useful tool for quantifying the processes underlying expectation. The SPN has been characterised in response to laser pain as a negative potential peaking at central electrodes and has been localised to the cingulate and anterior insula, which is implicated in affective processing (Brown et al., 2008; Caria, Sitaram, Veit, Begliomini, & Birbaumer, 2010). The SPN in response to electrical pain has been observed in posterior areas of the cortex (Berns et al., 2006; Hoflle et al., 2013; Lin, Hsieh, Yeh, Lee, & Niddam, 2013), and centroparietal electrodes (Seidel et al., 2015); however, other studies have failed to show the SPN in electrical compared to laser pain (Babiloni et al., 2003, 2007). Accordingly, the SPN to electrical pain is yet to be reliably quantified, which is likely due to the lack of research into the modulation of electrical pain by expectation.

The paucity of research into the modulation of electrical pain by expectation may be because electrical stimulation activates Aβ somatosensory fibres alongside Aδ nociceptive fibres which could add sensory noise to the signal (Perchet et al., 2012), activating a larger number of thalamo-cortical units than laser stimulation, and resulting in higher-amplitudes and EEPs compared to LEPs (Garcia-larrea et al., 2003; Gingold, Greenspan, & Apkarian, 1991; Detlef Treede et al., 1999). This has likely led to the concern that the representation of somatosensory processes by the resultant EEP could interfere with measurement of expectation-induced pain modulation, as activity related to innocuous somatosensory activity could increase the noise of the EEP. Yet electrical stimulation has obvious advantages over laser stimulation, so it is important to understand whether we can study pain expectations with this technique. Laser stimulation can result in heating of

the skin leading to sensitisation, which limits the number of trials in a study. Skin heating by the laser is also associated with the risk of skin lesions and therefore has additional ethical implications. The setup is expensive, may not be portable depending on the type of laser, requires operators to undertake substantial training, and requires the wearing of safety goggles which can be uncomfortable and distracting for participants. TENS is arguably a more practical method of pain assessment. It does not heat the skin so there is no limit to the number of trials. There are fewer ethical implications in using electrical stimulation to elicit pain, as the activation is transient and there is no risk of skin damage. Electrical stimulators are cheaper, portable and available commercially, and require no specific training to use so can be used more widely and potentially in conjunction with physiological phenotyping in clinical trials to identify placebo responders. If EEPs are modulated by expectation equivalently to LEPs, this would allow generalisation across the literature, and future studies could use electrical stimulation. The second aim of this study was to compare the electrical and laser-evoked pain ERP and the anticipatory SPN, and intensity ratings of laser and electrical pain within the same participants.

4.2. Methods

4.2.1. Design

The study was a 2 (stimulator: laser/electric) x 2 (pain expectation: high/low) x 3 (pain intensity: high/low/medium) within-subjects design.

4.2.2. Participants

Twenty participants aged 18-35 (9 females, mean age 23 years) were recruited via newspaper and university advertisements and received £30 compensation for participation in the study. Participants had normal or corrected-to-normal vision. They had no history of neurological or psychiatric conditions, did not take medications which could affect their neurotransmitter levels, or take analgesics, and did not have a history of chronic pain. Ethical approval was granted by the University of Manchester, where the study took place.

4.2.3. Apparatus

Visual stimuli were presented on a desktop computer screen 1 metre from the participant. The laser stimuli were generated by a class 4 thulium laser (IPG Photonics Corporation, US/ TLR-30-2050, wavelength 2050nm/ 30 watt). The laser stimuli were of 150ms duration and a beam diameter of 5 millimetres. Energy delivered at 50% intensity was

2.25 joules. See table 4.1 for a summary of the energy densities of the laser stimulator. The electrical stimuli were delivered by two silver/silver chloride cup electrodes attached to a TENS machine (maximum voltage 477 Volts; maximum current 81 milliamps) (Medical Physics, Salford Royal NHS Foundation Trust) and were of 230 to 300 microsecond duration. Maximum voltage was 477 volts; maximum current was 81 milliamps, and maximum output power 37 watts. All pain stimuli were delivered by Matlab which interfaced with the laser via a program built in Labview (Medical Physics department, Salford Royal NHS Foundation Trust). Participants submitted their intensity ratings of the pain using a keypad.

Table 4.1. *Fluence. Energy density values expressed as fluence for each pain level.*

Energy density in Joules/cm^2			
	Low	Medium	High
Mean	0.2	0.32	0.47
Minimum	0.14	0.18	0.13
Maximum	0.31	0.46	0.75

4.2.4. Procedure

Participants underwent two blocks: the laser and the electrical block. These two blocks differed only in the instrument used in stimulus administration, the location of the stimulation, and the fact participants wore safety goggles during the laser block of the experiment. Blocks were counterbalanced across participants. Stimulus timing was kept consistent between the two blocks. In the laser block, fibre laser stimuli were delivered to the dorsum of the participant's right forearm. A 3cm by 4cm grid was drawn on the arm before beginning the study, and the laser stimulus was aimed at a new box in every trial, to decrease sensitisation or skin damage from the laser heat. In the electrical block, electrical stimuli were delivered to the middle phalanx of the right index finger.

Before applying the EEG cap, participants underwent a pain calibration procedure to determine their subjective response to increasing stimulus intensities, separately for the laser and electrical stimulation. The order was counterbalanced between participants. The first stimulus was very low, generally an intensity which would be below the threshold for pain in most people, and the intensity of the stimuli increased in a ramping procedure. We used a 0-10 NPS for the pain response, where a level 3 was when the stimuli became painful, and level 7 was at the point where the participant did not wish to experience a higher level of stimulation in the experimental session. Consequently, we attained a 'low' pain level 3, a 'medium' pain level 5, and a 'high' pain level 7. We repeated this procedure

three times to determine the average intensities corresponding to these intensity ratings. We then ran a procedure where participants identified the intensity (low, medium or high) of 18 pulses to ensure that they were experiencing the pain stimuli as they had rated in the pain calibration procedure. If participants could not identify the intensity of at least 75% of the pulses, the ramping procedure was repeated until they performed above 75%.

Trials were pseudorandomised. On each experimental trial, participants viewed a fixation cross for 500-750ms, and then a veridical probability cue which signalled the outcome likelihood and magnitude for that trial for 500ms. The cue, presented for 500ms, either signalled a 75% likelihood of high intensity pain stimulation and a 25% likelihood of medium intensity pain stimulation, or a 75% likelihood of low intensity pain stimulation and a 25% likelihood of medium intensity pain stimulation. This was followed by presentation of a blank screen for 1500ms, and then the stimulus was delivered, with the blank screen continuing for 1000ms. A screen was then presented which prompted participants to numerically rate their subjective pain intensity on a NPS using a keypad. Inter-stimulus interval was maintained at a 10 second minimum. See figure 4.1 for a timeline for each trial. Over the experimental session, the 75% likelihood of high intensity pain stimulation cue was followed by 90 high intensity stimuli and 30 medium intensity stimuli. The 75% likelihood of low intensity pain cue was followed by 90 low intensity stimuli and 30 medium intensity stimuli. This meant on the relatively rare trials in which a medium intensity stimulus was administered, participants were expecting a high probability of either high or low pain, depending on the cue, which was crucial to our research question. At the end of every 7 minute block, participants rated the unpleasantness of the high, medium and low pain, on a 10-point NPS (see Appendix A), following previous research (Pascalis, Chiaradia, & Carotenuto, 2002). In a subset of trials we presented a cue signalling 100% likelihood of receiving a medium pain, but these trials were not included in the analysis. We did not analyse these trials as they were relevant to a different analysis. Participants had a 2 minute break every 7 minute block.

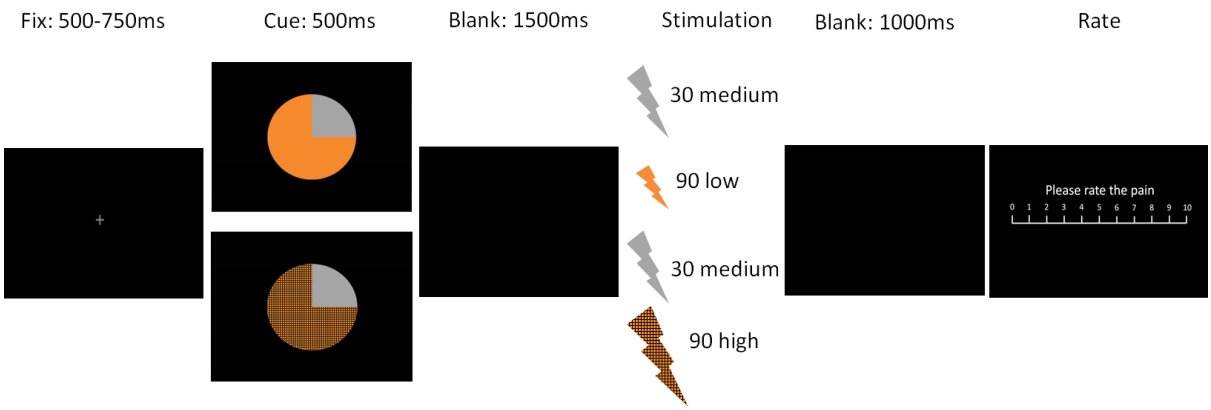

Figure 4.1. *Trial timeline. Participants viewed a fixation cross for 500-750ms, followed by a veridical probability cue signalling the probability of receiving low (light orange) or medium (grey) pain in the low pain expectation trials, and viewed the same cue signalling the probability of receiving high (dark orange) or medium pain (grey) in the high pain expectation trials, for 500ms. After presentation of a blank screen for 1500ms, the painful stimulus was delivered, followed by presentation of a blank screen for 1000ms. Finally, participants were prompted to rate the NPS of the painful stimulus using a keypad.*

4.2.5. EEG recording

Continuous EEG recording was acquired at a sampling rate of 1000 Hertz (Hz) using a 64 electrode Active-Two amplifier system (Biosemi, Amsterdam, Netherlands) with Biosemi acquisition software (BioSemi, Netherlands). An active and passive electrode replaces the ground electrode to create a feedback loop that drives the average potential of the subject (the common mode voltage) as close as possible to the analogue-to-digital converter reference voltage in the analogue-to-digital box. Impedances were kept at 20 KΩ or less. The experiment was conducted in a quiet room.

4.2.6. Behavioural data analysis

We firstly aimed to test for any psychophysical differences between the laser and electrical pain stimulation. We conducted a t-test on the number of 5% stimulation intensity increases required to reach a NPS 7 pain intensity rating per participant between laser and electrical pain in the pain calibration procedure. A significant difference would indicate a difference in the increase of perceived pain per 5% increase in stimulation intensity between the two conditions. We also used Pearson's correlation to test for correlations between average pain intensity rating and stimulation intensity across participants, where

a significant correlation would indicate a consistent relationship between a 5% increase in intensity and a corresponding increase in pain intensity rating.

Next we conducted two 2 (stimulator: laser/electric) x 2 (pain intensity: high/low) within-subjects ANOVAs, one for the pain intensity ratings and one for the pain unpleasantness ratings. Additionally, we conducted a 2 (stimulator: laser/electric) x 2 (pain expectation: high/low) within-subjects ANOVA for the pain intensity ratings of medium pain trials. Interactions were followed up with post-hoc Bonferroni-corrected t-tests. Finally, we conducted a linear mixed model in each laser and electrical pain condition separately, with trial as a predictor variable, to test for any effect of time on pain intensity rating. Here, participant was treated as a random effect.

4.2.7. EEG data analyses

Four participants were removed from the analysis because there was not a clear N2-P2 laser component, defined as a negative trough followed by a positive peak within 150-1000ms post-stimulus. One participant withdrew from the study, and one participant was detected as an outlier using Tukey's method of outlier detection, which holds the advantage that it does not depend upon a mean or standard deviation, and so is resistant to extreme values in the data (Tukey, 1977). This participant was removed from the data. The EEG signal for the remaining 14 participants was preprocessed using SPM12 (Ashburner et al., 2013; Litvak et al., 2011). Separate pre-processing pipelines were carried out for the pre-stimulus SPN and post-stimulus P2. Extracted data were analysed using SPSS.

4.2.7.1. Difference-wave analysis of the FRN

We conducted a difference-wave analysis by extracting voltages from vertex electrodes Fz, FCz and Cz, 240-340ms after the delivered pain, following a recent meta-analysis which identified these channels and latencies window as most likely to express the FRN, and following the difference-wave technique described in Chapter 3 (Taste FRN). Difference waves were computed using the standard technique where better-than-expected outcomes are subtracted from worse-than-expected outcomes. As pain is inherently aversive, higher-than-expected pain was defined as worse-than-expected, and lower-than-expected pain was defined as better-than-expected. We conducted a one-sample t-test on this signal, following Chapter 3 and the analysis protocol of a recent study (Heydari & Holroyd, 2016).

4.2.7.2. SPN

The signal was referenced to the mean of all scalp electrodes, downsampled to 200 Hz and filtered with a lowpass Butterworth filter (30 Hz). Data were not highpass filtered as this could remove low frequency slow-wave anticipatory potentials. Epochs were extracted 200ms before the presentation of the probability cue to 1000ms after delivery of the pain stimulus. An absence of a highpass filtering step could introduce noise into the data and lead to an unnecessarily high artefact rejection rate. To avoid this, artefact rejection with a threshold of 60uV was applied to highpass (0.1Hz) filtered data to create a list of artefacts in the data. This artefact information was then applied to the actual non highpass-filtered data. In the laser pain conditions, the following trial numbers remained across participants: Cue low get low intensity pain (M=70.4, SD=13.5), cue low get medium intensity pain (M=24.9, SD=4), cue high get high intensity pain (M=66.9, SD=13.9), cue high get medium intensity pain (M=23, SD=4.2). In the electrical pain conditions, the following trial numbers remained across participants: Cue low get low intensity pain (M=70, SD=11.8), cue low get medium intensity pain (M=23.4, SD=4.1), cue high get high intensity pain (M=66.5, SD=14.4), cue high get medium intensity pain (M=24, SD=3.3). Averaged across conditions, the difference in number of remaining trials between laser versus electrical pain conditions was less than one trial. Accordingly, we do not anticipate any influence of trial number on results. Single-trial data were averaged separately for the eight conditions using the "robust averaging" method in SPM12b (Litvak et al., 2011). Based on previous research, data were extracted from the time-window 500ms to 0ms before pain delivery (Brown, Seymour, Boyle, et al., 2008a). We ran a 2 (stimulator: laser/electric) x 2 (pain expectation: high/low intensity) within-subjects ANOVA on these data. We also used Pearson's correlation to test for correlations between the average SPN and EEP amplitude, NPS and unpleasantness ratings across participants. Finally, we conducted a linear mixed model in each laser and electrical pain condition separately, with trial as a predictor variable, to test for any effect of time on SPN amplitude. Participant was treated as a random effect.

4.2.7.3. P2

The re-referenced, downsampled signal was filtered with a Butterworth filter between 0.5 and 30 Hz. Epochs were extracted 700ms before the pain delivery to 1000ms after. Data underwent artefact rejection at a threshold of 60uV. In the laser pain conditions, the following trial numbers remained across participants: Cue low get low intensity pain (M=74.2, SD=15.8), cue low get medium intensity pain (M=24.4, SD=4.2), cue high get high intensity pain (M=66.1, SD=15), cue high get medium intensity pain (M=23.1,

SD=5.2). In the electrical pain conditions, the following trial numbers remained across participants: Cue low get low intensity pain (M=71.5, SD=12.3), cue low get medium intensity pain (M=23.6, SD=4.1), cue high get high intensity pain (M=68.3, SD=11.8), cue high get medium intensity pain (M=22.9, SD=5.4). Averaged across conditions, the difference in number of remaining trials between laser versus electrical pain conditions was less than one trial. Accordingly, we do not anticipate any influence of trial number on results. Single-trial data were averaged separately for the eight conditions using the "robust averaging" method in SPM12b (Litvak et al., 2011), and filtered with a lowpass Butterworth filter (30Hz) to remove high frequency noise introduced by robust averaging, in order to use individual average data files to identify pain-evoked P2 latencies and topographies. The latency of 50% of the maximum amplitude of the grand average across all conditions and participants was identified (Luck, 2005). This was 267-450ms post-stimulus for the EEP, and 410-645ms for the LEP. Though the LEP latencies are late, similar latencies have been reported in previous studies in response to both CO_2 (Brown et al., 2014) and thulium laser stimulation (Almarzouki et al., 2017). Each participant's maximum within this latency was identified, and ±40ms window was extracted around this, to include the peak of the maximum. See figure 4.2 for an illustration of the electrodes extracted in the analysis. The average amplitudes across this time window in each condition were analysed. We conducted two within-subjects ANOVAs to examine the extracted data, one with the factors 2 (stimulator: laser/electric) x 2 (pain intensity: high/low), and one with the factors 2 (stimulator: laser/electric) x 2 (pain expectation: high/low intensity). We used Pearson's correlation to test for correlations between the average P2 amplitude and pain intensity rating across participants. Finally, we conducted a linear mixed model in each laser and electrical pain condition separately, with trial as a predictor variable, to test for any effect of time on ERP amplitude. Participant was treated as a random effect.

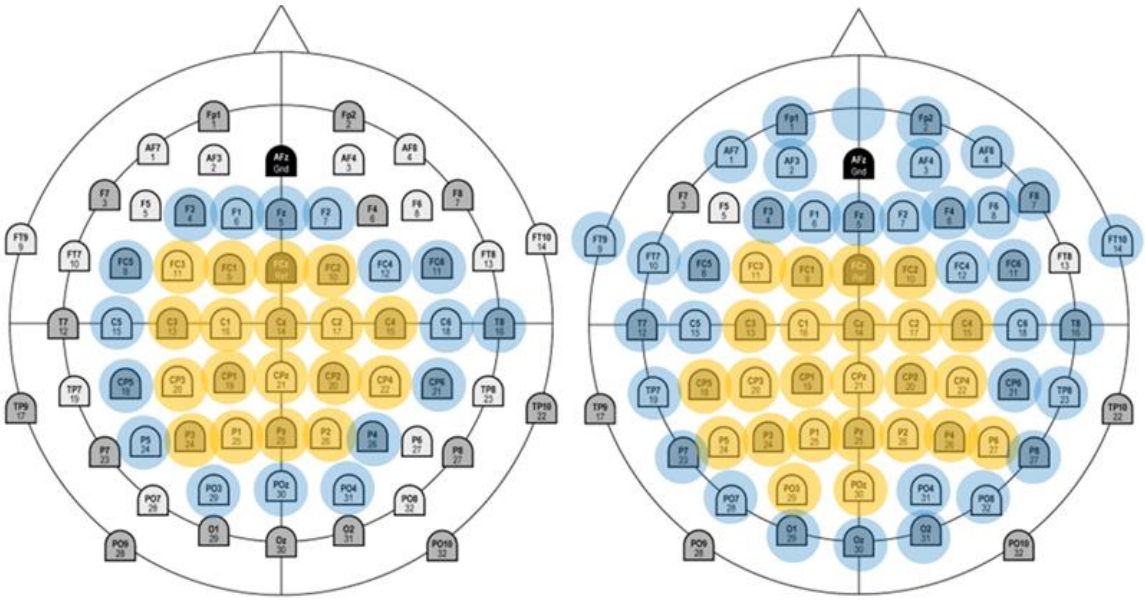

Figure 4.2. *Electrodes expressing the P2 and selected for analysis for the electrical(left) and laser condition. Electrodes extracted in the majority (at least 50%) of participants are highlighted yellow, and other electrodes extracted in blue. Across participants, the P2 peak was expressed at centroparietal electrodes, but more peripheral activation varied in topography between participants.*

4.3. Results

4.3.1. Behavioural results

We first tested for any psychophysical differences between the laser and electrical pain stimulation. In the pain calibration procedure, a paired samples t-test showed that across conditions, the number of 5% intensity increases from the lowest stimulation intensity to an intensity which elicited an NPS 7 pain response was significantly higher in response to electrical pain (M=8.33, SD=3.75) than to laser pain (M=5.4, SD=1.89) (t=-3.47, p=0.004, d=-1.085). The fact that a greater number of 5% intensity increases were required to elicit a level seven pain intensity rating to electrical pain suggests that participants were more sensitive to increases in laser stimulation than electrical stimulation. Pain intensity rating significantly positively correlated with stimulator intensity in response to both electrical pain (r=.49, p<.001, n=124) and laser pain (r=.82, p<.001, n=87) but in the laser pain condition the correlation coefficient was larger. The larger correlation coefficient in the laser pain condition further suggests that pain intensity ratings more closely reflected the 5% intensity increases in the laser pain condition compared to the electrical pain condition because the pain intensity rating correlated more closely with the intensity increase.

For the main experiment, we assessed the effects of pain intensity and stimulator on intensity rating. A 2 (stimulator: laser/electric) x 2 (pain intensity: high/low) within-subjects ANOVA revealed a main effect of stimulator (laser>electric) ($f(1, 13)=6.81$, $p=0.02$, $\eta_P^2=.344$) and a main effect of intensity (high>low pain) ($f(1,13)=248.75$, $p<.001$, $\eta_P^2=.95$), but no interaction ($f(1,13)=.31$, $p=.59$, $\eta_P^2=.02$), suggesting NPS score was overall higher in response to laser pain, but there was no difference between laser and electrical pain in terms of how pain intensities were differentiated (see figure 4.5).

We next assessed the effects of pain cue and stimulator on intensity rating of the medium intensity pain trials. A 2 (stimulator: laser/electric) x 2 (pain expectation: high/low) within-subjects ANOVA revealed a main effect of stimulator (laser>electric) ($f(1,13)=11.786$, $p=0.004$, $\eta_P^2=.476$) and a main effect of pain expectation (high>low pain cue) ($f(1,13)=80.219$, $p<.001$, $\eta_P^2=.861$), but no interaction ($f(1,13)=78$, $p=.393$, $\eta_P^2=.057$), suggesting ratings were overall higher in response to laser pain, but that the high pain cue increased ratings equally compared to the low pain cue in both the laser and electrical block (see figure 4.6).

We assessed the effects of pain intensity and stimulator on unpleasantness rating. We only collected unpleasantness ratings for the low and high intensity stimulation, because the medium intensity stimulation was rare (30 trials) and preceded by a high or low intensity cue so could be biased by the cue. A 2 (stimulator: laser/electric) x 2 (pain intensity: high/low) within-subjects ANOVA revealed a main effect of stimulator (laser>electric) ($f(1,13)=25.27$, $p<.001$, $\eta_P^2=.66$) and a main effect of intensity (high>low pain) ($f(1,13)=124.41$, $p<.001$, $\eta_P^2=.91$), and an interaction ($f(1,13)=13.686$, $p=0.003$, $\eta_P^2=.51$). We executed post-hoc Bonferroni-corrected t-tests with a significance criterion of $p<0.0125$. They showed the effect of intensity to be significant in both the laser ($t=12.93$, $p<.001$, $d=3.74$) and electrical ($t=7.43$, $p<.001$, $d=1.93$) condition, and the effect of instrument to be significant at high ($t=5.668$, $p<.001$, $d=1.33$) but not low intensities at the significance threshold ($t=2.821$, $p=0.014$, $d=0.83$). In other words, high intensity laser pain was rated as more unpleasant than high intensity electrical pain, but ratings for low intensity laser and electrical pain did not differ.

Finally, we conducted a linear mixed model to test the effect of time on pain intensity rating within each condition, separately for laser and electrical pain (table 4.2). We included the predictor trial number, and the outcome variable was NPS rating. In almost all conditions, there was a significant relationship between trial number and NPS rating. Beta values were positive across the laser pain conditions, indicating an increase in pain

intensity ratings over time, and possible sensitisation. Beta values were negative in the electrical pain conditions, indicating a decrease in pain intensity ratings over time, and possible habituation. In support of this, the average pain intensity rating across participants increased from the first ten experimental trials to the final ten trials across laser conditions, and decreased from the first ten trials to the final ten trials across electrical conditions (table 4.2). These results may be related to the relatively high trial numbers of this experiment. In Chapter 5 (PE size and variability), we also had high trial numbers. To address habituation to electrical pain, we included trial as a predictor in those analyses.

Table 4.2. *Effect of trial on NPS. Results of the linear mixed model, showing a positive relationship between laser pain intensity rating and trial, and a negative relationship between electrical pain intensity rating and trial. Significant results are in bold.*

Stimulator type	Cued pain intensity	Delivered pain intensity	R^2	*B*	Standard error	*p*	95% Confidence interval		Average (SD) NPS of first ten trials	Average (SD) NPS of final ten trials
Laser	Low	**Low**	**.05**	**.004**	**.004**	**<.001**	**.003**	**.004**	**2.85 (1.07)**	**3.59 (1.16)**
		Medium	**.01**	**.002**	**.001**	**.01**	**.0004**	**.003**	**4.46 (.81)**	**4.72 (.71)**
	High	**High**	**.01**	**.001**	**.0004**	**<.001**	**.001**	**.002**	**6.56 (.91)**	**6.79 (.87)**
		Medium	**.02**	**.002**	**.001**	**.003**	**.001**	**.004**	**5.82 (.91)**	**6.19 (1)**
Electric	Low	**Low**	**.01**	**-.01**	**.003**	**.01**	**-.001**	**-.0002**	**2.74 (.77)**	**2.52 (.73)**
		Medium	.01	-.001	.01	.05	-.003	.0002	3.94 (0.74)	3.76 (1.04)
	High	**High**	**.04**	**-.002**	**.0003**	**<.001**	**-.003**	**-.002**	**6.4 (.87)**	**5.89 (1.49)**
		Medium	**.04**	**-.004**	**.001**	**<.001**	**-.004**	**-.002**	**5.33 (.93)**	**4.83 (1.21)**

4.3.2. Electrophysiological results

4.3.2.1. FRN

We did not observe a significant FRN in either the laser (M = -.1, t (13) = -.27, p =.79), or electrical condition (M = -.25, t (13) = -.91, p = .38) (figure 4.3).

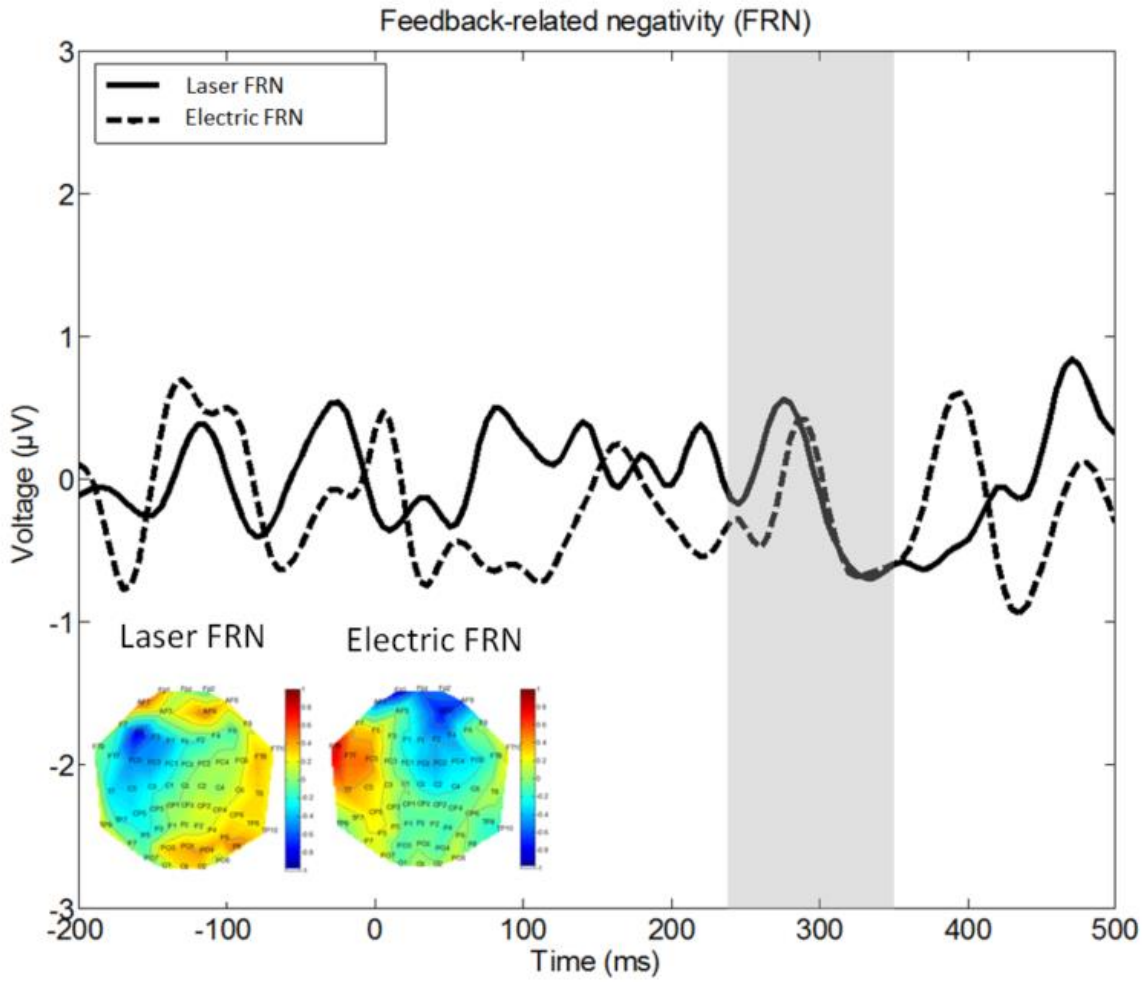

Figure 4.3. *Difference-wave FRN. The higher-than-expected minus lower-than-expected difference-wave FRN to pain, at electrodes Fz, FCz and Cz, averaged across an average of 24 trials in the laser pain condition and 23 trials in the electrical pain condition. The shaded area represents the 240-340ms time-window from where amplitudes were extracted. Topographic maps show the scalp topography for the higher-lower than expected pain stimulus difference wave.*

4.3.2.2. SPN

We subtracted the average response to the low pain expectation cue from the average response to the high pain expectation cue, and inspected the 500ms period preceding the pain stimulus in the average "difference" topography for each of the laser and electrical stimulators. In the electrical conditions we observed a negative difference across central-parietal electrodes which peaked at CP3 and CP5 (see figure 4.4), congruent with parietal anticipatory activity to electrical pain shown in previous work (Berns et al., 2006; Hoflle et al., 2013; Lin et al., 2013). This was supported by a negative potential across conditions at

these same electrodes. In the laser condition we observed a negative difference at C2 and Cz (see figure 4.4). We therefore extracted amplitudes from electrodes CP3 and CP5 for the electrical condition, and C2 and Cz for the laser condition. A 2 (stimulator: laser/electric) x 2 (pain expectation: high/low) within-subjects ANOVA revealed a main effect of stimulator (electric>laser) ($f(1,13)=16.79$, $p=0.001$, $\eta_P^2=.56$), a main effect of cue (high>low) ($f(1,13)=12.17$, $p=0.004$, $\eta_P^2=.48$), and an interaction ($f(1,13)=5.92$, $p=0.03$, $\eta_P^2=.31$) (see figure 4.4). Bonferroni-corrected paired samples t-tests with a significance criterion of $p<0.025$ showed the effect of cue to be significant in the laser ($t=3.42$, $p=0.004$, $d=1.09$) but not the electrical pain condition ($t=.81$, $p=0.43$, $d=0.22$). These results suggest the laser SPN differentiated pain intensity (cue low average amplitude was 4.41 μV; cue high: 2.12 μV), whilst although there was a numerical difference in the electricalSPN, it was not significant (cue low average amplitude was -3.07 μV; cue high: -3.4 μV). SPN amplitude did not correlate with unpleasantness in either the laser ($p=.84$, $r=-.041$, $n=28$) or the electrical condition ($p=.61$, $r=-.101$, $n=28$), or with pain intensity rating in either the laser ($p=.26$, $r=-.152$, $n=56$) or the electrical condition ($p=.08$, $r=-.24$, $n=56$). The SPN did not correlate with P2 amplitude in either the laser ($p=.33$, $r=-.133$, $n=56$) or the electrical condition ($p=.89$, $r=.018$, $n=56$).

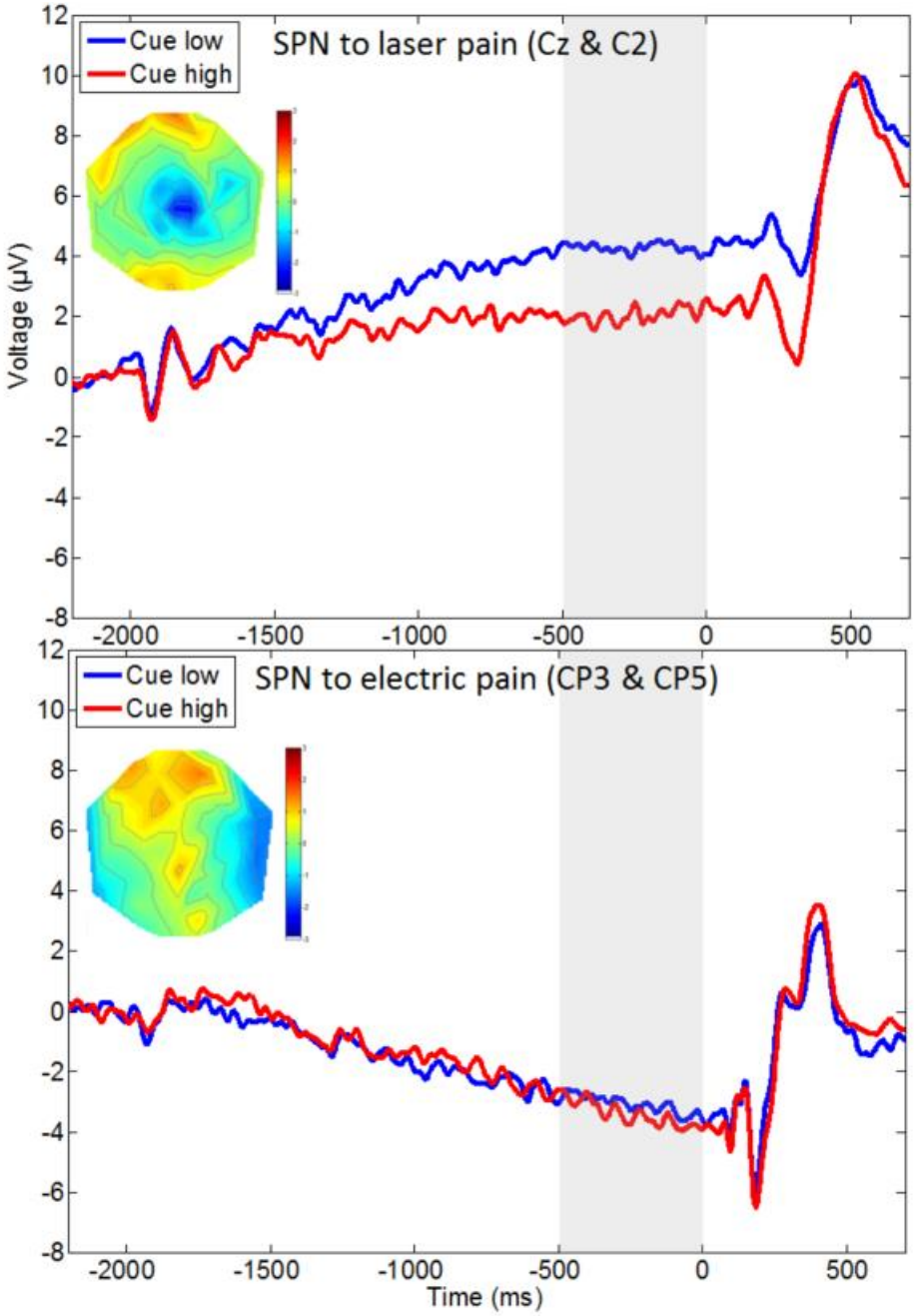

Figure 4.4. *Anticipation. Stimulus-preceding negativity for high (red) and low (blue) pain cues for laser (upper plot, averaged across electrodes C2 and Cz over an average of 46 trials) and electrical (lower plot, at electrodes CP3 and CP5 over an average of 46 trials) pain. The probability cue was presented at -2000ms, and the pain stimulus delivered at 0ms. We analysed the signal from 500ms before pain stimulation (shaded grey). Topographic plots show topographies for the difference between high and low intensity pain expectation across the 500ms anticipatory period, scaled from -3 to 3 microvolts. Data were baseline-corrected to 200ms before presentation of the visual cue.*

Finally, we conducted a linear mixed model to test the effect of time on SPN amplitude within each condition, separately for laser and electrical pain (table 4.3). We included the predictor trial number, and the outcome variable was SPN amplitude. Where results were significant in the laser pain condition, beta values were positive, suggesting SPN amplitude became less negative over time. In support of this, the average SPN amplitude over participants decreased from the first ten experimental trials to the final ten trials (table 4.3).

Table 4.3. *Effect of trial on SPN. Results of the linear mixed model, showing a positive relationship between SPN amplitude and trial. Significant results are in bold.*

Stimulator type	Cued pain intensity	Delivered pain intensity	R^2	*B*	SE	*p*	95% Confidence interval		Average (SD) SPN amplitude of first ten trials	Average (SD) SPN amplitude of final ten trials
Laser	Low	**Low**	**.001**	**.04**	**.02**	**.01**	**.01**	**.08**	**-5.54 (5.47)**	**3.03 (6.38)**
		Medium	**.14**	**.16**	**.05**	**<.001**	**.07**	**.25**	**-17.12 (11.53)**	**-8.07 (5.88)**
	High	High	.001	.03	.02	.09	-.01	.07	6.99 (7.83)	23.66 (18.91)
		Medium	**.01**	**.13**	**.06**	**.04**	**.003**	**.25**	**4.14 (6.88)**	**5.66 (7.44)**
Electric	Low	Low	-.0002	.01	.12	.48	-.02	.03	-3.88 (2.3)	-1.9 (4.91)
		Medium	.0001	.01	.02	.69	-.03	.04	-3.74 (2.64)	-2.84 (2.48)
	High	High	.002	-.001	.01	.91	-.02	.02	-4.15 (3.68)	-2.37 (4.04)
		Medium	-.01	.03	.046	.48	-.06	.12	-4.07 (2.23)	-3.06 (3.73)

4.3.2.3. Effects of delivered pain intensity on P2

Here we report ERP results from the veridically cued pain conditions, where a high pain intensity cue was followed by a high pain stimulus, and a low pain intensity cue was followed by a low pain stimulus. Peak latency of the EEP was shorter than LEP. The average peak latency across participants was 303ms (min=235ms, max=355ms, SD=43.3) to 383ms (min=325ms, max=455ms, SD=43.3) for EEPs, compared to 464ms (min=395ms, max=580ms, SD=55.3) to 544ms (min=475ms, max=660ms, SD=55.3) for LEPs. A 2 (stimulator: laser/electric) x 2 (pain intensity: high/low) within-subjects ANOVA on the voltages revealed a main effect of stimulator (electric>laser) ($f(1,13)=24.69$, $p<.001$, $\eta_P^2=.66$) and a main effect of intensity (high>low pain) ($f(1,13)=43.23$, $p<.001$, $\eta_P^2=.77$), but no interaction, ($f(1,13)=1.97$, $p=0.18$, $\eta_P^2=.13$) which indicates ERPs were higher in response to electrical pain, but the effect of intensity was equal in response to both laser and electrical pain (see figure 4.5). Pain intensity ratings significantly positively correlated with ERP amplitude in the laser ($r=.69$, $p<.001$, $n=28$) but not the electrical condition ($r=.31$, $p=.11$, $n=28$).

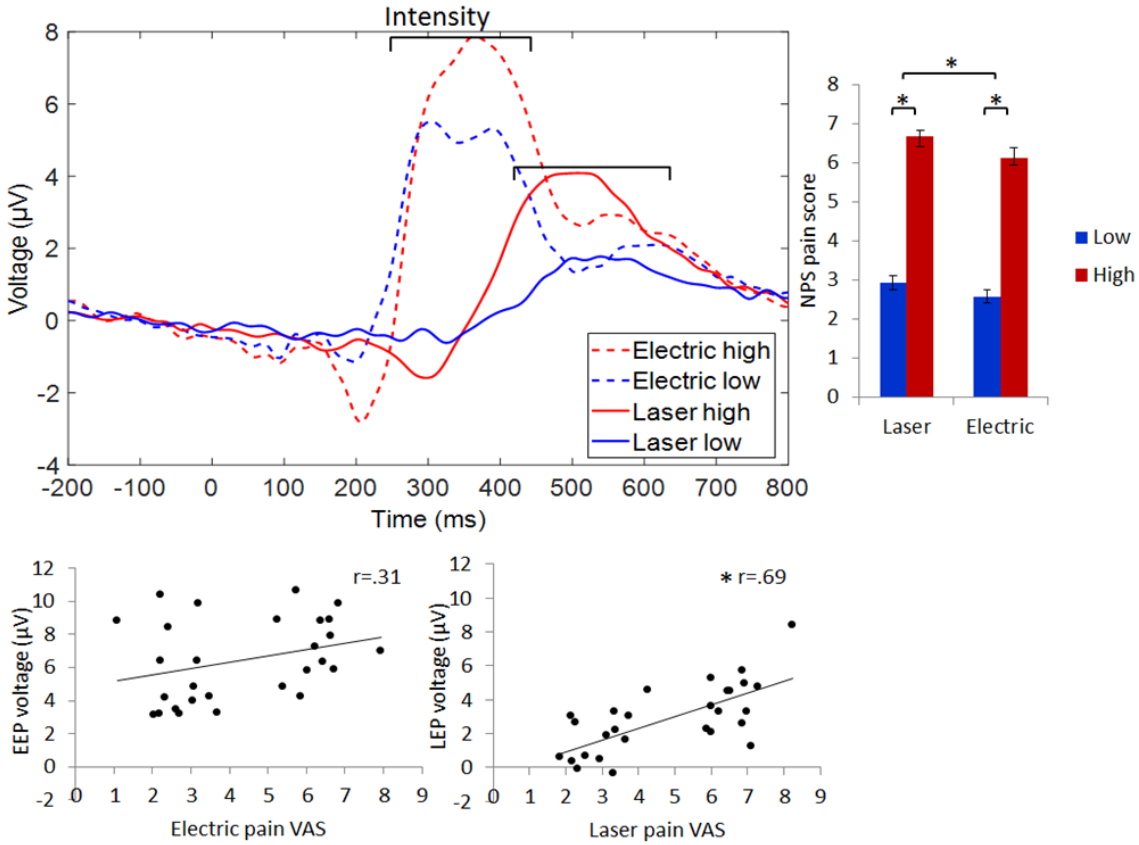

Figure 4.5. *Effect of intensity. Upper left panel: pain-evoked potentials for high (red) and low (blue) electrical (dashed line) and laser (solid line) pain-evoked potentials at peak electrodes for each participant, averaged over an average of 69 trials in the laser pain condition and 68 trials in the electrical pain condition. The painful stimulus was delivered at oms. Bars show the window of analysis for each stimulator type. Upper right panel: average NPS ratings of the stimuli for the laser and electrical pain. Red bars depict average NPS rating for high intensity stimulation, and blue bars average NPS rating for low intensity stimulation. Error bars represent standard error of the mean. Lower panel: scatterplots showing positive correlations between EEP amplitude and NPS (left), and the LEP amplitude and NPS (right), with lines of best fit.*

4.3.2.4. Effects of expectations on medium pain stimuli on P2

Here we report ERP results from the conditions where high and low pain intensity cues were followed by a medium intensity pain stimulus. A 2 (stimulator: laser/electric) x 2 (pain expectation: high/low) within-subjects ANOVA on the medium stimulus intensity pain-evoked potentials revealed a main effect of stimulator (electric>laser) (f(1,13)=15.6,

p=0.002, η_P^2=.55) and a main effect of cue (high>low) (f(1,13)=5.71, p=0.033, η_P^2=.31), but no interaction (f(1,13)=0.84, p<0.78, η_P^2=.01), suggesting ERPs were higher in response to electrical pain (as already noted in the section above), but the effect of cue was equal in response to both laser and electrical pain (see figure 4.6). Pain intensity rating did not correlate with ERP amplitude in either the laser (p=.26, r=.22, n=28) or the electrical condition (p=.39, r=-.17, n=28).

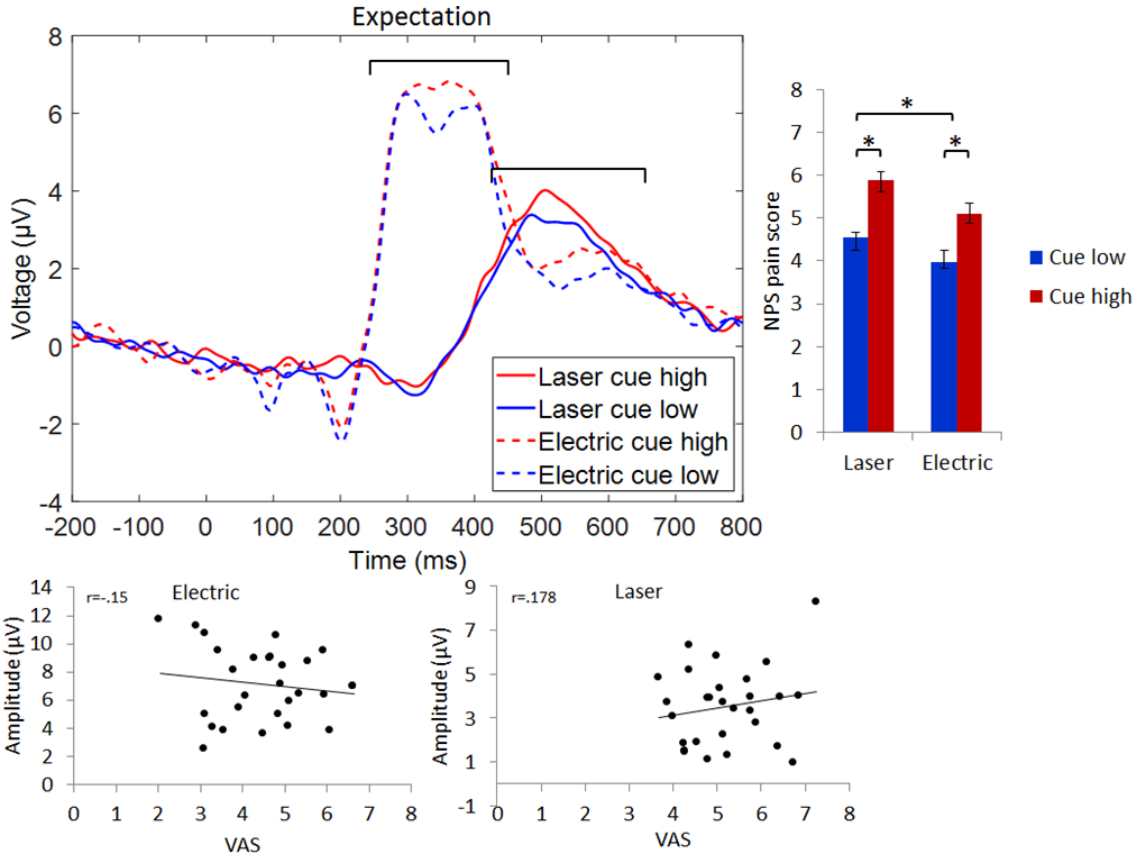

Figure 4.6. *Pain expectation effects on LEPs. Upper left panel: effect of high (red) and low (blue) pain cue on medium electrical (dashed line) and laser (solid line) pain-evoked potentials at peak electrodes for each participant averaged over an average of 24 trials in the laser and the electrical pain condition. The painful stimulus which in this case was always of medium intensity was delivered at oms with low and high intensity cues. Bars show the window of analysis for each stimulator type. Upper right panel: Average NPS intensity ratings of the stimuli for the laser and electrical pain, high and low pain expectation. Error bars represent standard error of the mean. Lower panel: scatterplots showing the positive correlation between EEP amplitude and NPS rating (left), and LEP amplitude and NPS rating (right), with lines of best fit.*

Finally, we conducted a linear mixed model to test the effect of trial on ERP amplitude within each condition, separately for laser and electrical pain (table 4.4). We included the predictor trial number, and the outcome variable was P2 amplitude. In all conditions, there was a significant relationship between trial number and P2 amplitude. Betas were negative in almost all conditions, suggesting both LEP and EEP amplitude decreased over time. In support of this, the average P2 amplitude over participants decreased from the first ten experimental trials to the final ten trials (table 4.4).

Table 4.4. *Effect of trial on ERP. Results of the linear mixed model, showing a negative relationship between LEP and EEP amplitude and trial.*

Stimulator type	Cued pain intensity	Delivered pain intensity	R^2	*B*	SE	*p*	95% Confidence interval		Average (SD) ERP amplitude of first ten trials	Average (SD) ERP amplitude of final ten trials
Laser	Low	Low	.01	-.02	.01	<.001	-.31	-.01	2.57 (3.16)	.48 (2.04)
		Medium	.06	-.15	.03	<.001	-.21	-.09	3.97 (3.53)	2.25 (1.7)
	High	High	.01	-.03	.01	<.001	-.05	-.02	3.93 (3.55)	1.7 (3.25)
		Medium	.04	-.13	.03	<.001	-.18	-.06	4.48 (2.85)	3.06 (2.69)
Electric	Low	Low	.01	-.02	.01	<.001	-.03	-.01	6.71 (3.08)	5.68 (2.5)
		Medium	.03	-.09	.03	<.001	-.14	-.04	7.61 (2.92)	6.34 (2.94)
	High	High	-02	-.02	.01	<.001	-.03	-.01	7.83 (2.49)	6.91 (2.34)
		Medium	.11	-.13	.02	<.001	-.17	-.08	8.09 (2.95)	6.53 (2.45)

4.4. Discussion

Results yielded three important findings. First, we did not see a significant FRN to laser or electrical pain. Second, our results show that electrical and laser stimulation elicits ERPs of different latencies and amplitudes, but these ERPs, and the corresponding subjective intensity ratings, were equally modulated by cue-evoked expectations. However, the intensity of the pain experience only correlated with the LEP and not the EEP. Third, we show morphological and topographical differences in the anticipatory SPN between the two stimulation types. The SPN only differentiates intensity expectation significantly in response to the laser but not the electrical pain stimulus.

4.4.1. Pain FRN and cue modulation of pain

Replication of the FRN analysis described in Chapter 3 (Taste FRN) did not reveal a significant FRN. Further, pain-evoked ERPs were modulated in the opposite direction to the aversive FRN captured in Chapter 3 (Taste FRN). In Chapter 3, when outcomes were unexpectedly delivered (worse or higher than expected) the signal was more positive than when they were unexpectedly omitted (better or lower than expected). Here, when pain

outcomes were higher than expected (cue low pain) the signal was more negative than when they were lower than expected (cue high pain).

The FRN and the pain-evoked potential share a similar spatiotemporal profile. Both are expressed between 240 and 340ms after an outcome at central electrodes, meaning the larger pain-evoked potential overlaid on the smaller FRN may have prevented capture of the FRN (Sambrook & Goslin, 2015a; Treede et al., 2003). Although the FRN to a cue signalling pain has been previously shown, our results indicate that the FRN is not a useful measure of PE directly to the delivery of pain. Our design addressed a key potential oversight in pain expectation research, that the amplitude of pain-evoked potentials could be influenced by the expression of the FRN. However, our results indicate that the FRN does not influence the amplitude of pain-evoked potentials. This novel finding is important for the remaining chapters of the thesis as it indicates that PE to pain cannot be measured using the FRN. In Chapters 5 (PE size and variability) and 6 (Cue and placebo effects on pain) we moved to other methods of investigating expectation and PE in pain perception.

4.4.2. Modulation of laser and electrical pain by expectation

4.4.2.1. Methodological discussion

The EEP, the marker for electrical pain, differed in several ways to that for laser pain LEP. These differences can be explained by the fact that electrical stimulation activates large myelinated somatosensory Aβ fibres as well as nociceptive Aδ fibres. Firstly, EEPs showed much shorter latencies than LEPs, consistent with previous studies comparing intracutaneous electrical stimulation with laser stimulation (Babiloni et al., 2007; Inui, Tran, Hoshiyama, & Kakigi, 2002). This suggests the method we used, transcutaneous electrical stimulation, elicits potentials to a similar latency to more invasive intracutaneous stimulation. The decreased latency of the EEP can be related to the fact that Aβ fibres have a faster conduction velocity of ~69 metres per second, compared to a conduction velocity of 10 metres per second in Aδ fibres (Tran et al., 2001). Secondly, EEPs were of a greater amplitude than LEPs, in line with intracutaneous electrical pain research, because Aβ fibre stimulation activates a larger number of thalamo-cortical units (Babiloni et al., 2007; Garcia-larrea et al., 2003; Gingold et al., 1991; Treede et al., 1999). This effect emerged despite the laser pain being rated as more intense and more unpleasant than EEPs.

When pain intensity was fully predicted by the cue, EEP amplitude did not correlate with intensity ratings, whereas LEP amplitude did. LEPs have been repeatedly shown to reflect

intensity rating (Beydoun, Morrow, Shen, & Casey, 1993; Iannetti, Zambreanu, Cruccu, & Tracey, 2005; Ohara, Crone, Weiss, Treede, & Lenz, 2004), however, as EEPs reflect somatosensory Aβ fibre activity alongside Aδ fibre nociceptive activity, it is unsurprising that they did not correlate with perceived pain intensity alone, as the 'noise' of the unrelated somatosensory related activity could prevent identification of a relationship. Also, EEP amplitude is known to habituate more than NPS ratings, which could also disrupt the relationship; this replicates previous EEP research (Babiloni et al., 2004; Christmann et al., 2007; Hoflle et al., 2013; Miltner et al., 1987; Rütgen, Seidel, Riečanský, et al., 2015). In summary, we show transcutaneous EEPs are earlier, higher in amplitude and do not correlate with pain intensity perception, in contrast to LEPs.

The laser pain stimulus was rated as more intense and more unpleasant than the electrical pain stimulus, despite participants undergoing a pain calibration procedure which was designed to calibrate stimulation to be equal between the two stimulator types. These differences in intensity rating are presumably due to changes between the pain calibration procedure and the main experiment. The most likely changes are differing habituation rates and skin temperature changes associated with the two stimulators.

LEPs and EEPs were modulated equally by participants' cued expectations. We propose that it is feasible to use either laser or electrical pain to study cued expectation and pain processing. We observed an anticipatory SPN with differing topography and morphology between laser and electrical pain, which differentiated pain intensity expectation during laser pain only. In this study, the SPN response to the intensity cue appears to be less sensitive prior to electrical stimulation than to laser stimulation. The differing topography, morphology and response to intensity of the SPN between conditions suggest they may originate from different neural generators.

We tested for effects of time on all measures of the pain response. Though significant results were small (see tables 4.2, 4.3 and 4.4), we did observe some differences between the two pain stimulation conditions. Laser pain perception increased over time, suggesting sensitisation to the stimulation, whereas electrical pain perception decreased over time, suggesting habituation. ERPs in both conditions decreased over time, suggesting ERP habituation to the pain stimulus. SPN amplitude decreased over time in the laser but not the electrical condition.

4.4.2.2. Theoretical discussion

LEPs and EEPs were modulated equally by cues despite the higher subjective unpleasantness of high intensity laser stimulation. Why did the higher affective impact of

the laser pain not interact with the expectation cue? Emotion plays a significant role in pain; negative emotion and catastrophizing increase perception of pain unpleasantness and intensity (Lin et al., 2013; Rainville, Bao, & Chrétien, 2005; Schupp, Berbaum, Berbaum, & Lang, 2005; Sullivan, Rouse, Bishop, & Johnston, 1997). The role of emotion in pain expectation is less clear, although studies suggest emotion plays a relevant role in expectation. For example, belief about the emotional impact of pain and confidence in the cue predicts the effect of expectation on pain (Brown, Seymour, El-Deredy, et al., 2008). Increased catastrophizing also leads to higher engagement even with inaccurate cues, possibly due to the increased threat status of the cue in high catastrophizers (Van Damme, Crombez, & Eccleston, 2002). Placebo analgesia itself has been modelled as a reduction in pain-related anxiety (Flaten, Aslaksen, Lyby, & Bjørkedal, 2011; Morton et al., 2009). In our study expectations of pain intensity may have modulated the sensory-discriminative rather than the affective-motivational dimension of pain. Future work could manipulate the affective dimension of pain whilst maintaining a consistent intensity, to further disentangle these two closely related qualities.

The SPN expressed different topography and morphology between laser and electrical pain, and differentiated between cue-evoked expectations of high and low laser but not electrical pain. This finding adds to a somewhat inconsistent literature, where some studies show an SPN in response to laser but not electrical pain, and others show an SPN to electrical pain (Babiloni et al., 2003, 2007; Seidel et al., 2015). The SPN to laser pain here differentiated between anticipation of high and low pain at central electrodes, as in previous studies showing sources in the anterior insula and cingulate in the laser pain SPN (Brown et al., 2008). However, the latter observations were only made for certain expectations and not for uncertain expectations. We observed an SPN to electrical pain at centroparietal electrodes contralateral to the site of stimulation, in line with previous studies showing activity in the posterior insula and posterior cingulate during anticipation of electrical pain (Berns et al., 2006; Hoflle et al., 2013; Lin et al., 2013). The contralateral topography of the electrical SPN suggests activity of the lateral sensory-discriminatory somatosensory cortices, rather than a medial affective-motivational response.

The differing topographies of the SPN across stimulators were accompanied by a difference in morphology, with greater negative amplitude to electrical rather than laser pain overall. This is surprising, as the anticipatory SPN should increase for events with greater impact, here the subjectively higher intensity and more unpleasant laser pain. Interestingly, the SPN amplitude did not correlate with unpleasantness ratings, in either condition. This suggests the SPN may, under certain conditions, reflect processes related to somatosensory rather than affective components of pain processing. However, as we did

not manipulate this, we cannot draw firm conclusions about this proposed role of the SPN, but it is a key direction for future work.

There are some limitations to the comparison between laser and electrical pain, because the two instruments require slightly different mode of stimulation, which could influence the results. First, the location of the laser stimulation was changed systematically between trials, but the location of the electrical stimulation was kept constant. This is a variable which is inherent to laser pain studies (e.g. Lorenz et al., 2005; Morton et al., 2009; Watson et al., 2007). The fact that a new area of skin was stimulated on each laser trial could influence participant's ability to discriminate the pain, although trial randomisation avoids any systematic bias associated with stimulation site and is therefore unlikely to affect the main results that we reported. The changes in the location of the laser stimulation also implied that it was less predictable than the electrical stimulation. We aimed to minimise any unpredictability by moving the laser in a systematic and predictable pattern across the skin. Further, the laser was visible to the participant so they were able to predict the position of the laser. It is also worth noting that the two instruments stimulated different locations in the body which could introduce differences between the two conditions. However, in terms of cortical topographic representation, the change in location is relatively small (Bingel et al., 2004; Saladin, 2012; Stippich et al., 1999), so is unlikely to influence the pain-evoked potential. It is also of note that participants were not instructed to attend specifically to either the location or the unpleasantness of the pain, and so this may have been a source of variability between participants. Attention to either of these aspects of pain can influence the neural networks responding to pain and this may be why we did not see any correlation between SPN amplitude and unpleasantness or intensity ratings (Kulkarni et al., 2005). Further, while the use of 75/25% probability cues was justified in this study because the key research question was about pain modulation by positive and negative expectation, the relationship between SPN and ERP amplitude has been observed only in response to fully predictable pain intensity cues (Brown, Seymour, Boyle, et al., 2008a). Our results support these findings in that all cues contained an element of unpredictability and we did not observe a relationship between SPN and ERP amplitude. This indicates that future studies should be designed with a fully predictable cue in order to examine the relationship between anticipatory SPN and pain-evoked potential amplitude.

Responses to laser and electrical pain were remarkably similar to one another, particularly in their modulation by cued expectation cues. When there is a reason to use many trials, our results suggest researchers should not hesitate to employ electrical stimulation if expectation is the effect of interest. See table 4.5 for advantages of laser and electrical

stimulation. For future studies we recommend that the use of a 75/25 reinforcement schedule and a minimum of 30 trials recorded per condition are adequate to capture modulation of pain-evoked potentials by expectation in either laser or electrical pain. Further research is required to test whether this effect can be captured across laser and electrical pain under different pain expectation manipulations, for example in a placebo analgesia manipulation. Larger anticipatory effect sizes may be obtained when employing a 'countdown' anticipation period (e.g. Brown, Seymour, Boyle, et al., 2008b). We also recommend that, as here, future studies limit the maximum number of delivered fibre laser pain stimuli, to avoid any skin heating or sensitisation. Finally, generally in pain expectation studies, it would be prudent to maximise the predictability of the pain stimulation location as we have done here. This minimises any confounding effect of location-related surprise on intensity expectation effects.

Table 4.5. *Laser versus electrical stimulation. A comparison of the advantages of laser and electrical stimulation.*

Advantages of laser stimulation	Advantages of electrical stimulation
Laser and electrical ERPs are modulated by stimulus intensity (section 4.3.2.3)	
Laser and electrical ERPs are modulated by intensity expectation (section 4.3.2.4)	
P2 reflects pain perception when pain intensity is expected (section 4.3.2.3)	ElectricalP2 amplitude is higher which may decrease statistical noise (figures 4.5 & 4.6)
Stimulation is perceived as more intense (section 4.3.1)	Stimulation is perceived as less unpleasant (section 4.3.1)
Anticipatory SPN reflects intensity expectation (section 4.3.2.2)	SPN does not habituate (section 4.3.2.2)
	No possibility of skin lesions
	Potential for a higher trial number without the risk of skin damage
	Does not require the wearing of safety goggles
	Portable, available commercially & less training required

In conclusion, we show that despite the absolute differences in intensity and unpleasantness ratings, and the latency and morphological differences of ERPs, intensity ratings and the marker for laser and electrical pain (EEP and LEPs) are modulated equally by cue-evoked expectancies. Further studies are required to explore the potential of using the two techniques to access different aspects of the processing of pain anticipation. In view of the powerful effects of placebo and nocebo effects, which are substantially driven by negative and positive expectation (Amanzio et al., 2013; Atlas & Wager, 2012; Colloca & Miller, 2011; Dodd, Dean, Vian, & Berk, 2017), both LEP and EEP methodologies have the potential to provide a physiological marker of individuals participating in clinical trials.

EEPs provide a more practical method for larger-scale studies, and the results of this study provide motivation for exploring this further.

Chapter 5: Prediction error and variability in human pain perception

Emily Hird designed the study, collected the data, analysed the data, and wrote the paper, with input at all stages from Deborah Talmi, Anthony Jones and Wael El-Deredy.

Abstract

There is burgeoning interest into the predictive mechanisms of pain perception, where expectations influence pain perception. This is captured in the predictive coding framework of pain perception. Here, pain perception is the result of the integration of expectations with pain stimulus intensity. We argue that it is likely that the influence of expectation on pain perception should reduce when expectations are strongly violated by pain, because highly erroneous expectations would otherwise lead to highly unrealistic perception. We test for the influence of expected intensity and pain stimulus intensity on perceived pain, and test whether highly unexpected pain stimulus intensities decrease the influence of expectations on perceived pain, using a pain cueing procedure and linear mixed modelling. Both expected intensity and pain stimulus intensity modulated perceived pain, and the influence of expectations on perceived pain decreased when pain was highly discrepant to expectation. We also test a previously unexamined prediction of the predictive coding framework, that the influence of pain stimulus intensity on pain perception is modulated by its certainty, or in other words, its inverse variance. The certainty of pain stimulus intensity did not modulate its influence on perceived pain. Our results provide evidence that the influence of expectation on pain is modulated by the discrepancy between expected pain and stimulus intensity, which has implications for chronic pain expectation-based therapies. We discuss the results within the framework of predictive coding.

5.1. Introduction

Expectations about pain intensity shift the perceived intensity of pain closer to the expected intensity. For example, in placebo analgesia expectations of low pain decrease pain perception and associated brain activity (Wager et al., 2004; Watson et al., 2007). Expecting high pain also increases the perceived intensity of pain, as in nocebo hyperalgesia (Blasini, Corsi, Klinger, & Colloca, 2017). This expectation effect can be accounted for in the predictive coding account of pain perception, where pain perception results from the integration of expectations with pain stimulus intensity. Although studies have shown the modulation of perceived pain by expectation, to our knowledge the influence of both expected pain intensity and pain stimulus intensity on perceived pain has not been formally tested (Almarzouki et al., 2017; Atlas et al., 2010; Brown et al., 2008; Brown et al., 2008; Keltner et al., 2006; Kong, Jensen, et al., 2013; Van Laarhoven & Evers, 2011; Yeung et al., 2014). This was our first aim.

One intriguing line of enquiry in pain perception relates to the limits of expectation. Although expectation is a powerful modulator of pain perception (Jonas et al., 2015), allowing highly unrealistic expectations to dictate perception could lead to inaccurate, even hallucinatory perceptions of the world. When there is clear sensory evidence that expectations are inaccurate, it is therefore likely that their influence reduces. We predict that there should an observable limit to the modulation of pain perception by expectation. The second aim of this study was to test the extent to which expectation modulates pain perception when these expectations are violated, by delivering pain stimulus intensities that violate expectations to increasing degrees and testing the effect on the resulting pain rating.

We cued expectations and delivered varying stimulus intensities which ranged from being fully expected to being highly discrepant to the cue. We formally tested the influence of cued intensity and stimulus intensity on pain intensity rating using a quantitative model, and tested whether highly unexpected stimulus intensities decreased the influence of cues on pain intensity rating. We manipulated PE size by manipulating the numerical discrepancy between cued intensity and stimulus intensity. The outcome variable was the numerical difference between the stimulus intensity and the pain intensity rating, which we term subjective error, or PE_{subj}. PE_{subj} provided a measure of how much pain intensity ratings were influenced by the cue. A polynomial relationship between PE and PE_{subj} would indicate that cued intensity influenced pain intensity rating up to a certain threshold, reflected in an increase in PE_{subj}, and this influence decreased when stimulus intensity was highly unexpected, decreasing PE_{subj}. This would suggest that there is a

'tipping point' where pain stimulation intensity is so unexpected that the influence of expectations on perceived pain intensity decreases (see figure 5.1).

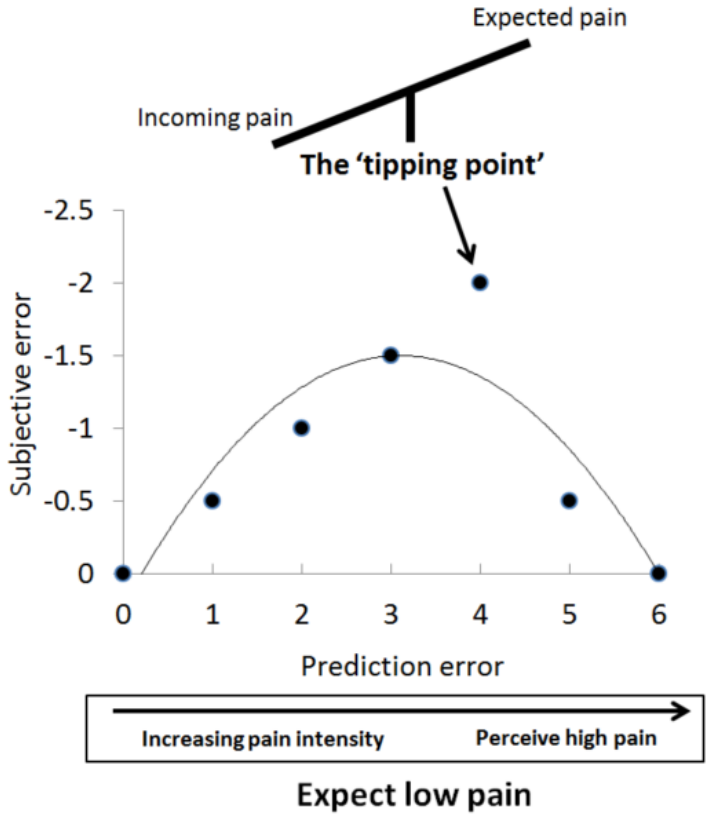

Figure 5.1. *Graphical representation of the potential polynomial relationship between* PE_{subj} *and PE. As the discrepancy between cued intensity and stimulus intensity (PE) increases, the discrepancy between stimulus intensity and pain intensity rating (*PE_{subj}*) also increases, as expectations influence pain perception. The 'tipping point' is reached where stimulus intensity is so unexpected that the influence of expectations decreases.*

5.2. Methods

5.2.1. Participants

31 participants aged 18-35 (19 females, mean age 23 years) were recruited via university advertisements. Participants received £15 compensation. Participants had normal or corrected-to-normal vision. They had no history of neurological or psychiatric conditions, had not taken analgesics on the day of the experiment, and did not have a history of chronic pain. Ethical approval was granted by the University of Manchester, where the study took place.

5.2.2. Apparatus

Visual stimuli were presented on a desktop computer screen one metre away from the participant. Painful stimuli were electrical pulses delivered via a concentric electrode by a constant current stimulator (Digitimer DS5 2000, Digitimer Ltd., Welwyn Garden City, UK). The pulse width of the electrical stimulation was 5 milliseconds. All stimuli were delivered on a Matlab platform (Mathworks) which interfaced with the pain stimulator via a digital-to-analogue convertor (Multifunction I/O device, National instruments, Measurement House, Berkshire, UK). Participants submitted their intensity ratings of the pain using a keypad.

5.2.3. Procedure

Upon arrival to the lab, participants were briefed by the experimenter, who introduced the study as a simple test of pain processing. After providing consent, participants washed both hands with soap and water.

Participants first underwent a pain calibration procedure on their left hand to determine their subjective response to increasing electrical stimulus intensities. The first stimulus was at a low intensity which is below the threshold for pain perception in most people. The stimulus intensity increased in a ramping procedure up to a maximum of five volts. We used a 0-10 NPS to measure the pain intensity rating, where a pain intensity rating of NPS 2 was when the stimulus became "just painful", NPS 5 was "medium pain", and NPS 8 was at the point where stimulus was "just tolerable", replicating previous research (Atlas, Wielgosz, Whittington, & Wager, 2014). We repeated this procedure three times and computed the average stimulus intensities over these three repetitions corresponding to NPSs 2, 3, 4, 5, 6, 7 and 8. Participants then underwent a pre-experiment test procedure: stimulus intensities corresponding to their pain intensity ratings NPS 2 to level 8 were delivered in a pseudorandom order four times and participants were instructed to identify the intensity of each pulse. Participants had to correctly identify 75% of stimulus intensities to continue to the main experiment. If they did not achieve this in the test procedure, the intensities were adjusted based on participants responses, and the test repeated until participants correctly identified 75% of stimulus intensities.

In the main experiment, participants were instructed that the cue predicted the stimulus intensity on each trial. The cue was a number on the computer screen which depicted the intensity of upcoming stimulation (figure 5.2), and then a stimulus intensity was delivered which either corresponded to the cued intensity or violated it at varying levels, in a partially reinforced cueing procedure (table 5.1). Only the NPS 2 ("just painful") and NPS

8 (“highest tolerable pain”) cues were followed by unexpected stimulus intensity; all other pain cues were veridical, or in other words the cued intensity matched the stimulus intensity. The veridical trials reinforced participant’s belief in the validity of the cues.

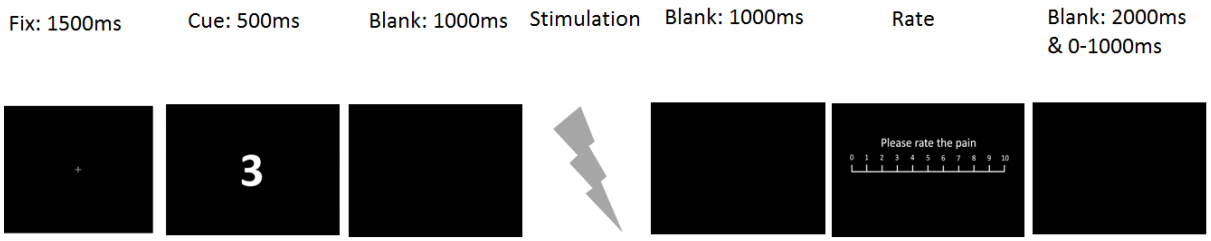

Figure 5.2. *Trial timeline. After viewing a fixation cross, participants viewed a number from 2 to 8 which depicted the cued intensity for that trial. After a blank screen, participants received the stimulus, followed by another blank screen. A rating screen was presented which prompted participants to rate the pain on a NPS scale. Finally, a blank screen was presented again.*

The cue depicted a cued intensity of NPS 2, 3, 4, 5, 6, 7 or 8 (figure 5.2 and table 5.1). In PE trials after presentation of the cued intensity NPS 2 or 8 participants could receive either NPS 2, 3, 4, 5, 6, 7 or 8 of stimulus intensity. This design allowed us to elicit a range of PEs. Participants were instructed to rate the intensity of the stimulus and were not informed that the cues were discrepant. See table 5.1 for a summary of all trials in the study.

Table 5.1: *A summary of all trials*

Cued intensity	Stimulus intensity	PE	Number of trials
2	2	0	5
2	3	1	5
2	4	2	5
2	5	3	5
2	6	4	5
2	7	5	5
2	8	6	5
3	3	0	10
4	4	0	10
5	5	0	10
6	6	0	10
7	7	0	10
8	8	0	5
8	7	-1	5
8	6	-2	5
8	5	-3	5
8	4	-4	5
8	3	-5	5
8	2	-6	5

On each experimental trial, participants viewed a fixation cross for 1500ms, a cue for 500ms, and then a blank screen for 1000ms. The stimulus was delivered and followed by presentation of a blank screen for 1000ms. A screen was then presented which prompted participants to numerically rate their subjective pain intensity on a 0-10 NPS using a keypad. There was no time limit on this response. Upon input of the rating, the screen was blank for 2000ms and a random amount of time up to 1000ms before commencement of the next trial. See figure 5.2 for a timeline for each trial.

5.2.4. Data analysis

First we aimed to quantify the influence of cued intensity and stimulus intensity on pain intensity rating. To achieve this, a mixed model multiple regression analysis was conducted across all conditions to test whether cued intensity and stimulus intensity predicted pain intensity rating, treating participant as a random variable, in Stata.

Second we aimed to test whether PE_{subj} expressed a relationship with PE. We tested for a linear or a polynomial relationship between PE and PE_{subj}. We analysed pain intensity ratings to stimuli which had a cued intensity of NPS 2 (low pain) or NPS 8 (high pain). PE was calculated as the numerical difference between the cued intensity and the stimulus intensity on a given trial, and so ranged from -6 (cue NPS 8 pain, deliver NPS 2 pain) to

+6 (cue NPS 2 pain, deliver NPS 8 pain). This provided a measure of how discrepant the stimulus intensity was compared with expectation. PE_{subj} was calculated as the numerical difference between the stimulus intensity and the pain intensity rating on a given trial. This provided a measure of how far expectations shifted the pain intensity rating away from the stimulus intensity.

Sensitivity to painful stimulation can vary over time, influenced both by habituation and by changes in motivation, boredom, and novelty (section 2.2.3) (Bingel et al., 2007). To account for this we tested for a relationship between trial and pain intensity rating. A significant result meant we included the effect of trial as a predictor in all above models.

5.3. Results

5.3.1. Influence of trial number on pain intensity rating

Across all trials, trial number negatively correlated with pain intensity rating, suggesting pain intensity rating habituated over time (B = -.002, SE = .001, p=0.03, 95% CI =-.004, -.0003). To account for this we included trial as a regressor in all subsequent analyses.

5.3.2. Analysis 1: Influence of cued and actual pain intensity on pain intensity rating

We first assessed the extent to which cued intensity and stimulus intensity influenced pain intensity rating. The averaged data plot suggested that as stimulus intensity increased, so did pain intensity rating. When stimulus intensities were preceded by a cue signalling NPS 2 (“just painful”), pain intensity ratings decreased compared to ratings for the same stimulus intensity when it was veridically cued. When stimulus intensities were preceded by a cue signalling NPS 8 (“highest tolerable pain”), pain intensity rating increased compared to the veridically cued stimulus. This effect suggested that low and high pain intensity cues shifted pain intensity ratings towards the cued intensity. This occurred at all stimulus intensities (figure 5. 3).

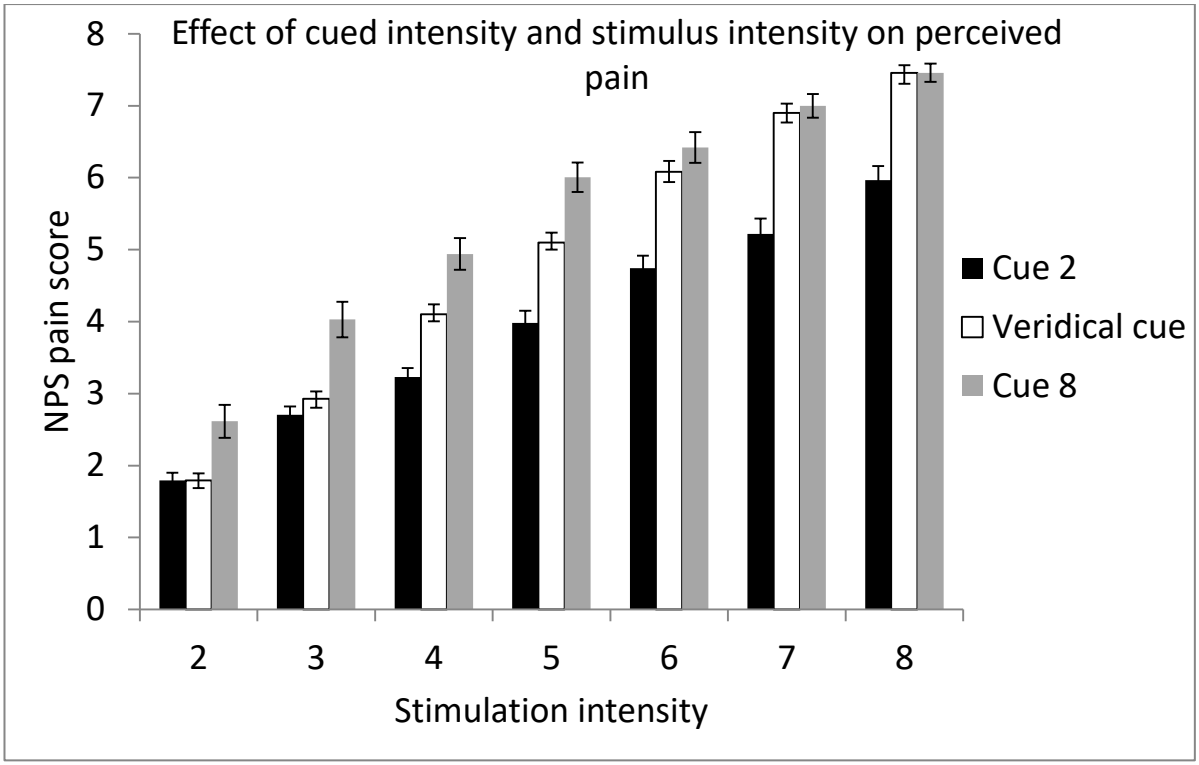

Figure 5.3. *Influence of cued intensity and stimulus intensity on NPS rating, across participants. Error bars represent the standard error of the mean. As stimulus intensity increased, pain intensity rating increased. A stimulus preceded by a level 2 cue (black) was rated as lower than the same intensity stimulus preceded by a level 8 cue (grey). Veridical ratings, where the cue accurately predicted intensity, are depicted in white.*

We used a mixed model multiple regression of pain intensity rating across all trials, with the predictors cued intensity, stimulus intensity and trial, and interactions between these predictors (table 5.2) to test whether cued intensity and stimulus intensity predicted pain intensity rating. This represented the influence of expectation (cued intensity) and actual stimulus intensity on pain perception. Participant effects accounted for 19% of the model's variability. The model explained 59% of the variance in pain intensity ratings. Stimulus intensity, cued intensity and the cued x stimulus intensity interaction were significant predictors of pain intensity rating. The results suggested that as cued intensity and stimulus intensity increased, so did pain intensity ratings, in line with the predictive coding framework, but the larger beta values for the effect of stimulus intensity (b=.62) indicated its relationship with pain intensity rating was stronger than the effect of cued intensity (b=.21). There was also evidence for habituation. Trial and the trial x cued intensity interaction were significant predictors of pain intensity rating, suggesting pain intensity rating and the effect of cued intensity decreased over time. The trial x stimulus intensity interaction was not significant.

Table 5.2. *Results of the linear mixed model described in analysis 1, testing the influence of cued intensity, stimulus intensity and trial on pain intensity rating. Grey shaded area shows the output for the intraclass correlation (ICC).*

Linear mixed model: Pain intensity rating										
Predictor	***b***	***SE***	***p***	**95% CI**		**R^2**	**ICC**	**SE**	**95% CI**	
Stimulus intensity	.62	.03	<.001	.56	.68	.59	.19	.04	.12	.29
Cued intensity	.21	.02	<.001	.17	.26					
Cued x stimulus intensity	.02	.004	<.001	.01	.03					
Trial	-.001	.001	.038	-.01	.002					
Trial x stimulus intensity	-.002	.003	.41	-.0004	-.001					
Trial x cued intensity	-.001	.0002	<.001	-.001	-.001					

5.3.3. Analysis 2: Relationship between PE and PE_{subj}

The first analysis showed that cued intensity and stimulus intensity modulate pain intensity ratings, but this did not show how the difference between these two variables influences pain intensity ratings. Next we tested for a relationship between the discrepancy between cued intensity and stimulus intensity (PE), and the influence of cued intensity on pain intensity rating (PE_{subj}). A mixed model regression analysis was employed to assess the extent to which the relationship between PE and PE_{subj} was linear, which would suggest the influence of expectations on pain intensity rating did not decrease when PE was large, or polynomial, which would suggest the influence of expectations on pain intensity rating decreased when PE was large. We included linear PE, trial number and the linear PE x trial number interaction as predictors in the linear model. In the polynomial model, we added the polynomial (PE^2) term for PE and the polynomial PE x trial number interaction.

The linear mixed model explained 31% of the variance in PE_{subj} (table 5.3). The addition of a polynomial term to the mixed model improved the fit, which now explained 32% of the variance in PE_{subj}. Participant effects accounted for 17% of the model's variability. The AIC score for the polynomial model was lower than for the linear model. Therefore, we concluded that the polynomial model fitted the data better than the linear model. In the polynomial model PE and PE^2 were both significant predictors of PE_{subj}, as were trial and the PE x trial and PE^2 x trial interactions.

Table 5.3. *Results of the linear (top) and polynomial (bottom) mixed model in analysis 2, testing whether the relationship between PE and PE_{subj} is linear or polynomial. Grey shaded area shows the output for the intraclass correlation (ICC).*

Linear mixed model: PE_{subj}										
Predictor	***b***	***SE***	***p***	**95% CI**		**R^2**	**ICC**	**SE**	**95% CI**	**AIC**
PE	.3	.02	<.001	-.33	-.27	.31	.17	.04	.11 .26	7466.58
Trial	-.01	.001	<.001	-.01	-.003					
PE x trial	-.001	.0002	.001	.003	.001					
Polynomial mixed model: PE_{subj}										
Predictor	***b***	***SE***	***P***	**95% CI**		**R^2**	**ICC**	**SE**	**95% CI**	**AIC**
PE	-.32	.02	<.001	-.35	-.29	.32	.17	.04	.11 .26	7428.74
PE^2	-.02	.004	<.001	-.032	-.01					
Trial	-.01	.001	<.001	-.01	-.004					
PE x trial	.001	.0002	<.001	.001	.001					
PE^2 x trial	.29	.13	.03	.04	.56					

To investigate the interaction between PE and trial, we broke down the data into the first half and second half of the trials. As shown in table 5.4, in trials 0-60 and trials 61-120, the polynomial term for PE was a significant predictor of PE_{subj}. The interaction thus does not qualify the relationship between polynomial PE and PE_{subj}.

Table 5.4. *Results of the polynomial mixed model investigating the interaction between PE and trial by testing whether the polynomial relationship between PE and PE_{subj} changed between the first and second half of the experimental session.*

Trial	**Polynomial mixed model:** PE_{subj}										
	Predictor	***b***	***SE***	***P***	**95% CI**		**R^2**	**ICC**	**SE**	**95% CI**	
1-60	PE	-.29	.01	<.001	-.31	-.27	.33	.19	.05	.12	.3
	PE^2	-.02	.003	<.001	-.02	-.01					
61-120	PE	-.23	.01	<.001	-.26	-.21	.26	.18	.04	.11	.27
	PE^2	-.01	.003	.002	-.02	-.003					

These results indicate that the relationship between PE and PE_{subj} was polynomial. As pain became more unexpected, pain intensity rating continued to be influenced by the cued intensity. When pain was highly unexpected, the influence of cued intensity decreased.

5.4. Discussion

We report a behavioural investigation into the influence of expected pain intensity, actual pain stimulus intensity and the discrepancy between them on perceived pain. This is the

first study to systematically vary pain PE size and test the influence on the resulting pain rating. The results yield two important findings. First, we observed a significant impact of both stimulus intensity and cued intensity on pain intensity rating, confirming the influence of these two parameters on pain perception. This is in agreement with previous work showing the influence of expectation on pain perception (Anchisi & Zanon, 2015; Watson et al., 2007; Wiech et al., 2014). Second, we show that PE size modulates the influence of expectations on pain intensity rating. When stimulus intensity was highly discrepant to cued intensity, pain intensity ratings were less influenced by the cued intensity. This result indicates that there is a 'tipping point' or a limit to the influence of expectations on pain perception: when pain stimulus intensities strongly violate expectation, the influence of that expectation on perceived pain intensity decreases.

This result is in line with the predictive coding framework. Here, pain stimulus intensities which do not strongly violate expectations are assimilated into expectation, but if pain stimulus intensity is highly discrepant to expectation, the influence of expectation on pain perception is reduced in order to better represent the stimulus intensity (Büchel et al., 2014; FitzGerald, Dolan, & Friston, 2014). Interestingly, our results show that this occurs in response to both higher than expected pain and lower than expected pain, implicating predictive coding in both placebo analgesia and nocebo hyperalgesia.

The neural expression of PE has been captured in the PAG, and the PAG-RVM-spinal cord pathway has been identified as a potential pathway for predictive coding (Büchel et al., 2014). In the context of our results, the difference between cued intensity and stimulus intensity is likely to be calculated in the PAG. If the stimulus intensity is acceptably similar to the cued intensity, the PAG may employ its descending modulatory pathways to modulate signalling of the stimulus intensity to better match expectations. When the stimulus intensity is highly discrepant to the cued intensity, the influence of expectations is reduced and this may be reflected in altered ascending signalling of pain from the PAG (Büchel et al., 2014; Hosobuchi et al., 1977; Johansen et al., 2010; Ritter et al., 2013). Future studies could test for this effect in the PAG using fMRI.

Chronic pain is a significant public health issue (Tabor et al., 2017). Almost two in three chronic pain patients report that their medication is inadequate to control their pain, and side-effects of medication are a major concern, which means that expectation-based therapies are a useful alternative (Breivik, Collett, Ventafridda, Cohen, & Gallacher, 2006). Our result is relevant for therapies using expectations to improve chronic pain outcomes, such as those using verbal suggestion and imagery (Peerdeman et al., 2016). We show that the influence of expectations on pain reduces when stimulus intensities are highly

discrepant, which suggests expectation-based therapies should not try to build expectations which are radically different to the sensory reality.

There are three key limitations to this study. First, we induced expectations of both high and low pain. The experience of expecting high pain is affectively different to expecting a low pain. For example, expecting low pain may decrease anxiety, whereas expecting high pain may increase anxiety, and anxiety is known to influence the perceived intensity of pain which could interact with the effect of expectation (Wager, 2005). Expecting high pain could also increase attention to the stimulus intensity, and attention is known to modulate responses to pain (Bantick et al., 2002; Brooks, Nurmikko, Bimson, Singh, & Roberts, 2002; Miron, Duncan, & Bushnell, 1989). Future studies could repeat the study while recording anxiety and attention to pain to test whether they influence the effects of pain expectations reported here. Second, there is a possible confound in that stimulus intensity was varied to elicit different levels of PE. A higher stimulus intensity is likely to be more salient, and thus have more importance assigned to it which could increase its influence on perceived pain (Borsook, Edwards, Elman, Becerra, & Levine, 2013). It would be useful to examine the effect of different pain intensity cues at a constant level of stimulus intensity, to test the effect of PE on ratings and remove the variable of pain intensity. Third, predictive coding argues that the influence of expectation on perceived pain is also modulated by the certainty (the inverse variability) of the pain stimulus intensity. For these results, this could mean that unexpected pain reduces the influence of expectation by reducing the certainty of expectation. This would confound our claim that PE size modulates the influence of expectation on pain. To test whether the variability of pain stimulus intensity modulates the influence of expectations on perceived pain intensity, we conducted a second experiment to test whether increasing the variability of pain stimuli changes the influence of cued intensities on pain intensity ratings (section 5.5)

To conclude, we explored parameters of pain perception using the intensity of painful stimulation itself as a manipulation in order to increase the real-world applicability of our design. We show that although both expectations and pain intensity modulate pain perception, when pain is very different to what was expected, perception moves closer to the pain stimulus intensity. This suggests the influence of expectations on pain perception is dependent on how discrepant the pain stimulus is to expectation. Pain perception is bewilderingly variable (Diatchenko et al., 2005) but our results provide insight into the influence of PE on pain perception.

5.5. Variability study

5.5.1. Introduction

Pain is dynamic, and is accompanied by somatosensory information, creating a noisy and variable signal (Moseley & Vlaeyen, 2015). Predictive coding argues that more variable pain stimulation should increase the influence of pain intensity expectation on pain perception, because stimulus intensity becomes less certain (Büchel et al., 2014). To test whether the higher levels of variability in the PE conditions could influence our results, we delivered a high variability and low variability series of pain stimulus intensities in two separate blocks within the same visit and using the same apparatus as the main experiment. We tested whether the influence of the cued intensity increased in the high stimulus variability block compared to the low stimulus variability block (table 5.5).

5.5.2. Methods

First, a pain calibration procedure which was identical to that described above (section 5.2.3) adjusted stimulation intensity for pain stimuli which were delivered to the right hand.

Next, participants took part in two blocks: in the high pain variability block, stimulus intensity varied from NPS 1 to NPS 8 (including the following intensities: 1, 2, 3, 4, 6, 8). In the low variability block, only the stimulus intensities of NPS 6 or NPS 2 were delivered (table 5.2). Blocks were counterbalanced. Participants viewed a cue which signalled a 75% likelihood of receiving NPS 2 and a 25% likelihood of receiving NPS 6, and then rated the stimulus intensity. Because the cue signalled a high likelihood of receiving NPS 2 pain, we tested the influence of expecting NPS 2 pain on pain intensity ratings when the stimulus intensity was more or less variable. In the low variability block, the visual cue was veridical and 90 stimulus intensities were at NPS 2, whilst 30 were at NPS 6. In the high variability block, the average stimulus intensity equalled NPS 2 and NPS 6, but they varied around this average. In the high variability block, for the NPS 6 trials we delivered 10 pulses at a NPS 6, but also 10 at a NPS 4 and 10 at a NPS 8. For the NPS 2 trials we delivered 30 at NPS 2, but also 30 at NPS 3 and 30 at NPS 1. The cue changed in shape (but not in meaning) between the high and low variability condition. See table 5.5 for a summary of all trials and figure 5.4 for a timeline for each trial. Participants were not explicitly made aware of any difference between the blocks; instead they were instructed that the stimulus intensities always followed the cue and that they would receive NPS 2 stimulus intensities in 75% of trials, and NPS 6 stimulus intensities in 25% of trials.

Table 5.5. *A summary of all trials in the variability session*

Cued likelihood of pain intensity	Condition	Actual pain intensity	Number of trials
75% NPS 2 25% NPS 6	Low variability	2	90
		6	30
	High variability	2	30
		1	30
		3	30
		6	10
		4	10
		8	10

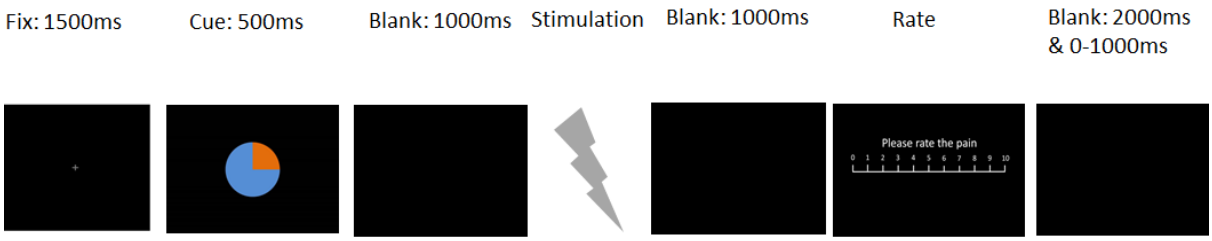

Figure 5.4. *Trial timeline. Participants viewed a fixation cross and then a probability cue, followed by a blank screen and a painful stimulus. Finally, a rating screen prompted participants to rate the pain from NPS 0-10.*

Frequency histograms and the Shapiro-Wilk test of normality showed that data were not normally distributed when participants rated NPS 2 stimulus intensities in the high variability condition (p=.02). We therefore entered participant averaged pain intensity ratings of the NPS 6 and NPS 2 stimulation into a Sign test, testing for an overall difference between pain intensity ratings in the high and low variability condition in SPSS.

5.5.3. Results

A sign test on the non-normally distributed data showed that there was no significant effect of variability on pain intensity ratings (p>0.05).

5.5.4. Discussion

Results showed that the influence of cued intensity on pain intensity rating did not change whether the variability of pain stimulus intensity was high or low. In the context of the main experiment (section 5.1 to 5.4), this suggests that increased variability associated with the PE manipulation did not change the influence of cued expectations on pain

intensity rating. This indicates that the reduced influence of expectation when PE was high was not dependent on the increased variability associated with higher PE size. Our results contradict a key prediction of the predictive coding framework of pain perception, that the weight of stimulus intensity is inversely related to its variance.

Chapter 6: Investigating cue and sham treatment as pain modulators

Emily Hird designed the study, collected the data, analysed the data, and wrote the paper, with input at all stages from Deborah Talmi, Anthony Jones and Wael El-Deredy.

Abstract

Placebo analgesia is an intriguing phenomenon which reveals the powerful impact of expectation on perception. Here the administration of a sham analgesic decreases the perceived intensity of pain. One account of placebo analgesia suggests that it is a response to the ritual of treatment administration. This is important for studies investigating the modulation of pain perception by cue, including Chapters 4 and 5 of this thesis, because it suggests the results of placebo and cue evoked expectation manipulations are not comparable. Study one of this chapter aimed to test whether cue-evoked expectations are comparable to placebo-evoked expectations by closely replicating the conventional placebo analgesia manipulation using visual cues. Study two aimed to test whether placebo analgesia responses can be elicited in response to electrical pain stimulation, which has methodological advantages but is less well-established than laser pain stimulation. We aimed to directly compare the cue-evoked pain modulation in study 1 with the placebo analgesic pain modulation in study 2. To achieve this, we kept all procedures consistent between the placebo and cue manipulations. Our hypothesis was that EEG and behavioural pain responses after a cue signalling low pain would be equivalent to pain responses after a placebo analgesia manipulation, further linking the results of cue manipulations with that of placebo manipulations. Participants were separated into cue and placebo groups. The manipulation was consistent between groups, except that in the cue condition, participants viewed a conditioned pain intensity cue, whereas in the placebo condition, participants received a conditioned sham treatment. We measured pain intensity ratings, the anticipatory SPN and the pain EEP. The key result of study one was that cues modulated pain intensity ratings and EEP amplitude similarly to placebo analgesia. In study two, we did not observe an effect of placebo analgesia on pain responses. We discuss the potential reasons for this and make suggestions for adjustments in future studies.

6.1. Introduction

6.1.1. Study 1: a cue replication of placebo analgesia

Expecting pain relief after a medical intervention can change how pain is perceived. This intriguing phenomenon is termed placebo analgesia (Amanzio & Benedetti, 1999; Benedetti & Amanzio, 1997; Petrovic, Kalso, Petersson, & Ingvar, 2002b; Zubieta et al., 2005). The magnitude of this response is strong, as evidenced in a meta-analysis showing sham analgesia surgery achieves a level of pain relief comparable to that of real analgesia surgery (Jonas et al., 2015). After placebo analgesia, perception of low pain persists even after expectations are violated by the high pain stimulus (Watson et al., 2007). With enough conditioning trials, this effect persists across the experimental session (Colloca, Petrovic, Wager, Ingvar, & Benedetti, 2010).

Placebo analgesia manipulations typically involve the administration of a sham analgesic alongside the covert reduction of pain stimulation intensity in a conditioning procedure (Morton et al., 2009; Watson et al., 2006, 2007). Perception of subsequent high pain stimulation intensities is reduced by the manipulation. Conscious expectations are important for the placebo response: awareness that the pain stimulation intensity was decreased in the conditioning procedure abolishes placebo responses (Montgomery & Kirsch, 1997). Conditioning also increases the magnitude of the placebo response (Colloca and Benedetti 2009). As well as conscious expectations and conditioning, the placebo response may also depend on 'non-specific' effects of treatment administration (Atlas & Wager, 2012). These 'non-specific' effects include the social interaction between a therapist and a patient and therapeutic touch and gestures (Atlas & Wager, 2012; Carlino, Pollo, & Benedetti, 2011; Finniss, Kaptchuk, Miller, & Benedetti, 2010; Flaten et al., 2011; Miller, Colloca, & Kaptchuk, 2009). For example, the social support offered in administering a treatment enhances placebo responses (Kaptchuk et al., 2008; Valentini, Martini, Lee, Aglioti, & Iannetti, 2014).

These non-specific effects are not a feature of the cued expectations studies reported in Chapters 4 and 5. In these studies, expectations of stimulus intensity were elicited by the presentation of a conditioned cue which was reinforced throughout the test session, where the pain outcome matched the cue most of the time. In contrast, in the test session of placebo analgesia studies expectations are consistently violated (e.g. Watson et al., 2007). Consequently, Chapters 4 and 5 of this thesis which show modulation of pain by reinforced cues may represent a different mechanism to placebo analgesia. The objective of study one was to check whether we can obtain a placebo analgesia-like effect while

manipulating expectations with the pain-intensity cues developed in Chapters 4 and 5. For this purpose, we replicated as closely as possible a classical placebo analgesia manipulation paradigm with the exception that we removed all aspects of the treatment ritual. We hypothesised that the cue manipulation would modulate pain perception and the associated anticipatory SPN and EEPs as we have seen before, and comparably to that of a placebo analgesia manipulation. This would indicate that it is possible to achieve placebo analgesia without 'non-specific' effects associated with the treatment ritual. Such a result would allow us to apply the findings of the thesis to placebo analgesia.

6.1.2. Study 2: placebo analgesia modulation of electrical pain

Although the aim of study 1 stands alone, a direct statistical comparison of this result with a placebo manipulation would reveal any differences in the modulation of pain by cue and sham treatment. The first aim of study 2 was to directly compare the cue-evoked pain modulation in study 1 with the placebo analgesic pain modulation in study 2.

Placebo analgesia has been well-established as a powerful effect using laser pain stimulation with a placebo cream (Colloca, Sigaudo, & Benedetti, 2008; Morton, Brown, et al., 2010; Morton, El-Deredy, Watson, & Jones, 2010; Morton et al., 2009; Watson et al., 2009b; Watson, Power, Brown, El-Deredy, & Jones, 2012; Watson, El-Deredy, Bentley, Vogt, & Jones, 2006; Watson et al., 2007). An alternative to laser pain stimulation is electrical pain stimulation. Electrical pain and its correlates are modulated by expectation cues equivalently to that of laser pain, as we showed in Chapter 4 (Pain FRN and cue modulation of pain). However, modulation of electrical pain-evoked potentials using a placebo cream, which is the best established placebo manipulation, has not been clearly shown. Studies which show placebo analgesia to electrical pain did not use a placebo cream, instead presenting a TENS stimulator or a pill as sham treatment (Colloca & Benedetti, 2006, 2009; Colloca et al., 2010; Rütgen, Seidel, Silani, et al., 2015; Rütgen, Seidel, Riečanský, & Lamm, 2015; Yeung et al., 2014). Although one study did elicit placebo responses to electrical pain with placebo cream, this was a behavioural study and so they did not show modulation of neural pain responses (De Pascalis et al., 2002). One fMRI study showed modulation of electrical pain by placebo, but here the conditioning trials were delivered at a different site to the test trials (Wager, 2004). The administration of a cream to a specific area of the body is likely to elicit the most powerful placebo response because responses are specific to the somatotopic target of the sham treatment, only expressing analgesia where the cream is applied (Benedetti, Arduino, & Amanzio, 1999). Electrical pain stimulation holds various methodological advantages to laser

stimulation, as discussed in detail in Chapter 4. The most salient point is that laser stimulation can result in heating of the skin, risking skin lesions and potentially leading to sensitisation, which could confound pain responses. Electrical stimulation does not heat the skin and there is no risk of skin damage. It is therefore useful to clearly show that electrical pain and corresponding evoked potentials are modulated by a placebo manipulation. We aimed to replicate the results of previous, well-established LEP cream placebo studies. Therefore, the aim of study two was to test whether it is possible to replicate the well-established three block placebo analgesia effect with cream described in laser pain studies with electrical stimulation (Morton, Brown, et al., 2010a, 2010b; Morton et al., 2009; Watson et al., 2012; Watson et al., 2009, 2006, 2007).

6.2. General methods

6.2.1. Design

Participants were randomly allocated into one of four groups: placebo experimental, placebo control, cue experimental and cue control. Responses were defined as the difference in pain intensity ratings and EEPs between the pre-conditioning (block one) and the post-conditioning (block three) for each group, which we expected would be larger in the experimental compared with a control group (figure 6.1). Studies one and two used mixed 2 (block: one vs. three) x 2 (session type: experimental vs. control) designs.

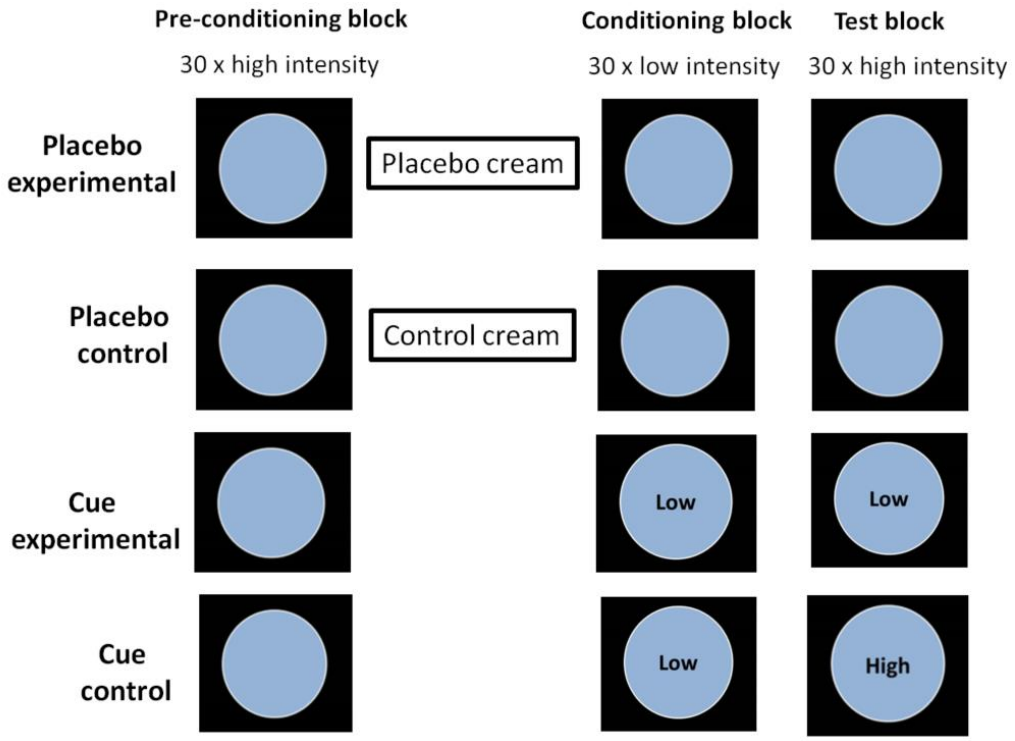

Figure 6.1. *Experimental structure of the placebo and the cue groups. Across all groups, participants received 30 high intensity painful stimuli in the pre-conditioning block, 30 low intensity painful stimuli in the conditioning block, and 30 high intensity painful stimuli in the post-conditioning block. A blank circle served as the fixation cross. In the placebo experiment group, a placebo cream was administered with verbal instruction that this would decrease the pain. In the placebo control group, the same cream was administered with verbal instruction that this was an inert cream which would not change the pain. In the cue experimental group, the low pain cue was conditioned in the conditioning block and presented again in the test block. In the cue control group, a high pain cue was presented in the test block.*

6.2.2. Participants

Participants aged 18-35 years were recruited via university advertisements and received £15 compensation for participation in the study. Participants had normal or corrected-to-normal vision. They had no history of neurological or psychiatric conditions, did not take medications which could affect their neurotransmitter levels, or take analgesics, and did not have a history of chronic pain. Ethical approval was granted by the University of Manchester, where the study took place. Placebo responses have been observed with a sample size of 30 per group so we aimed to collect 30 participants per group, totalling 120 participants (Morton et al., 2010, 2009).

A manipulation check, planned to be carried out halfway through data collection, revealed a procedural problem in study 2 (section 6.4.1.3). Consequently, we continued collecting data for study 1, and restarted study 2 with new participants after additional work was carried out to improve the methodology. Therefore, we analysed a sample of N=30 participants per group for study 1 (33 females, mean age 22 years) and N=22 per group for study 2 (29 females, mean age 22 years).

In the pain intensity ratings analysis, two outlying participants were identified using Tukey's method of outlier detection (Tukey, 1977). We reasoned that if participants perceived the pain stimulus intensity unusually, their EEG data would not be reliable, so we removed these participants from the whole analysis. Five participants were removed from the whole analysis because no pain intensity ratings were recorded. In the SPN data, five outlying participants were identified using Tukey's hinges and removed from the SPN analysis. In the EEP data, one outlying participant was identified using Tukey's hinges and removed from the EEP analysis. Three participants were removed from the EEP analysis because no EEG triggers were recorded during the experiment, and two were removed because they blinked on almost every trial which meant too few trials were available for analysis.

6.2.3. Apparatus

Visual stimuli were presented on a desktop computer screen one metre away from the participant. Painful stimuli were electrical pulses generated by a constant current stimulator and delivered by a concentric electrode (Digitimer DS5 2000, Digitimer Ltd., Welwyn Garden City, UK). All pain stimuli were delivered on a Matlab platform (Mathworks) which interfaced with the pain stimulator via a digital-to-analogue convertor (Multifunction I/O device, National instruments, Measurement House, Berkshire, UK). The painful stimuli were of 5 millisecond duration and were delivered to the dorsum of the right hand. The intensity of the pulse was limited to a 5 volt maximum. Participants submitted their intensity ratings of the pain using a keypad on a 0-10 NPS, where a level three was when the stimuli became painful, and level seven was at the point where the participant felt pain as high as it could be for them while still remaining tolerable.

6.2.4. Procedure

Participants were randomly allocated to either the experimental or control condition, to which the experimenter was blinded until the end of block one. Whether a particular participant was allocated to the placebo or cue groups was predetermined, to allow set-up of either before the participant arrived.

Upon arrival to the lab, participants were briefed by the experimenter with a scripted introduction (appendix B). After providing consent and applying the EEG cap, participants washed their right hand with soap and water and a conductive paste was applied to the stimulation site. Participants underwent a pain calibration procedure to determine their subjective response to increasing electrical stimulus intensities. The first pulse was very low, generally an intensity which would be below the threshold for pain in most people, and the intensity of the stimuli increased in in a ramping procedure. This procedure was repeated three times to determine the average stimulation intensities required to elicit NPS 4 and 7 pain responses.

6.2.5. Trial timeline

In all groups, on each experimental trial, participants viewed a circle or cue, in the placebo or cue condition respectively, for 2000ms. The pulse was delivered, and this was followed by presentation of a blank screen for 1000-1250ms. The pulse width of the electrical stimulation was 5ms. A screen was then presented which prompted participants to numerically rate their subjective pain intensity on a NPS using a keypad, which did not have a time limit. Upon input of the rating, the screen was blank for 2 seconds before commencement of the next trial. See figure 6.2 for a timeline for each trial.

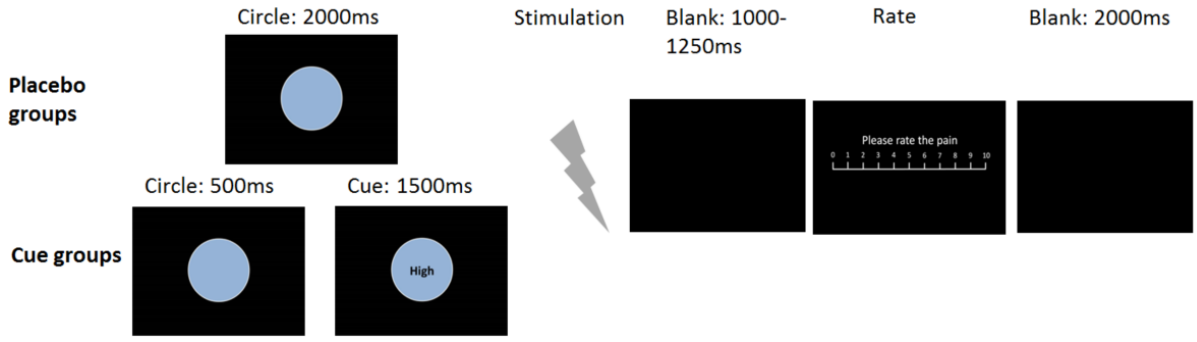

Figure 6.2. *Time-course of each trial. In the placebo groups, a blank circle was followed by the painful stimulation and a pain intensity rating prompt. In the cue groups, a blank circle was followed by a pain intensity cue, followed by the painful stimulation and a pain intensity rating prompt.*

6.2.6. EEG recording

Continuous EEG recording was acquired at a sampling rate of 512 Hertz using a 64 electrode Active-Two amplifier system (Biosemi, Amsterdam, Netherlands) with Biosemi acquisition software (BioSemi, Netherlands). Impedances were kept at 20 KΩ or less. The

experiment was conducted in a soundproofed, electromagnetically shielded, dimly lit room.

6.2.7. Behavioural data analysis

Average pain intensity ratings for each participant and condition were entered into a 2 (group: experimental vs. control) x 2 (block: 1 vs. 3) mixed-model ANOVA, for study one and study two separately. Significant interactions were broken down using planned comparisons paired T-tests. Average NPS, threat, unpleasantness ratings and manipulation ratings for each participant were entered into independent samples t-tests comparing the control vs. experimental condition, separately for study one and two. We did not compare the results of study 1 and study 2 in a single statistical model.

6.2.8. EEG data analyses

6.2.8.1. SPN

The SPN was preprocessed using SPM12. The signal was referenced to the mean of all scalp electrodes, downsampled to 200 Hertz (Hz), and filtered with a lowpass Butterworth filter (30 Hz). Epochs were extracted from 200ms before the presentation of the probability cue, across the anticipatory and pain delivery period, to 500ms after delivery of the pain stimulus. Data were filtered with a highpass Butterworth filter (0.1Hz) and underwent artefact rejection with a threshold of 80uV. This artefact information was then applied to the previous data which had not been filtered with a lowpass Butterworth filter. On average, 16% of trials were removed across participants and conditions (average remaining (SD): cue control 26.23 (.91); cue experimental 27.12 (.71); placebo control 25.22 (1.07); placebo experimental 22.59 (1.37). Single-trial data were averaged separately for the eight conditions using the "robust averaging" method in SPM12b (Litvak et al., 2011). Weights were computed by condition to account for differing number of trials between conditions. Averages were then filtered with a high-pass filter to remove high frequencies introduced by the robust averaging method. As we could not see a clear topographic negativity in the 500ms preceding pain delivery, participant voltages were extracted from this time-window at central electrodes CPz, Cz, FCz, C1, C2, FC1 and FC2 (Brown et al., 2008). We ran a 2-way mixed model ANOVA with factors Block (1 vs. 3) and Group (control vs. experimental) on these data, separately for the cue and placebo groups. Correlations between SPN amplitude and pain intensity rating, threat and unpleasantness rating or EEP amplitude were tested using Pearson's coefficient.

6.2.8.2. P2

For the remaining data, the ERP time-locked to pain delivery was preprocessed using SPM12 (Ashburner et al., 2013; Litvak et al., 2011). The signal was referenced and downsampled as in the SPN analysis, and was then filtered with a Butterworth filter between 0.5 and 30 Hz. This time, epochs were extracted 200ms before the pain delivery to 800ms after. Data underwent artefact rejection at a threshold of 80uV. On average, 17% of trials were removed across participants and conditions (mean trials remaining (SD): cue control 21.02 (1.86); cue experimental 27.29 (.81); placebo control 25.33 (1.47); placebo experimental 25.75 (1.33). The data were averaged and filtered as in the SPN analysis. The latency of 50% of the maximum amplitude of the grand average across all conditions and participants was identified, as previously reported in analysis of the peak (Chapter 4, Pain FRN and cue modulation of pain; Luck, 2005). This was 175-375ms post-stimulus for the placebo experimental, 175-380ms post-stimulus for the placebo control, 180-375ms post-stimulus for the cue control, and 175-375ms post-stimulus for the cue experimental group. Each participant's maximum within this latency was identified, and ±40ms window was extracted around this to include the peak of the maximum, at peak electrodes within each participant (figure 6.3). Data were entered into a 2 (group: experimental vs. control) x 2 (block: 1 vs. 3) mixed-model ANOVA, for study one and study two separately. Correlations between EEP and pain intensity rating were tested using Pearson's coefficient.

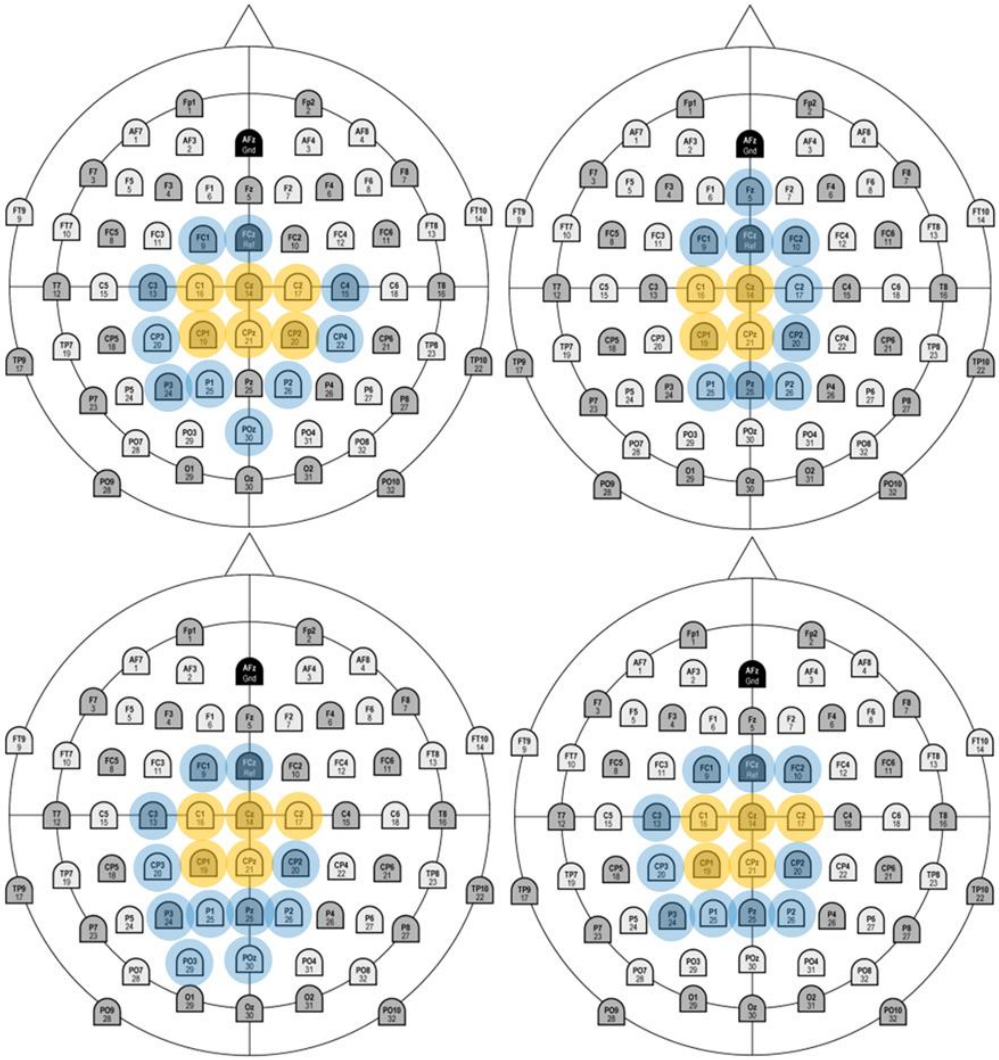

Figure 6.3. *Electrodes selected for voltage extraction, across participants. Yellow highlights show electrodes selected for extraction in more than 50% of participants, blue highlights show electrodes selected for extraction in fewer than 50% of participants. Across participants, the P2 peak was expressed at centroparietal electrodes. Clockwise, from upper left: Cue control; cue experimental; placebo experimental; placebo control.*

6.3. Study 1: a cue replication of placebo analgesia

6.3.1. Methods

See section 6.2 for the methodological details which spanned studies 1 and 2.

6.3.1.1. Cue experimental

Participants viewed a circle on the computer screen identical to the placebo condition, except that it contained verbal instruction of the stimulus intensity (figure 6.1). In block one, the word 'high' indicated that the incoming pulse was high. Participants viewed this cue, received an electrical pulse at an intensity corresponding to their NPS 7 and rated the pain intensity 30 times. After picking out a piece of paper from an envelope (unbeknown to the participant, both sheets of paper said 'pain perception study'), the electrode was removed and a mark was placed at the location of the electrode to ensure it was replaced in the same location, to avoid any variability in afferent stimulation sites. Participants then had a 10 minute break, after which the electrode was reapplied to the hand. In block two participants were informed that they would receive pain at a low intensity, and each low intensity pulse was preceded by a visual cue indicating low intensity pain, participant's NPS 4. Finally, participants underwent a third block where the pulse intensity was increased to the participant's level seven, but the pulse was again preceded by a visual cue indicating low intensity pain, replicating the experimental group procedure of placebo analgesia paradigms (Watson et al., 2007).

6.3.1.2. Cue control

Here, the same procedure was carried out, but in the third block, the visual cue indicated high intensity pain, replicating the control group procedure of placebo analgesia paradigms (Watson et al., 2007).

In both groups, after each block, participants rated how unpleasant and threatening they found the pain stimulation using a NPS. At the end of the session, participants underwent a manipulation check where they rated how accurately they felt the cue predicted the pain intensity using a NPS.

See figure 6.1, section 6.2.1 for a summary of the experiment structure.

6.3.2. Results

6.3.2.1. Behavioural results

Pain intensity ratings expressed a main effect of block ($F(1,54)=28.97$, $p<.001$, $\eta_P^2=.35$) (block 1>block 3), qualified by an interaction with group ($F(1,54)=36.79$, $p<.001$, $\eta_P^2=.41$). Paired samples t-tests revealed a significant effect of block in the experimental group,

where pain was decreased in block 3 compared to block 1 (t (27) = 6.41, p<.001) but not the control group (t (27) = -.76, p=.45) (table 6.1).

There was not a significant difference in the threat ratings for cue experimental and cue control conditions (t(57)=.57, p=.57), nor was there a significant difference in the unpleasantness ratings for cue experimental and cue control conditions (t(35)=1.02, p=.31) (table 6.1).

As predicted, there was a significant difference in the cue accuracy ratings for cue experimental and cue control conditions (t(54)=3.26, p=0.002), suggesting participants rated the control cue as more accurate at predicting the pain intensity than the experimental cue (table 6.1).

Table 6.1. *Mean ratings of NPS, threat, unpleasantness and cue accuracy for study 1*

Ratings	**Group**	**Block 1 Mean (sd)**	**Block 3 Mean (sd)**
NPS	Experimental	6.04 (.58)	4.86 (1.12)
	Control	6.58 (.51)	6.65 (.77)
		Mean (sd)	
Threat	Experimental	1.33 (.44)	
	Control	1.41 (.6)	
Unpleasantness	Experimental	4.22 (1.5)	
	Control	4.65 (.9)	
Cue accuracy	Experimental	4.2 (2.83)	
	Control	6.7 (2.89)	

6.3.2.2. Electrophysiological results

6.3.2.2.1. SPN

A 2-way ANOVA revealed a trend towards a main effect of block (F(1,50)=2.95, p=0.09, η_P^2=.06), and no interaction (F(1,50)=.79, p=0.38, η_P^2=.02), suggesting there was no statistically significant effect of block on SPN amplitude, or a difference between the experimental and control group. The SPN did not correlate with pain intensity rating in either the control (r=.02, n=69 p=.9) or the experimental condition (r=-.11, n=81, p=.34), nor did it correlate with EEP in either the control (r=-.19, n=69, p=.11) or the experimental condition (r=.06, n=81, p=.61). Finally, the SPN correlated negatively with unpleasantness rating in the control (r=-.34, n=43, p=.03) but not the experimental condition (r=.07, n=59, p=.59), but it did not correlate with threat rating in the control (r=-.22, n=61, p=.09) or experimental condition (r=-.09, n=77, p=.42). See figure 6.4.

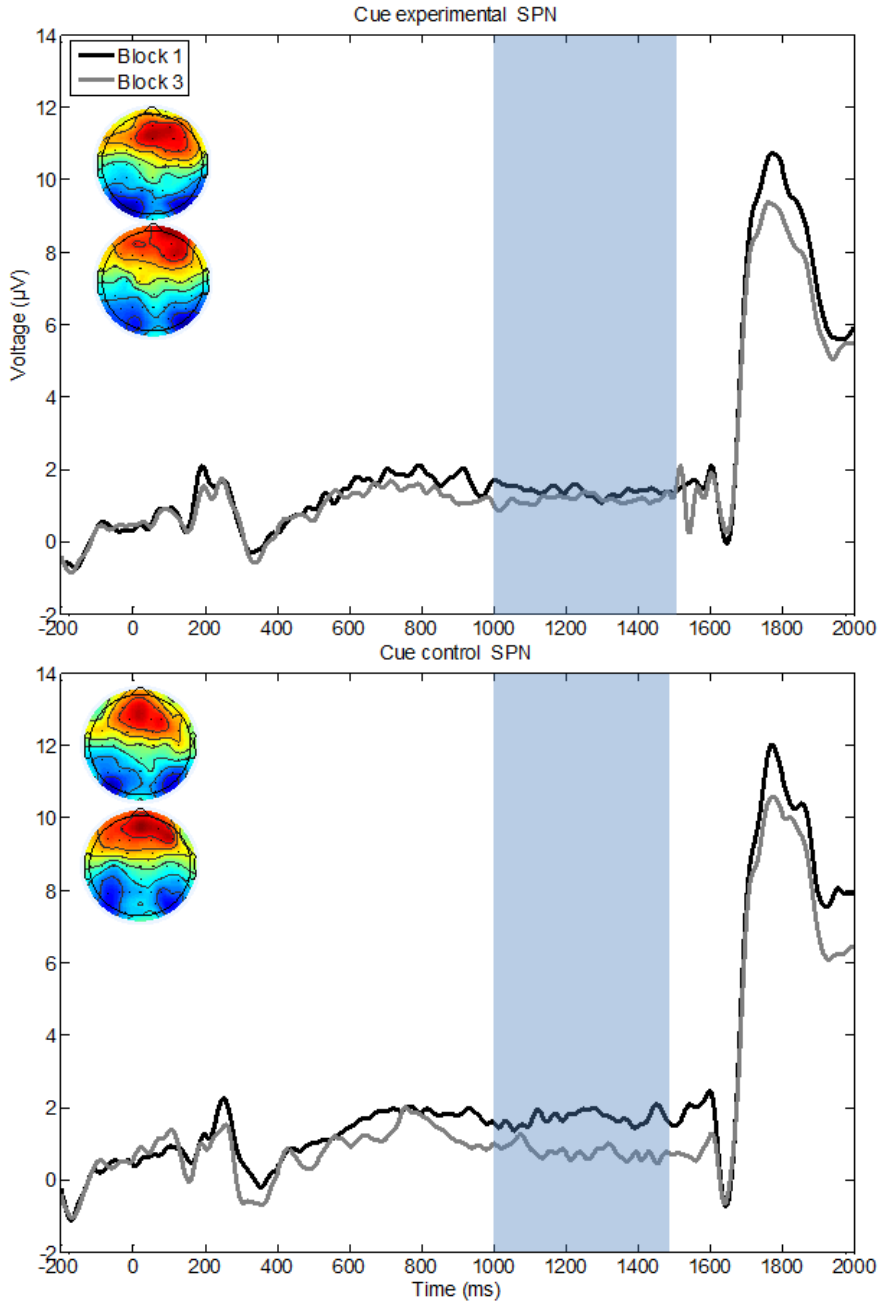

Figure 6.4. *Anticipatory SPN for the experimental (upper plot) and control (lower plot) groups at electrodes CPz, Cz, FCz, C1, C2, FC1 and FC2, over an average of 27 trials per condition. The visual cue was presented at 0ms, and the pain stimulus was delivered at 1500ms. The shaded area shows the 500ms time-window which was analysed. Data were corrected to 200ms before the cue. Topographic plots show scalp activity for the 500ms time-window before pain delivery: upper topographic plot corresponds to block 1, and lower topographic plot corresponds to block 3.*

6.3.2.2.2. P2

There was no main effect of block (F(1,53)=1.55, p=0.22, η_P^2=.03), but there was a significant block by group interaction (F(1,53)=4.17, p=.05, η_P^2=.07). Paired samples t-tests showed block 1 amplitude to be significantly higher than block 3 amplitude in the experimental (block 1 M=9.9, SD=3.26; block 3 M=8.87, SD=2.63, t (28) = 3.08, p=0.005) but not the control group (block 1 M=9.82, SD=3.15; block 3 M=10.07, SD=3.27, t (28) = -.46, p=.65). Pain intensity rating significantly positively correlated with EEP amplitude in the experimental (r=.36, n=81, p=.001) but not the control group (r=.13, n=69, p=.31). See figure 6.5.

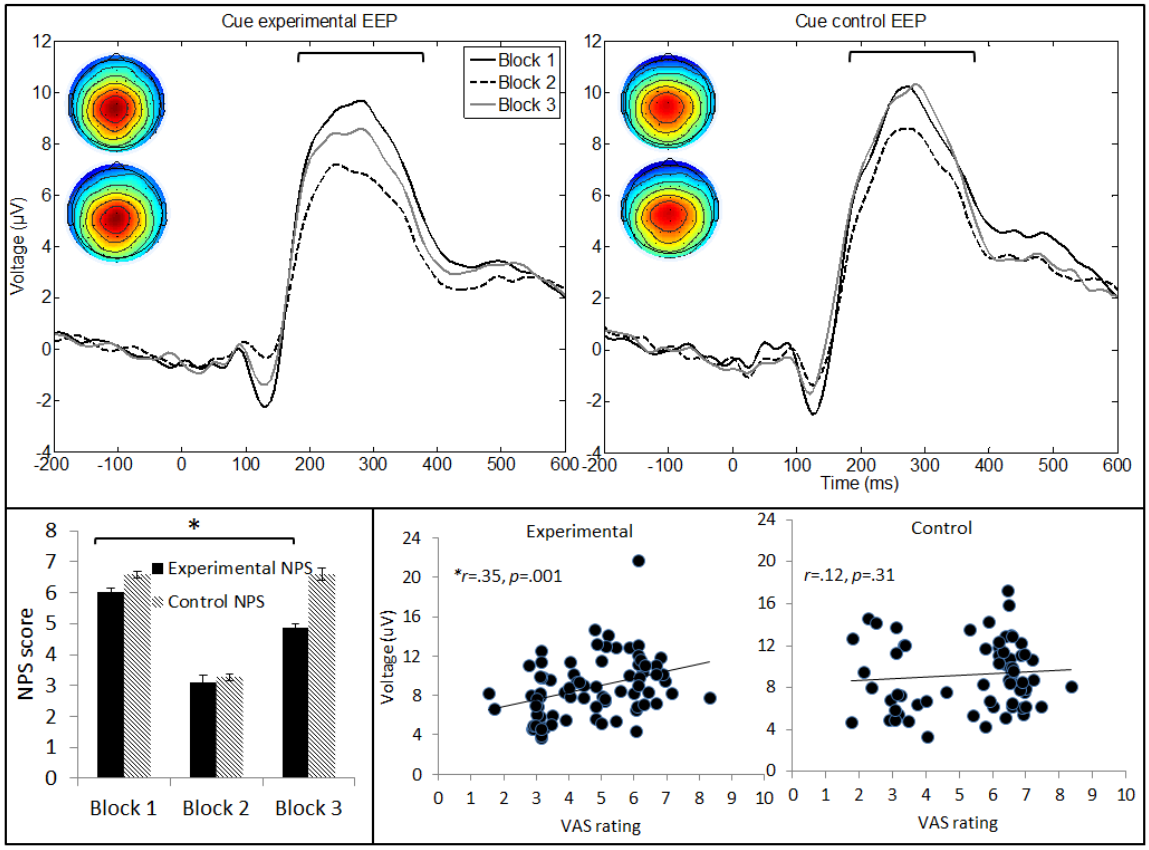

Figure 6.5. *Cue groups. Upper plots: EEPs to Block 1 (black solid), Block 2 (black dashed) and Block 3 (grey), in experimental (left) and control (right) cue groups, at peak electrodes for each participant over an average of 24 trials per condition. Upper topographic plots show block 1 scalp topography, lower show block 3 scalp topography. Lower left plot: Average NPS pain intensity ratings for block 1, 2 and 3 in the experimental (black) and control (grey) group. Error bars represent standard error of the mean. Lower right plots: scatterplots showing the positive correlation between EEP voltage and pain intensity rating in the experimental (lower left) and control (lower right) condition, with lines of best fit.*

6.3.3. Discussion

We successfully reproduced the results of previous placebo analgesia studies (e.g. Morton, Brown, et al., 2010; Morton, El-Deredy, Watson, & Jones, 2010; Morton et al., 2009; Watson et al., 2009b; Watson, Power, Brown, El-Deredy, & Jones, 2012; Watson, El-Deredy, Bentley, Vogt, & Jones, 2006; Watson et al., 2007), using a simple cue presentation procedure with no treatment ritual. Though previous research has shown reinforced probability cues modulate pain perception, our design was novel in that we replicated the exact procedure of a placebo analgesia manipulation. Previous cue studies, including Chapters 4 (Pain FRN and cue modulation of pain) and 5 (PE size and

variability) of this thesis, used a partial reinforcement procedure, where most pain stimuli were at the cued intensity, but sometimes pain was higher or lower than expected (Atlas et al., 2010; Hird et al., 2017; Kong et al., 2013). Hence, these studies are not directly comparable to placebo analgesia studies, as extinction rates differ according to the amount and type of reinforcement delivered (Wagner, 1961). A recent paper, published in the final stages of data collection for this study, showed a behavioural effect of cue similar to ours, although that study used fewer extinction trials than a standard placebo analgesia study, which means that any differences in the response to pain may not have been detected in the few trials delivered (Corsi & Colloca, 2017). We show a modulation of EEPs by cue which is similar to the responses in these studies (e.g. Watson et al., 2007). This suggests the results of Chapter 4 and 5 can be discussed in the context of placebo analgesia as well as cue-evoked expectation modulation.

The manipulation did not exert a significant effect on SPN amplitude, nor did the SPN correlate with threat rating, or unpleasantness rating other than in the cue control group, similarly to the lack of correlation shown in Chapter 4 (Pain FRN and cue modulation of pain). Literature on the SPN to electrical pain is mixed, with some studies showing no SPN in response to electrical pain, and others showing an SPN for electrical pain (Babiloni et al., 2003, 2007; Seidel et al., 2015). Hence, we cannot draw firm conclusions about the null result in the SPN data.

These results are important because we eliminate previously termed 'non-specific factors' which result from the social interaction in placebo analgesia studies, and which could contribute to pain responses (Atlas et al., 2014). We show that modulation of pain by expectation can occur without any treatment ritual, but with simple cue-evoked stimulus expectancies. Furthermore, this effect was evident despite there being no differences in threat or unpleasantness ratings between the control and the experimental group, suggesting the response was not due to a decreased threat status of the pain.

The key finding of this study is that the presentation of a pain intensity cue, without continuous reinforcement or a treatment ritual, can elicit modulation of the pain response both behaviourally and at the neural level. These results suggest placebo analgesia can be attributed to the combined effects of expectation and conditioning, and the ritual of sham treatment administration is not necessary for this response. This is clinically relevant, as it suggests an improvement in pain symptoms is entirely possible through cue conditioning procedures, and this could be extrapolated to psychological therapies to improve pain outcomes without the deception inherent to the administration of a sham treatment. This result is particularly relevant for this thesis, as it contextualises the results of cue-evoked

expectation manipulation in Chapters 4 and 5 in both placebo analgesia and cue expectation manipulations.

6.4. Study 2: placebo analgesia modulation of electrical pain

6.4.1. Methods

See section 6.2 for the methodological details which spanned studies 1 and 2.

6.4.1.1. Placebo experimental

The pain intensities delivered in all three blocks were identical to the cue conditions. First, the procedure for block one was identical to the cue conditions, except that participants viewed a blank circle identical in colour and shape to that viewed by the cue groups (figure 6.1). After block one, the participant picked out the piece of paper which read 'pain-reducing cream study', and the experimenter put on a pair of latex gloves and applied the placebo cream to the hand of the participant. The placebo cream was a colourless and odourless paste, as used in a previous electrical pain placebo study (Wager et al., 2004). This was the same conductive paste used to connect the stimulation electrode to the skin, decanted into a new container. Manufacturer advice was that the stimulator adjusted output intensity based on the conductivity levels of the skin, so using conductive paste for the sham treatment would not influence sensitivity to the stimulation. The experimenter then gave the following verbal instructions: 'This cream has been shown in preliminary studies to be highly effective at reducing pain caused by electrical stimulation. It begins to take effect within a minute but we are leaving it on for ten minutes, which allows the pain reducing ingredients to fully soak into the skin'. The cream was wiped off after ten minutes and the electrode reapplied to the hand. Block two followed, where the participant was informed that the same set of painful stimuli was being delivered, but the stimulation was surreptitiously decreased to the participant's level four pain intensity rating. Finally, participants underwent block three, where the stimulation intensity was returned to the participant's level seven pain intensity rating.

6.4.1.2. Placebo control

Here, the same procedure was carried out, with the only difference being in the verbal instructions. After picking out the piece of paper which read 'pain-reducing cream study: inactive cream' participants were informed that cream was not analgesic and that the pulses in block two were at their level four NPS intensity, which would be increased to their level seven in block three. Hence, the participants were aware that the decrease in

pain experience in block two was because the stimulation was decreased, not because of any effects of treatment.

In both groups, participants gave the same unpleasantness and threat ratings as in the cue groups, and in the manipulation check they rated how effectively they felt the placebo treatment decreased the pain intensity using a NPS.

See figure 6.1, section 6.2.1 for a summary of the experiment structure.

6.4.1.3. Manipulation check and procedural adjustments of the placebo analgesia manipulation

Midway through data collection we carried out a routine manipulation check. This check included 41 participants in the cue groups and 41 participants in the placebo groups. While the pain intensity ratings looked as they would be expected in the cue groups (a reduction between block 1 and block 3), both the experimental and control placebo groups showed surprising and statistically significant increases in block three relative to block one of the placebo arm, which suggested an aspect of the procedure was causing an increase in sensitivity to the stimulation. See section 2.5.3 for details of this check. As a result the procedure was adjusted and all previous participants in the placebo groups were excluded. Advice from the stimulator manufacturer was that any increased skin conductivity from the placebo cream would not increase pain perception. However, the application of a placebo cream was the only physical difference between the placebo groups and the cue groups, who were not expressing this behavioural effect. Consultation with Salford Royal NHS Foundation Trust Medical Physics indicated that application of the cream after block one could increase the depth of penetration of the moisture, thus increasing perception of pain intensity. Analysis of the pain intensity ratings revealed that ratings were not at NPS 4 in the conditioning block two of the experimental group, suggesting an increase in pain sensitivity after application of the paste. To counter this issue, three adjustments were made to the placebo administration procedure. First, before commencement of block two, ten pulses at the participant's 'low' intensity (level four on the NPS) were delivered and the participant was asked to rate these. If they were rated as higher than NPS 4, the output of the stimulator for that block only was adjusted until stimulation was rated as NPS 4. Second, specific instructions were given about the duration of the analgesic effect of the cream when it was applied, that it would be effective for one hour, to ensure placebo analgesia was not being abolished by a reduction in expectation of pain relief. Third, a controlled amount of cream, the minimal amount to convincingly suggest a medication, was applied to decrease the likelihood of it penetrating deeper into the tissue of the hand.

All other aspects of the experimental set-up remained identical to the procedure described above, and the same randomisation procedure continued to be followed. All previously collected participants in the placebo experimental and control group were discarded and data were collected anew. Because of the delay between collection of the original data and collection of the new data, this meant there was a time difference of months between collection of the majority of the cue data and the majority of the placebo data. For this reason we did not directly statistically compare the placebo and the cue groups in a single model.

6.4.2. Results

6.4.2.1. Behavioural results

For the pain intensity ratings, a 2-way ANOVA yielded a trend towards a main effect of block ($F(1,41)=3.04$, p =.09, η_P^2=.07) but no significant interaction with group ($F(1,41)=2.35$, p=.13, η_P^2=.05).

There were no significant differences between the experimental and control groups for threat ($t(40)=.84$, p = .4) or unpleasantness ratings $t(40)=1.26$, p =.22). There was a significant difference in the manipulation ratings for placebo experimental and placebo control conditions ($t(35)=-4.41$, $p<0.001$), suggesting participants rated the placebo treatment as more effective in the experimental than the control group (table 6.2).

Table 6.2. *Mean ratings of NPS, threat, unpleasantness and manipulation for study 2*

Ratings	**Group**	**Block 1 Mean (sd)**	**Block 3 Mean (sd)**
NPS	Experimental	6.34 (.94)	6.38 (1.49)
	Control	6.16 (.84)	6.78 (.89)
		Mean (sd)	
Threat	Experimental	1.19 (.33)	
	Control	1.29 (.42)	
Unpleasantness	Experimental	4.03 (1.61)	
	Control	4.58 (1.23)	
Manipulation	Experimental	4.45 (2.46)	
	Control	1.17 (1.98)	

6.4.2.2. Electrophysiological results

6.4.2.2.1. SPN

A 2-way ANOVA revealed a main effect of block ($F(1,37)=10.13$, $p=0.003$, $\eta_P^2=.215$)(block 3>block 1), but no interaction ($F(1,37)=.18$, $p=.67$, $\eta_P^2=.005$), suggesting the SPN was more negative in block 3 across the experimental and control group (figure 6.6). The SPN did not correlate with pain intensity rating in either the control ($r=.01$, n=53, $p=.95$) or the experimental condition ($r=-.14$, n=60, $p=.29$), nor did it correlate with EEP in either the control ($r=-.1$, n=53, $p=.47$) or the experimental condition ($r=.18$, n=60, $p=.16$). Finally, the SPN did not correlate with unpleasantness rating in either the control ($r=.19$, n=52, $p=.16$) or the experimental condition ($r=.04$, n=55, $p=.79$), nor did it correlate with threat rating in the control ($r=.05$, n=53, $p=.71$) or experimental condition ($r=.06$, n=52, $p=.69$).

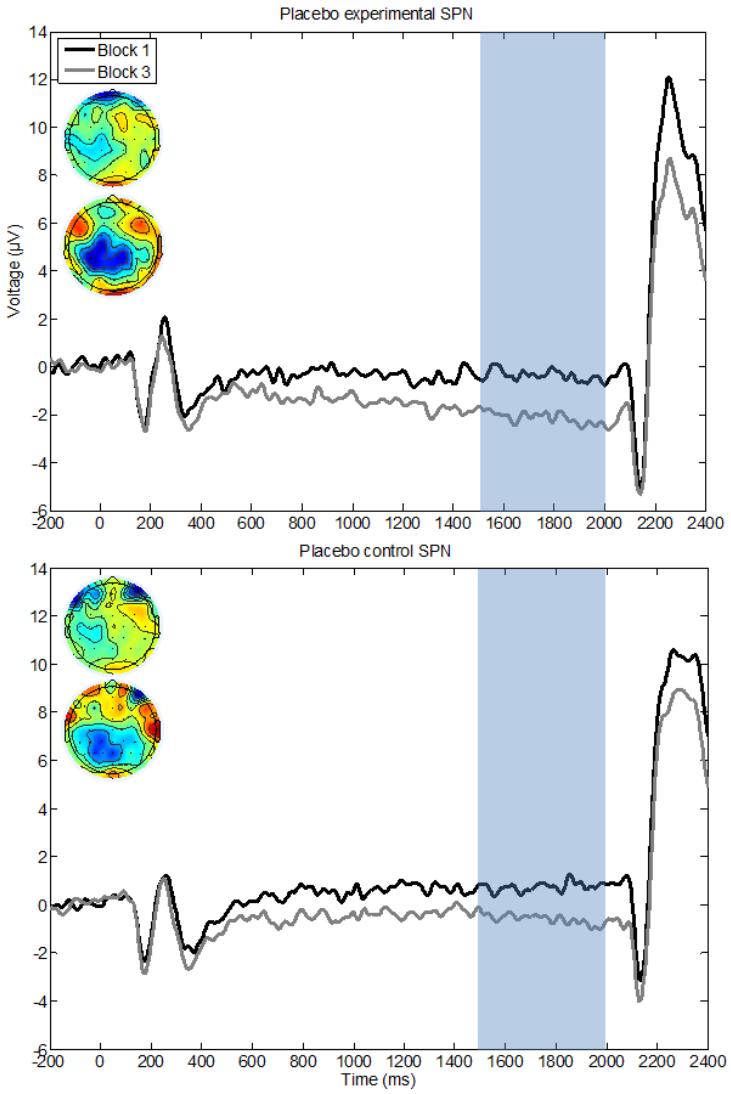

Figure 6.6. *Anticipatory SPN for the experimental (upper) and control (lower) groups at electrodes CPz, Cz, FCz, C1, C2, FC1 and FC2, over an average of 24 trials per condition. The visual fixation cue was presented at 0ms, and the pain stimulus was delivered at 2000ms. Although the anticipatory period appears to be 500ms longer here than in the cue groups, this is because the cue groups were presented with a fixation cue identical to the placebo groups for 500ms before viewing the anticipatory cue, which was not included in the plot. The shaded area shows the 500ms time-window which was analysed. Data were corrected to 200ms before the fixation cue. Topographic plots show scalp activity for the 500ms time-window before pain delivery: upper plot corresponds to block 1, and lower plot corresponds to block 3.*

6.4.2.2.2. P2

We did not observe a main effect of block (F(1,38)=.00, p=.983, η_P^2=.0), but the block by group interaction was significant (F(1,38)=4.56, p=0.04, η_P^2=.11). A paired-samples t-test showed the difference between block 1 and 3 to be non-significant in both the control (t(17)=-1.55, p=.14) and the experimental group (t (21)=-1.5, p=.15). An independent samples t-test showed the difference between control and experimental group to be non-significant in both block 1 (t(38)=-1.24, p=.23) and block 3 (t (38 =-.002, p=.99). The interaction was driven by the fact that in the control group, block 1 (M=9.89 μV, SD=2.96 μV) was lower than block 3 (M= 10.62 μV, SD=2.95 μV), but in the experimental group, block 1 (M=11.34 μV, SD=4.18 μV) was higher than block 3 (M=10.62 μV, SD=3.31 μV), but these differences were not statistically significant when tested with a t-test. Pain intensity rating significantly correlated positively with EEP amplitude in the experimental (r=.32, n=60, p=.01) and the control group (r=.31, n=53, p=.03). See figure 6.7.

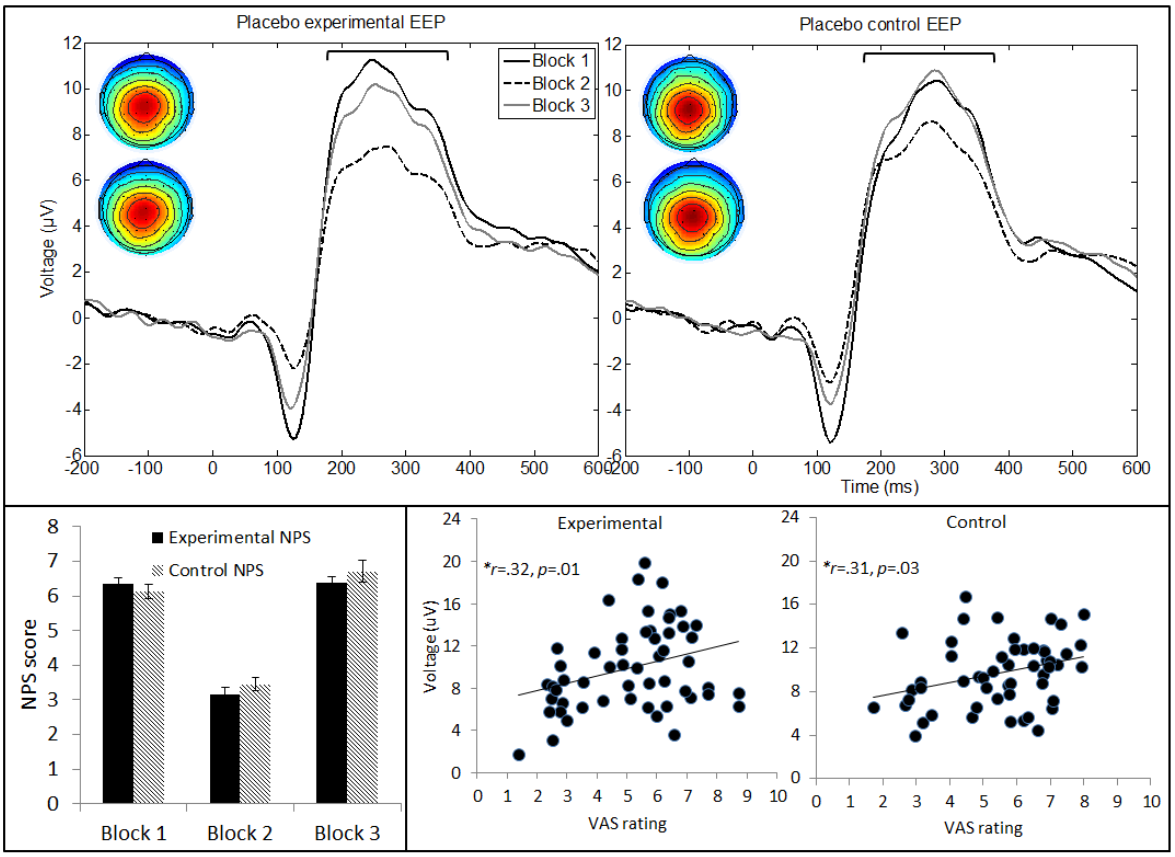

Figure 6.7. *Placebo groups. Upper plots: EEPs to Block 1 (black solid), Block 2 (black dashed) and Block 3 (grey), in experimental (left) and control (right) placebo groups, at peak electrodes for each participant over an average of 26 trials per condition. Upper topographic plots show block 1 scalp topography, lower show block 3 scalp topography. Lower left plot: Average pain intensity ratings for block 1, 2 and 3 in the experimental (black) and control (grey) group. Error bars represent standard error of the mean. Lower right plots: scatterplots showing the positive correlation between EEP voltage and pain intensity rating in the experimental (lower left) condition and the control (lower right) groups, with lines of best fit.*

6.4.3. Discussion

We did not see significant behavioural or EEP effects of the sham treatment on pain, though a trend in the predicted direction did occur in the EEP, and the manipulation check revealed that participants in the experimental group rated the sham treatment as significantly more effective than those in the control group. Placebo analgesia has been posited as a reduction in anticipation to pain (Watson et al., 2009), but here, the SPN increased in amplitude (becoming more negative) in block three compared to block one, suggesting participants anticipated the pain more in block three than block one.

The failure to achieve a placebo effect could be due to a physiological issue with the stimulation. The only physiological difference between the successful cue manipulation and the unsuccessful placebo manipulation was the administration of the placebo cream. Despite manufacturer instructions that the output of the stimulator was automatically adjusted to match skin conductivity, it is possible that administration of the cream increased the penetration of the conductivity area and stimulated more afferent nociceptors, increasing the perceived intensity of the pain, which would explain why participants rated block 2 stimuli as higher than we expected. Electrical pain stimulation activates a larger area of nociceptive and somatosensory afferents than laser pain stimulation (Perchet et al., 2012), and so may be more liable to interference from conductivity changes.

The lack of placebo response is unlikely to be due to peripheral sensitisation which is an inflammatory response which occurs after injury (Rocha et al., 2007), or central sensitisation which is usually only seen in chronic pain populations (Xu, Ge, & Arendt-Nielsen, 2010). When we made further adjustments to the procedure, by carefully controlling the amount of placebo cream administered and adjusting the intensity of block 2 stimulation, pain intensity ratings did not increase between block 1 and block 3 in the pilot (section 2.5.3), suggesting that these adjustments resolved any physiological issues in the procedure.

One explanation for the failure to replicate the placebo effect is that there were limitations in the psychological effectiveness of the manipulation. Placebo analgesia administration requires close experimenter-participant contact. Though the experimenter followed well-established placebo protocols closely, and was trained in placebo administration by experienced placebo researchers, we cannot rule out the possibility that an unidentified variable in the experimenter-participant interaction influenced the placebo response. For example, the level of empathy displayed by the experimenter has been shown to affect the placebo manipulation (Kaptchuk et al., 2008). Also, the study was conducted chiefly on undergraduate students in a psychology and neuroscience building. Although the session was introduced as a pain perception study, it has been previously shown that students, who may be more suspicious of a study conducted in a psychology building, do not show the same placebo response as non-students (Wager, 2004).

Finally, the lack of placebo responses could be because the sample size was smaller in the placebo than in the cue groups, which was a consequence of the procedural issues described above. However, although ERP placebo responses require a higher sample size, behavioural placebo responses have been shown with sample sizes similar to ours, and

even as low as N=12, meaning we could expect to at least see a behavioural placebo response at this sample size if it had been effective (Colloca et al., 2008; Colloca & Benedetti, 2009; Pascalis, Chiaradia, & Carotenuto, 2002; Yeung et al., 2014).

We aimed to replicate the three-block placebo conditioning procedure using sham cream with electrical pain (Watson et al., 2007). Though through piloting we ensured any obvious physiological issues were removed, the manipulation was not successful. In future studies, various adjustments could be made to maximise the likelihood of achieving a placebo response. The study could be repeated on non-students, or in a clinical environment, to maximise the possibility that participants believe in the manipulation. These adjustments are likely to improve the outcome of the manipulation. If they failed to improve the response, the manipulation could also be compared alongside a placebo TENS electrode or a placebo pill, which have been previously effective (Colloca et al., 2008; Colloca & Benedetti, 2006, 2009; Colloca et al., 2010; Rütgen et al., 2015; Yeung et al., 2014).

6.5. General summary

Results showed a statistically significant effect of cue but not of sham treatment on electrical pain intensity rating and associated EEPs. In study 1, pain intensity ratings and EEP amplitudes indicated the low pain intensity cue decreased responses to painful stimulation. In study 2, participants rated the treatment as more effective in the experimental group than the control group, but there was no statistically significant effect of block on either pain intensity ratings or EEPs. The SPN, a marker for anticipation (Böcker et al., 2001), increased in amplitude in block three relative to block one across cue and placebo groups, though this was significant in study 2 only. Overall, our results indicate that pain intensity cues modulate pain perception and EEP responses, and this effect is similar to the results of previous placebo analgesia manipulations. This suggests that the well-documented placebo effect is not chiefly the result of non-specific effects of sham treatment, and indicates that the findings of this thesis can be applied to placebo analgesia.

Chapter 7: General discussion

Abstract

This thesis aimed to probe the influence of expectation on perception by measuring electroencephalography and behavioural responses to unexpected aversive outcomes. Specifically, the aim was to explore the mechanisms underlying aversive prediction error and how prediction error and expectation modulate pain perception. This final chapter summarises the results of the experimental work and discusses them in the context of the key predictions of the thesis. Overall, the results of this thesis indicate that aversive PE can be captured at the scalp in response to a visual cue, but not in response to pain. Pain expectations elicited by a cue modulate pain intensity ratings and associated pain-evoked potentials, across laser and electrical pain, and PE modulates this effect, as hypothesised by the predictive coding account of pain perception. Finally, we demonstrate that cue-evoked expectation modulates pain comparably to placebo-evoked expectation, which provides an empirical link between the studies of this thesis and the placebo analgesia literature.

7.1. Summary of experimental work and implications

A series of studies investigated the expression of aversive PE and how this influences pain perception. First, the function of the EEG marker of PE, the FRN, was characterised. The second study explored EEG markers of PE and pain after an expectation manipulation. Third, two studies probed the parameters of behavioural expression of PE to pain. The final study of the thesis tested the effects of cue-evoked expectation and sham treatment-evoked expectation to try to apply understanding of cue-evoked expectation to the placebo analgesia literature. These studies are summarised below.

Chapter 3 (Taste FRN) investigated the EEG correlates of PE by characterising the FRN, the most likely electrophysiological correlate of PE. We recorded ERPs to a cue signalling expected and unexpected appetitive and aversive taste in a Pavlovian conditioning procedure. Being a primary reinforcer, taste is motivationally close to pain. This allowed us to test whether the FRN signals utility PE across modalities, or reward or aversive PE. We did not observe a signal for utility across outcomes, but we did capture correlates of salience and aversive PE. Although previous studies suggested the FRN signals salience PE, this is the first study to use the same modality across the domains of reward and aversion, and to show unequivocally that this ERP does not signal utility PE. This presents a challenge to the utility PE interpretation of the FRN, and suggests instead that this ERP may reflect a response to aversive PE and salience, which is an important finding for ERP studies of reinforcement learning (Holroyd & Coles, 2002).

Chapter 4 (Pain FRN and cue modulation of pain) probed the EEG correlates of pain PE and the modulation of different pain-evoked potentials by cue. Again a Pavlovian conditioning procedure was used, but in this study the outcome was painful stimulation. We manipulated the expected intensity of painful stimulation using pain intensity cues and tested the response to a painful stimulus which was lower or higher than expected. At the latency and topography of the FRN, ERPs did not express pain PE. Instead, pain-evoked potentials expressed a modulation which was in the opposite direction to the aversive PE signal captured in Chapter 3. In Chapter 3 when outcomes were unexpectedly delivered the signal was more positive than when they were unexpectedly omitted. In Chapter 4 (Pain FRN and cue modulation of pain) when pain outcomes were higher than expected, the signal was more negative than when they were lower than expected. Therefore, pain-evoked potentials were modulated by expectation, reflecting the integration of the cued intensity into perceived pain. PE was not expressed as we would expect at the scalp, as a more positive response to higher than expected pain intensities. This result indicates that the FRN is not overlaid on pain-evoked potentials, and should

not be a concern for studies into expectation and pain. Because ERPs did not express pain PE, for the remaining studies we measured the amplitude of pain-evoked potentials and behavioural pain responses to examine the influence of expectation and PE on pain.

This study held further methodological value because it showed that electrical-evoked pain is modulated to an equal degree to laser-evoked pain by cued expectations. This is important because some of the cueing procedures used in this thesis required a higher number of trials than those typically used by laser pain studies, because cues were reinforced. Delivering a high number of laser stimuli holds an increased risk of sensitisation and skin lesions, but electrical pain does not carry these risks. The results demonstrated the feasibility of using electrical pain stimulation to explore the modulation of pain by expectation. This is relevant for future studies examining the modulation of pain by expectation, as it shows electrical pain stimulation, which is safer, can be used to study the modulation of pain-evoked potentials by cue. The results also suggested that the anticipatory SPN does not differentiate electrical pain intensity but does differentiate laser pain intensity. We noted this as a limitation of using electrical pain stimulation. However, the advantage of being able to deliver more trials without confounding results with sensitization outweighed the limitation of the SPN not reflecting pain intensity, as the key focus of the thesis was on PE rather than anticipation. The remaining experiments used electrical pain stimulation.

Chapter 5 (PE size and variability) describes two behavioural studies where we manipulated pain expectations and pain stimulation intensities and tested the resulting pain intensity rating to explore the effect of PE size and pain variability on pain perception (Büchel et al., 2014; Jones et al., 2013). First, we tested the limits of the integration of expectations into pain perception by delivering increasingly unexpected pain to test whether large pain PE decreases the weight of expectation on perceived pain. Results indicated that cued intensity influenced the perceived pain intensity at each level of PE, but that when PE was large, the influence of expectations was reduced. This result indicates that there is a limit to the influence of expectations on pain. This is in line with the predictive coding framework which proposes that when stimulus intensity is highly discrepant to expectation, the weight of expectations is decreased to better represent the sensory data (Büchel et al., 2014; Friston, 2009). This result also has implications for therapies using expectations to improve chronic pain outcomes, such as those using verbal suggestion and imagery, because it suggests that expectation-based therapies should only manipulate expectations away from reality to a moderate degree in order to influence perception of pain, as expectations which are strongly violated will have less influence on perception (Peerdeman et al., 2016). Second, predictive coding argues that the certainty of

expectations and pain stimulus intensity modulates their respective influence on pain perception. Although there is evidence that the certainty of expectations modulates their influence on perceived pain, the effect of the certainty of stimulus intensity has not been previously shown (Brown, Seymour, Boyle, et al., 2008b; Brown et al., 2008; Colloca & Benedetti, 2006). We showed that increasing the uncertainty of stimulus intensity did not decrease its influence on pain perception, which contradicts predictive coding.

On a neural level, the expected level of pain is signalled from the PFC to the PAG and presumably compared against the stimulus intensity signal in the PAG, which signals PE. The PAG is known to project down to the RVM and spinal cord to modulate pain (Bee & Dickenson, 2009; Eippert, Finsterbusch, et al., 2009; Heinricher & Fields, 2013; Ren & Dubner, 2009; Roy et al., 2014). Based on the results of Chapter 5 (PE size and variability), we speculate that if expectations are highly certain, the PAG modulates the pain signal via signalling from the PFC to better fit expectations through its descending projections to the RVM and spinal cord (section 1.2.5). Indeed, this has already been identified as a candidate pathway for predictive coding (Büchel et al., 2014). However, according to the results of Chapter 5, the certainty (inverse variability) of the stimulus intensity does not appear to influence this process. Our results indicate that the difference between cued pain intensity and the pain stimulus intensity changes the influence of expectation on pain. If the stimulus intensity signal is similar enough to the cued intensity, it may be modulated by the descending projections of the PAG, reflected in the modulation of pain intensity ratings by cued intensity in Chapter 5. The PAG is also involved in the ascending signalling of pain (Büchel et al., 2014; Johansen et al., 2010; Ritter et al., 2013). We speculate that if the stimulus intensity is highly discrepant to the expected intensity, the PAG alters ascending signalling of the pain signal to reduce the influence of expectations on pain perception and better represent the stimulus intensity.

Whereas Chapters 3 (Taste FRN), 4 (Pain FRN and cue modulation of pain) and 5 (PE size and variability) used cues to explore mechanisms of aversive PE and expectation, the final Chapter 6 (Cue and placebo effects on pain) was an investigation of the effect of cue versus a sham treatment on pain perception. We aimed to replicate a placebo analgesia procedure with cues instead of a sham treatment and also to replicate the well-established three block sham treatment effect described in laser pain with electrical stimulation. Placebo analgesia is a remarkable phenomenon which reflects the powerful impact of expectation on perception, and has an extensive literature; we aimed to test whether the results of Chapters 4 and 5 could be discussed in the context of placebo analgesia (Atlas & Wager, 2012a; Benedetti, 2014; Fabrizio Benedetti & Frisaldi, 2013; Colloca & Miller, 2011a; Enck, Benedetti, & Schedlowski, 2008; Murray & Stoessl, 2013; Peciña & Zubieta,

2015). We did not elicit a placebo response using sham treatment. However, as expected, the results of the cue conditioning procedure resembled those of previous chapters and those of placebo analgesia manipulations, showing a clear modulation of pain by cue-evoked expectation. This result suggests that placebo analgesia results from the integration of expectation with pain, rather than being a specific response to the treatment ritual. This result is important because it eliminates a key potential confound in the pain expectation literature: the non-specific effects of the treatment ritual, such as the social interaction between a practitioner and patient, which are not accounted for when comparing between cue and placebo studies (Atlas & Wager, 2012). These results also indicate that the findings of Chapters 4 and 5 can be applied to the placebo analgesia literature, which suggests first that electrical pain should be modulated by a sham treatment equally to laser pain, and second that the modulation of pain by sham treatment will be reduced in effectiveness when pain stimulus intensity is very different to what was expected. Although we did not see a modulation of electrical pain by sham treatment in Chapter 6, we attribute this to the procedural issues discussed in that chapter.

7.2. Relationship of results to hypotheses

7.2.1. Hypothesis 1: the FRN is a marker for pain PE

Chapters 3 and 4 showed that although the FRN is a marker for aversive PE, it is not a marker for PE to pain. The practical implication of this is that the FRN is not a suitable marker for PE in placebo studies measuring the response to pain, where expectations of pain intensity are manipulated.

Chapter 3 (Taste FRN) revealed an EEG correlate of aversive PE to cues signalling unexpected taste which matched the spatiotemporal profile of the FRN. We also captured salience responses before and after the PE signal which indicates ERPs expressed salience as well as PE. Our results showed that at the latency and topography of the FRN, ERPs responded positively to a cue signalling the delivery of aversive taste compared to a cue signalling the omission of aversive taste. These results indicated the FRN can be used to study aversive PE.

Chapter 4 (Pain FRN and cue modulation of pain) did not reveal a pain PE overlaid on the pain-evoked signal. Replication of the FRN analysis described in Chapter 3 (Taste FRN) did not reveal a significant FRN. Instead, pain-evoked potentials shifted towards the expected intensity. This suggested pain-evoked PE was not expressed at the scalp.

There are two potential reasons why PE to pain was not captured in Chapter 4 (Pain FRN and cue modulation of pain). First, the FRN and the pain ERP share a similar spatiotemporal profile, both being expressed between 240 and 340ms post-outcome at central electrodes (Sambrook & Goslin, 2015a; Treede et al., 2003) . It is possible that noise associated with the relatively larger pain-evoked potential overlaid the more subtle FRN. Component overlap is an issue commonly recognised in ERP studies (Luck, 2005). Second, the descending influence of a pain intensity expectation on pain perception means pain signals may be modulated as low as the spinal cord, so a PE difference-wave is not detectable at the scalp when manipulating expected magnitude, as we did in this study, rather than expected delivery or omission of an outcome, as in Chapter 3 (Taste FRN) (see figure 1.6) (Matre et al., 2006). The manipulation of expected pain intensity described in Chapter 4 has also been carried out in fMRI (Atlas et al., 2010). That fMRI study did not observe cue effects on the PAG, which is known to be a pain PE encoder, which suggests PE was not expressed (Roy et al., 2014). Instead there was greater activity in pain processing areas for high intensity cues than low, similarly to the ERP amplitudes in Chapter 4 (Atlas et al., 2010). As in that fMRI study, in Chapter 4, the expectation that was violated was whether an outcome would be high or low whereas in Chapter 3 (Taste FRN), we showed a PE response to taste which was unexpectedly delivered or omitted. We speculate that we saw an FRN to taste because this manipulation involved expectations about whether an outcome would be delivered or omitted, as in previous research which has shown an FRN to the unexpected delivery or omission of primary reinforcers (Garofalo et al., 2014; Talmi et al., 2013). It is possible that expecting more or less of an outcome such as taste or pain shifts responses towards the expected outcome, as in placebo analgesia whereas the unexpected delivery of an outcome may elicit an FRN.

Though both studies examined PE, there were some differences between the two studies which should be acknowledged. In Chapter 4 (Pain FRN and cue modulation of pain) we did not test for the FRN to pain using the axiomatic model of PE described in Chapter 3 (Taste FRN) (Rutledge et al., 2010). This is for two reasons. First, one of the axioms of the axiomatic model of PE is that responses to expected outcomes should not differ from one another. When measuring the PE response to an outcome cue, the physical stimulus (the visual cue) can be kept constant, while the meaning (the outcome signified by the cue) changes, but this is not possible when measuring the PE response to outcome receipt, as we did in Chapter 4. The expected outcomes in this analysis were high and low intensity pain. As shown in Chapter 4, high intensity pain elicits greater P2 amplitudes than low intensity pain regardless of any effects of expectation, and so by definition the ERP responses to these expected outcomes would differ from one another. Second, for the axiomatic analysis described in Chapter 3, outcomes were unexpectedly delivered or

omitted. We did not deliver or omit outcomes in Chapter 4. Instead, in keeping with pain expectation studies and placebo manipulations which generate expectations of low pain rather than expectations of pain omission, in Chapter 4 we measured responses to violation of expected pain intensity (Atlas et al., 2010; Brown, Seymour, Boyle, El-Deredy, & Jones, 2008a; Wager, 2004a; Watson, El-Deredy, Vogt, & Jones, 2007). Although in Chapter 4 we did not operationalise PE using the axiomatic model described in Chapter 3, we did replicate the difference-wave analysis at the latency and topography described in Chapter 3. Another difference between the two studies comes in the task. Chapter 3 used a passive Pavlovian conditioning task, whereas in Chapter 4 participants were prompted to rate the intensity of the pain at the end of every trial, which could elicit motor preparation or evaluative processes (Nagai et al., 2004). Despite this, the ERP matched the spatiotemporal profile of the P2, meaning it is likely to chiefly reflect pain-evoked activity rather than motor preparation or evaluative processes. Future studies could use a passive observation task to rule out the confounding effects of motor planning and actions.

The FRN literature focuses around the response to a visual cue signalling unexpected monetary gain and loss. Although one study showed responses to errors on a task signalled by an auditory cue, to our knowledge the FRN has not been shown in any modality other than to visual cues (Miltner et al., 1997). A concern about the FRN literature is that the FRN is characterised in response to a visual cue which means that the FRN literature is specific to the visual domain. Chapters 3 (Taste FRN) and 4 (Pain FRN and cue modulation of pain) reflect efforts to diversify understanding of the FRN to taste and pain. One useful avenue for research is to measure the FRN to auditory, gustatory or somatosensory outcomes, to test whether modality influences the FRN. This could show for example whether the FRN still increases in amplitude to the receipt of delivered aversion and decreases in amplitude to omitted aversion in other modalities. If the FRN continues to respond in the same way, this supports perspectives on the FRN as a PE signal. Further, the FRN to the receipt of delivered and omitted reward in other modalities would show whether this ERP also signals reward or salience in other modalities. However, we did not see an FRN in Chapter 4 which indicates that this signal does not express PE to pain. Although we showed the FRN to taste reflects aversive PE, we did not capture an FRN response to the direct receipt of pain. For the remaining studies we concluded that the FRN was not an effective marker to investigate the effect of placebo analgesia on PE.

7.2.2. Hypothesis 2: Pain perception reflects the integration of expectation and incoming pain stimulation

The pain intensity ratings and corresponding ERPs in this thesis clearly support the hypothesis that pain perception reflects the integration of expectation and incoming pain stimulation. The results of this thesis provide mixed support for the electrically-evoked SPN as a marker of pain anticipation.

7.2.2.1. The influence of expectation and pain intensity on pain intensity ratings and evoked potentials

The influence of expectation and pain stimulus intensity on perceived pain is clearly reflected in the pain intensity ratings and pain-evoked potentials in this thesis. Pain intensity ratings consistently reflected cue-induced expectation modulation in Chapters 4 (Pain FRN and cue modulation of pain), 5 (PE size and variability) and 6 (Cue and placebo effects on pain). Importantly, Chapters 4 and 6 clearly demonstrate that EEPs are modulated by cue-evoked expectation. Chapter 4 suggested cued expectation influenced pain perception and corresponding potentials regardless of stimulation type or unpleasantness. Chapter 6 revealed cued expectation effects on pain intensity ratings and EEP amplitude in a blocked conditioning paradigm, even when expectations were consistently violated in the test block.

However, the expectations elicited by the placebo manipulation in Chapter 6 did not modulate pain responses. It is possible that the psychological placebo manipulation was not effective, for example because the study was conducted in a lab in a psychology and neuroscience department which could cause suspicion in participants that this was a psychological manipulation rather than a true analgesia experiment. However, participants rated the placebo cream as significantly more effective in the experimental group than the control group, and this was reflected by the experimental group rating the effectiveness of the cream at 4 points out of 10 on the NPS, compared to 1 point out of 10 on the NPS in the control group, suggesting they believed it to work to some extent. It is also possible that peripheral physiological changes associated with the placebo cream influenced pain perception, which abolished placebo responses.

Though in Chapter 6 (Cue and placebo effects on pain) we replicated a placebo analgesia paradigm as closely as possible, we cannot say whether this manipulation influenced neural systems previously implicated in placebo analgesia or in cue manipulation studies. Placebo analgesia and cue-evoked expectation do modulate similar areas of pain

processing regions, with both reducing activity in pain processing regions such as the thalamus, insula, and somatosensory cortex (Atlas et al., 2010; Price, Craggs, Verne, Perlstein, & Robinson, 2007; Wager, 2004; Watson et al., 2009b). Interestingly, in a cue manipulation these effects are modulated by activity in the medial OFC and the ventral striatum, areas which are associated with valuation and PE (Atlas et al., 2010). This has not been shown in placebo analgesia. This suggests cue-elicited pain modulation may more directly modulate prediction error mechanisms alongside top-down expectation mechanisms. However, it is possible that the tonic expectation elicited by a treatment ritual influences pain by different mechanisms to the trial-by-trial expectation elicited by a cue (Atlas et al., 2010). There is substantial evidence that placebo analgesia involves the endogenous opioid system (Amanzio & Benedetti, 1999; Bingel. et al., 2006; Eippert, Bingel, et al., 2009; Scott et al., 2008). It is possible that our cued procedure activated neural systems implicated in placebo analgesia, due to the tonic nature of the expectations we elicited as the cue did not vary from trial to trial. It would be of interest to repeat the study with Positron Emission Tomography (PET), to assess the involvement of the tonic opioid system to the cue manipulation, and understand in more detail the mechanisms involved.

The results of Chapter 5 (PE size and variability) go further in illustrating the contribution of expectations on perceived pain because we formally tested the influence of cue-evoked expectation and pain stimulation intensity on pain intensity rating in a multiple regression. Results showed a role for both. The pain intensity ratings of Chapter 5 show that at high levels of PE the influence of expectation on perceived pain was decreased. This result demonstrates the limit of the influence of expectation on pain, and shows that the size of PE is important for determining how much expectations influence pain.

Although the pain intensity ratings and pain-evoked potentials procured in this thesis provide clear support for the role of ascending stimulus intensity and descending expectations in pain perception, we did not observe a reliable EEG correlate of pain anticipation.

7.2.2.2. The SPN as a marker for pain anticipation

The role of descending expectations on pain can be probed with EEG by studying the relationship between pain anticipation and perception. In this thesis the SPN was not a clear marker of pain intensity anticipation in the context of electrical pain stimulation. In Chapter 4 (Pain FRN and cue modulation of pain) the SPN tracked laser stimulation intensity but not electrical stimulation intensity, in line with previous studies showing SPN responses to laser but not electrical pain (Babiloni et al., 2003, 2007). This could be

related to the physical properties of the two stimulation types. Laser heat pain is naturally occurring so biologically relevant, and will cause skin damage if delivered above safe limits. Electrical pain is not encountered naturally, and participants know it is highly unlikely to cause skin damage. It is possible that the increase in electrical pain from low to high intensity did not hold the same anticipatory relevance for participants as the same increase in laser pain. In support of this, in Chapter 4 high intensity laser pain was rated as more unpleasant than high intensity electrical pain, suggesting it had a higher emotional relevance. This is an important consideration for the value learning literature, which mainly uses electrical pain as an aversive reinforcer (e.g. Garofalo et al., 2014; Heydari & Holroyd, 2016; Talmi et al., 2013), and a potential limitation of this thesis. However, Chapter 4 also showed that high intensity electrical pain was rated as more unpleasant than low intensity electrical pain, so although the emotional impact of electrical pain may be smaller, there is still a differentiation between intensities. Interestingly, despite the SPN not reflecting pain intensity expectation for electrical pain in Chapter 4, the EEP was modulated by expectation. This indicates dissociation between the anticipatory SPN and the influence of expectations on perceived electrical pain.

The SPN also did not track perceptions of low pain intensity in the cue study of Chapter 6 (Cue and placebo effects on pain), which suggests it did not represent anticipated pain intensity. In the placebo conditions of Chapter 6 the SPN increased in amplitude after the placebo manipulation, even though pain intensity ratings did not change after the manipulation. This again suggests the SPN does not reflect anticipated pain intensity. A previous placebo analgesia manipulation failed to show a reduction in SPN amplitude post-treatment and attributed this to the SPN being increased by the reward of a placebo treatment (Morton et al., 2010). Another potential influence on the SPN in this thesis is that expectations were uncertain, because of the 75/25% likelihood cues in Chapter 4 (Pain FRN and cue modulation of pain), and because expectations were consistently violated in the test blocks of Chapter 6. The SPN is more pronounced to cues that are 100% certain, and correlates with pain-evoked potentials only when intensity is predictable (Brown, Seymour, Boyle, et al., 2008b; Seidel et al., 2015). Cues were not certain, and intensity was not predictable in any experiments in this thesis. Overall, the results of this thesis suggest that in studies where pain is not predictable, the SPN is not a reliable marker of electrical pain anticipation.

Another surprising characteristic of the SPN results of this thesis is that although it is described as a negative potential, amplitudes were not always negative (Chapter 4, Pain FRN and cue modulation of pain). There are three points to note here which could account for this surprising result. First, the polarity of a component could be influenced by other

factors beyond whether the signal is excitatory or inhibitory, such as the orientation of the signalling neurons and the location of the reference electrode (Luck, 2012). This means that the polarity of an EEG signal is not necessarily meaningful in itself. Second, signal amplitude is relative to the amplitude included in the baseline correction procedure. In Chapter 6, the inter-stimulus interval was shorter because electrical stimulation does not heat the skin so there was no requirement for a long interval. This means participants may have been in a more constant state of anticipation despite the low pain intensity cue, which would influence the amplitude of the baseline correction period of the SPN, and thus could increase the amplitude of the SPN. Third, pain anxiety increases activity in the insula. If participants were anxious, insula activity could overlay the SPN and decrease its negative polarity (Lin et al., 2013). This is particularly relevant in the SPN results of Chapter 4, where the SPN was positive to laser pain, which was rated as more unpleasant (and presumably more anxiety-inducing) than electrical pain, which elicited a negative SPN. Overall, the results of this thesis did not reflect a consistent SPN to anticipation of pain so it is difficult to draw conclusions about specific anticipatory processes that it reflects, beyond the general point that it does not afford a direct read-out of expectations of pain intensities across modalities.

7.2.3. Hypothesis 3: the integration of expectation into pain is modulated by certainty

According to predictive coding, the certainty, or inverse variance, of expectations and stimulus intensity dictates the influence of either on pain perception (figures 1.8 and 1.9). In Chapter 5 (PE size and variability) we examined for the first time whether the certainty of stimulus intensity dictates its weighting on pain perception. Here, low variance incoming pain stimulus intensity did not influence pain intensity ratings any more than high variance incoming pain stimulus intensity. This presents a potential challenge to the predictive coding account of placebo analgesia (Büchel et al., 2014). However, there could be methodological issues with this study which prevented the manipulation from taking effect. First, we incorporated the pain variability into the test trials themselves rather than in a pre-test block. Participants might have required more trials to detect different levels of variability. Second, the effect of certainty of pain stimulation may have interacted with the certainty of the cued expectation. The same cue was presented on each trial in this study. This could mean that the expectation elicited by this cue was very certain and outweighed any effect of the pain stimulation variability on pain perception. Indeed, the results of Chapter 6 (Cue and placebo effects on pain) could also reflect the impact of a highly certain cued expectation. Here, expectations of low pain intensity were elicited with

high certainty in the conditioning procedure, where the pain intensity cue was presented as 100% certain, and was paired with low intensity pain on every conditioning trial. Despite the PE elicited on every trial by a discrepancy between the low pain cue and high stimulation intensity in the test block, expectations modulated pain perception and corresponding evoked potentials, suggesting the highly certain expectation may have outweighed the discrepant pain stimulation intensity.

To summarise, the results of this thesis indicate that although the FRN signals aversive PE, it is not a marker for pain PE. The thesis also provides substantial evidence that pain perception reflects the integration of expectation and pain stimulation, also showing a role for PE in this process, although the SPN was not a clear marker of pain anticipation. Finally, results did not support a key principle of the predictive coding account of pain perception, that the influence of expectation on pain is modulated by its certainty.

7.3. Other findings

7.3.1. EEG markers of pain

Overall, the EEG responses to pain were somewhat variable in this thesis. The SPN to electrical pain did not reflect intensity in Chapter 4 (Pain FRN and cue modulation of pain) or in Chapter 6 (Cue and placebo effects on pain). See section 7.2.2.2 for a detailed discussion of the SPN results. The relationship between subjective pain intensity ratings and the marker for pain perception, P2, was also inconsistent. In Chapter 4, pain intensity ratings correlated positively with P2 amplitude in response to veridically cued laser pain, as shown previously (Carmon, Friedman, Coger, & Kenton, 1980). In Chapter 4, the relationship between pain intensity rating and P2 amplitude was absent in response to electrical pain, but in Chapter 6, it was present to electrical pain in three out of four conditions, despite the added somatosensory noise associated with electrical stimulation (Perchet et al., 2012). It is possible that because there was more explicit uncertainty associated with the 75% probability cues in Chapter 4 compared to the (albeit misleading) 100% probability cues in Chapter 6, the relationship between pain perception and EEP amplitude was abolished in Chapter 4 because participants were more variable in their pain responses. Indeed, there is a dissociation in the brain areas activated by certain and uncertain pain: when pain is uncertain, anticipation activates areas commonly associated with attention, whereas when pain is certain, areas more commonly associated with semantic and prospective memory are activated (Brown, Seymour, Boyle, et al., 2008a). The lack of a relationship between pain perception and P2 amplitude is not novel, and it has also been proposed that the P2 may reflect salience alongside pain, which could also

abolish the relationship between pain perception and evoked potential amplitude (Fiorio et al., 2012; Legrain, Iannetti, Plaghki, & Mouraux, 2011; Wager et al., 2006). Furthermore, habituation of the EEP but not the pain intensity rating could abolish the relationship between EEP amplitudes and pain intensity ratings (section 2.2.3). Electrical pain intensity ratings significantly decreased over time in both Chapters 4 and 5 (PE size and variability), and EEP and SPN amplitudes decreased over time in Chapter 4. These results indicate some habituation of pain responses to electrical pain. Overall, our results indicate that the SPN is not a clear marker for electrical pain anticipation, and EEPs are not a clear marker for electrical pain perception when that pain is highly unpredictable.

7.3.2. Is the modulation of pain by expectation an affective process?

The value of outcomes is central to computational models of reinforcement learning (Sutton & Barto, 1990; Wagner, 1972). While PE research highlights the importance of outcome value, placebo research is often focused around perceived pain intensity. On one hand, PE research defines outcomes in terms of their affective value (better or worse than expected) and has shown PE signalling in value-encoding areas such as the midbrain, ventral striatum, orbitofrontal cortex, amygdala and PAG (Hollerman & Schultz, 1998; Li et al., 2011; O'Doherty, Dayan, Friston, Critchley, & Dolan, 2003b; Roy et al., 2014b; Schultz, Dayan, & Montague, 1997; Seymour et al., 2004, 2007). On the other hand, expectation manipulations typically define outcomes in terms of their intensity (higher or lower than expected). However, pain is an aversive outcome, which means that value and intensity are highly correlated, which suggests that the affective value of pain could play a role in expectation.

The results of this thesis suggest the modulation of pain by cued expectation is an emotionally neutral perceptual mechanism. First, the cue manipulation in Chapter 6 (Cue and placebo effects on pain) decreased perceived pain intensity but did not change the perceived unpleasantness of pain. Second, the SPN did not correlate with unpleasantness in Chapter 4 or 6.

There is evidence for a role for emotion in the placebo modulation of pain perception. The value of a sham treatment can influence its effectiveness: a placebo treatment with higher perceived monetary value decreases pain more effectively (Waber, Shiv, Carmon, & Ariely, 2008). Placebo analgesia decreases the perceived unpleasantness of pain, and placebo analgesia has been modelled as a reduction in negative emotion (Flaten et al., 2011; Vase, Robinson, Verne, & Price, 2003; Verne et al., 2003). It may be that sham treatment

decreases pain unpleasantness but cues do not. Had the placebo manipulation of Chapter 6 been effective, perhaps this would have modulated the perceived unpleasantness of the pain. Future research could repeat these studies with the adjustments suggested in Chapter 6 to maximise the likelihood of achieving a placebo response, to ascertain whether administration of a sham treatment changes unpleasantness ratings compared to a cue manipulation. This would contradict our conclusions that placebo analgesia can be replicated exactly with a cue and would suggest that placebo manipulations modulate affective responses more than cue manipulations. To extend this, future studies could manipulate the expected affective impact of the pain and compare this with a manipulation of the expected sensory impact of the pain. A difference in pain intensity ratings and corresponding brain activity would suggest two potential mechanisms of expectation modulation of pain perception: one involving the modulation of expected pain intensities and one involving the modulation of the expected value of pain.

7.4. Strengths and limitations

As well as the limitations discussed in each experimental chapter, there are some general limitations to this thesis.

Some important limitations are related to the limits of EEG. Pain signals are modulated from the spinal cord upwards (Eippert et al., 2009; Fields, 2004; Liebeskind, Guilbaud, Besson, & Oliveras, 1973). EEG measures the cumulative activity of neurons expressed at the scalp (section 2.1), so it is unlikely to reliably capture pain modulatory processes occurring lower down in the pain processing hierarchy. FMRI would allow a more holistic insight into modulation of activity at all levels of the hierarchy, but is limited in its temporal sensitivity, which makes it difficult to distinguish between anticipatory activity and activity elicited by the receipt of pain when pain is predictable. Using EEG allowed us to distinguish between pain-anticipatory and pain-evoked responses, but it should be acknowledged that this only captures the overall cortical product of pain perception. There are other analysis techniques in EEG, such as frequency analysis, which have been looked at in the context of PE before, but these are not as well-established as ERP analysis (Cohen, Elger, & Ranganath, 2007).

Another point of criticism relates to the usefulness of self-reported pain as a way to measure pain experiences. As previously acknowledged, any study involving subjective reports cannot exclude report participant bias as a factor (Brown et al., 2008). It is possible that pain intensity ratings were influenced by the desire to comply with perceived desires of the experimenter, which could either be to incorporate the cued intensity, or to rate the stimulation intensity and disregard the cued intensity. This is particularly salient

in Chapter 5 (PE size and variability), where no measure of neural responses to pain was taken. We aimed to avoid bias by instructing participants to rate the pain as however they felt it at that time in the experiment. Participants did not rate their pre-stimulus expectations of pain intensity, or their trial-by-trial confidence in their expectations, so we cannot make any conclusions about what participants expected. We avoided this because taking ratings of expectations may have directed participant's attention towards their expectations which could confound the manipulation.

Finally, the direct application of the thesis findings to clinical populations is limited, because we used short phasic pain stimulation and presented cues to elicit expectation. Clinically, pain is more likely to be tonic (although it can also be recurrent acute pain) and expectations are likely to be built over a longer period of time and will involve a wider variation of associations (Von Korff, Dworkin, & Le Resche, 1990). The results are more useful to understand the mechanisms behind perception of acute pain, although there is the potential to incorporate the role of PE and expectation into behavioural therapies for chronic pain, which work by changing beliefs about pain for example through verbal suggestion or mental imagery, and can improve pain outcomes (Morley, Eccleston, & Williams, 1999; Peerdeman et al., 2016).

There are some general advantages to the thesis which should be acknowledged.

We employed a variety of primary reinforcers, namely bitter and sweet taste, and laser and electrical pain. We also used three different expectation manipulations (75/25% reinforcement, 100% reinforcement, and sham treatment). This is important because the FRN is chiefly characterised as the response to a visual cue signalling unexpected monetary gain and loss which means literature on the FRN is specific to the visual domain. The results of this thesis provide a more multi-modal understanding of the EEG correlates of aversive PE and its expression to pain.

We resolved some inherent issues in the literature. First, as discussed in Chapter 3 (Taste FRN), reward and outcome delivery have been consistently confounded in the FRN literature. We resolved this in Chapter 3 by delivering and omitting both appetitive and aversive taste. Second, Chapter 4 (Pain FRN and cue modulation of pain) was partly motivated by the observation that studies which examine the modulation of LEPs by expectation do not account for expression of the FRN which has a similar spatiotemporal profile. As we did not see an FRN, results suggest that pain expectation studies should not be concerned about the influence of the FRN on pain-evoked potentials. Third, though modulation of pain by expectation is widely discussed, to our knowledge the contribution of expectation and pain intensity to pain perception had not been formally quantified,

which was achieved in Chapter 5 (PE size and variability), showing that both cued intensity and stimulus intensity influence pain intensity ratings. This is important because the literature assumes that expectation influences pain perception, for example predictive coding assumes pain perception is the result of the integration of expectation and pain stimulus intensity, but the influence of both within a single experiment had not been formally shown. Fourth, much of the placebo literature discusses expectation as a placebo mechanism without differentiating this from effects of the treatment ritual. In Chapter 6 (Cue and placebo effects on pain) we separated these two things to show that the treatment ritual is not necessary for pain modulation.

The results of the thesis also held methodological value. Chapters 4 (Pain FRN and cue modulation of pain), 5 (PE size and variability) and 6 (Cue and placebo effects on pain) confirmed the feasibility of using electrical pain stimulation in pain expectation studies. Chapter 6 revealed the difficulties of achieving a placebo response using electrical pain. Chapters 4 and 6 provided insights into the SPN to different pain stimulation types, which is particularly useful to electrical pain for which SPN responses are poorly understood.

7.5. Future studies

The findings of this thesis yielded some follow-on studies which would extend these results.

It would be useful to examine why a reward PE was not expressed in response to appetitive taste in Chapter 3 (Taste FRN). Our interpretation of this result is that ERPs prioritised expression of salience over expression of reward PE. It is possible that anticipation of the aversive taste in future blocks distracted participants from the reward value of the appetitive taste. This could be investigated by repeating the reward PE manipulation alone, without an aversive condition. It is also possible that the sweet taste was not appetitive, despite titration of the hedonic intensity of the taste to individual participant's preferences. Recording trial-by-trial ratings of the appetitive value of sweet taste would test this. If sweet taste does not elicit high ratings of appetitive value, the study could be repeated a somatosensory modality, with pain as an aversive reinforcer and hedonic touch as an appetitive reinforcer, which has been recently shown to be appetitive (Pawling, Trotter, McGlone, & Walker, 2017). The study could also be repeated using within-block delivery and not omission of appetitive and aversive outcomes, as when confronted with the possibility of two equally salient outcomes, ERPs may revert to reflecting utility. This result would challenge our results and generate new perspectives on the functional significance of the FRN.

Aversive PE to pain has been characterised in fMRI studies (Boll, Gamer, Gluth, Finsterbusch, & Büchel, 2013; Ploghaus et al., 2000; Roy et al., 2014). One aim of Chapter 4 (Pain FRN and cue modulation of pain) was to extend this understanding to ERPs. Because we measured responses to pain, we could not employ the axiomatic approach described in Chapter 3 (Taste FRN), and so could not include the effect of expectation. One way to examine the effect of expectation in the pain-evoked potential would be to compare ERPs to expected and unexpected delivery of pain, and expected and unexpected omission of pain. This has been done in fMRI, and the difference between ERP responses to unexpected and expected omission of pain has already been demonstrated (Garofalo et al., 2014; Roy et al., 2014). We did not employ this paradigm in Chapter 4 because we aimed to capture a magnitude PE signal as would be elicited in previous pain expectation studies, rather than a signal of delivery and omission (Atlas et al., 2010). However, this may be one way to capture an aversive pain PE signal using EEG.

One interesting avenue of research is directed by the results of the unsuccessful variability manipulation in Chapter 5 (PE size and variability). Predictive coding emphasises the role of certainty in the weighting of expectation versus stimulus intensity. However, we did not see an effect of certainty on the cues in Chapter 5. This may be because participants did not have the opportunity to implicitly perceive how variable the pain was because the pain was delivered during the test block. Also, the cue did not change between trials, meaning expectations associated with the cue may have been highly certain, abolishing any influence of pain stimulation intensity. It would be useful to systematically vary the certainty of expectations and of incoming pain stimulation to clearly understand the role of certainty in pain perception. Variability of pain stimulation could be manipulated in a pre-test block to ensure participants have the opportunity to perceive the variability. A pre-test block where the participant receives 30 stimuli which are highly variable in one group versus 30 stimuli which are consistently the same intensity in another group would achieve this. Certainty of expectations could also be changed within the same study, either by manipulating participant's confidence in a sham treatment or by using cues with different predictive qualities, as a probability cue of 100% likelihood is more certain than a probability cue of 75% likelihood. Further, recording EEPs would reveal the influence of the painful stimulation on cortical activity. We would predict the highest EEP response in the group with low certainty of low pain expectation and high certainty of stimulus intensity, and the lowest EEP response in the group with high certainty of low pain expectation and low certainty of stimulus intensity, in line with predictive coding.

Finally, aversive PE is not as well investigated as appetitive PE. Some research suggests aversive PE is signalled by serotonin in the dorsal raphe system (Daw, Kakade, & Dayan,

2002) and other research shows a dopamine response to aversive outcomes (Brischoux et al., 2009; Bromberg-Martin et al., 2010; Joshua et al., 2008). There is a clear need to better quantify aversive PE, in terms of the neurotransmitter systems, neuroanatomical basis, and the psychological parameters of aversive PE. An important avenue for future research is the role of negative expectation in aversive states such as chronic pain and how aversive PE is expressed here.

7.6. Conclusion

This thesis explored the expression of aversive PE and how this influences pain perception. We characterised the FRN, the EEG correlate for aversive PE to taste, challenging perspectives on this ERP as a utility PE signal, but did not observe FRN to pain, suggesting this ERP is not visibly overlaid on the pain-evoked signal. Results supported previous research showing that expectations of pain intensity are integrated into pain. We show that the influence of expectation appears to be modulated by PE size, in line with predictive coding, but not by the variability of painful stimuli, which contradicts predictive coding. We also extend previous research by showing that expectation modulation of pain can be elicited using cues and various reinforcement schedules, and a sham treatment is not necessary to elicit this response. This indicates that the results of the thesis appear to be generalizable to placebo analgesia, although there may be differences in the affective impact of sham treatment and cues which need to be explored further. Overall our results open new avenues for the study of aversive learning using the FRN, and indicate that cued modulation of pain is an affectively neutral perceptual mechanism which is generalizable across pain sensation types and is sensitive to PE.

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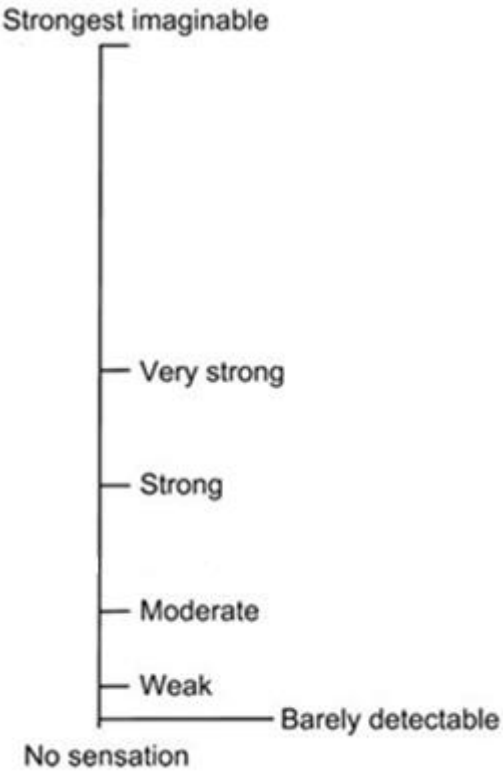
Appendices

Appendix A: Rating scales

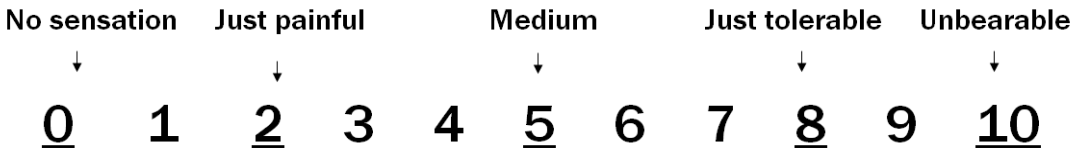
Labelled magnitude scale (LMS) (Chapter 3)

Please rate how strong you found the taste, from *no sensation* to *strongest imaginable* taste.



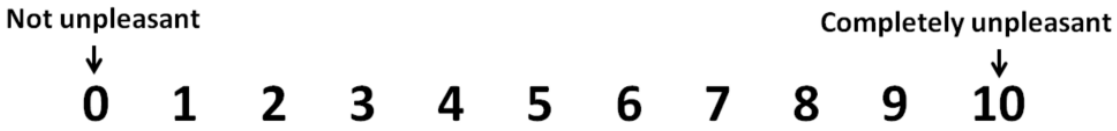
Numerical pain scale (NPS) (Chapters 4, 5 and 6)

Please rate the intensity of the pain stimulation, from *0 (no sensation)* to *10 (unbearable pain)*. You will not receive a higher stimulation than what you rate as *8 (just tolerable)*.



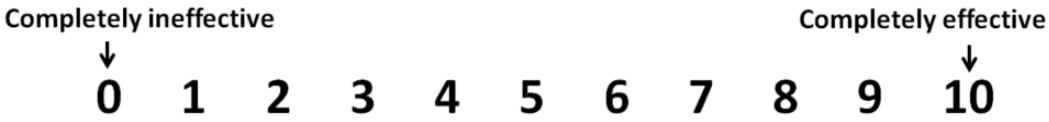
Unpleasantness (Chapters 4 and 6)

Please rate the unpleasantness of the pain stimulation, from *0 (not unpleasant)* to *10 (completely unpleasant)*.

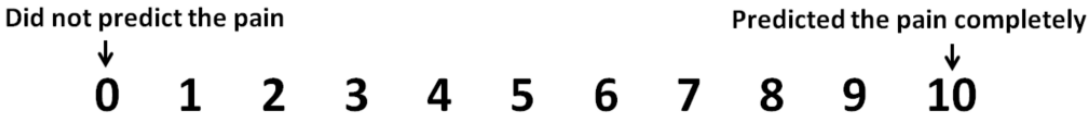


Manipulation check (Chapter 6)

Please rate the effectiveness of the analgesic cream, from *0 (completely ineffective)* to *10 (completely effective)*.



Please rate how accurately the cue predicted the pain, from *0 (did not predict the pain)* to *10 (predicted the pain completely)*.



Appendix B: Chapter 6 experimenter script

“This study is interested in your experience of pain. In this study you will receive brief, painful electrical pulses to the back of your hand. After setting up the EEG cap, we will test your response to different intensities of electrical stimulation, as everybody responds differently, and the intensity will be adjusted to suit you. Then, you will receive 30 electrical pulses. After this, you will pick a piece of paper out of an envelope which will decide whether you will be in one of two studies: either testing the effect of a pain reducing treatment, or testing your pain processing. Different people experience pain differently, and your experience of pain can change over weeks, days, and over the course of this experiment. You should rate the pain as however you feel it at that time in the experiment”. The final two sentences were intended to avoid any effect of report bias on behaviour.