AN EEG INVESTIGATION OF PAIN PROCESSING IN PARKINSON’S DISEASE

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in Faculty of Biology, Medicine and Health

2019

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School of Biological Sciences
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# Abbreviations

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<tr>
<td>AAL2</td>
<td>Anatomical Automatic Labelling 2</td>
</tr>
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<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BEM</td>
<td>Boundary Element Model</td>
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<tr>
<td>BL</td>
<td>Baseline</td>
</tr>
<tr>
<td>CBT</td>
<td>Cognitive Behavioural Therapy</td>
</tr>
<tr>
<td>D2R</td>
<td>Dopamine D&lt;sub&gt;2&lt;/sub&gt; Receptor</td>
</tr>
<tr>
<td>EBR</td>
<td>Eye Blink Rate</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
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<tr>
<td>ERP</td>
<td>Event Related Potential</td>
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<tr>
<td>HADS</td>
<td>Hospital Anxiety and Depression Scale</td>
</tr>
<tr>
<td>HC</td>
<td>Healthy Control</td>
</tr>
<tr>
<td>ICA</td>
<td>Independent Component Analysis</td>
</tr>
<tr>
<td>LEP</td>
<td>Laser Evoked Potential</td>
</tr>
<tr>
<td>LORETA</td>
<td>Low Resolution Electromagnetic Tomography</td>
</tr>
<tr>
<td>MCC</td>
<td>Mid-Cingulate Cortex</td>
</tr>
<tr>
<td>MDS-UPDRS</td>
<td>Movement Disorder Society Unified Parkinson’s Disease Rating Scale</td>
</tr>
<tr>
<td>MoCA</td>
<td>Montreal Cognitive Assessment</td>
</tr>
<tr>
<td>PAG</td>
<td>Periaqueductal Gray</td>
</tr>
<tr>
<td>PCS</td>
<td>Pain Catastrophising Scale</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s Disease</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal Cortex</td>
</tr>
<tr>
<td>PwPD</td>
<td>People with Parkinson’s Disease</td>
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<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>SASICA</td>
<td>Semi-Automatic Selection of Independent Components for Artifact correction</td>
</tr>
<tr>
<td>S1/S2</td>
<td>Primary/Secondary Somatosensory Cortex</td>
</tr>
<tr>
<td>SMA</td>
<td>Supplementary Motor Area</td>
</tr>
<tr>
<td>SPM</td>
<td>Statistical Parametric Mapping</td>
</tr>
<tr>
<td>TWOI</td>
<td>Time Window of Interest</td>
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<tr>
<td>VAS</td>
<td>Visual Analogue Scale</td>
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Abstract

Chronic pain in Parkinson’s disease (PD) is significantly higher than in the general population, and is often overlooked to be a side effect of the impaired movement and stiffness associated with the disease. There is scientific rational to link the degeneration which occurs in the PD brain to the increased prevalence of chronic pain. Although acute pain thresholds have been shown to be lower in PD, there is a lack of neuroimaging research which investigates the role of central processing in PD. Therefore, this thesis documents one of the first neuroimaging studies to investigate whether central pain processing is abnormal in people with PD.

The perception of pain can be modulated by subject driven processing such as attention, salience assignment and cognition, which is regarded as top-down modulation and is often abnormal in chronic pain conditions. A technique to investigate top-down processing is to study the neural activity during the anticipation of acute pain stimulus. Hence, in this thesis, we used EEG to record the anticipatory response to acute pain stimuli delivered by a CO₂ laser.

Our first experiment compared people with PD whilst off their medication to a healthy age-matched cohort and concluded that the PD patients showed an amplified anticipatory response and a higher sensitivity to the laser. In contrast, following dopamine increasing medication, no difference was shown between the PD and healthy controls.

To investigate the role of dopamine in altering pain perception in PD, the second study investigated the effect of manipulating the dopamine D2 receptor in young healthy volunteers. The study reported that the agonist and antagonist induced reductions in neural activity in the parietal and temporal lobes during pain perception, and the antagonist induced a reduction in the activity of the insula during pain perception, and intensified the effect of certainty during anticipation on the rating of pain.

The patient and the D2R manipulation studies demonstrated that dopamine may play a role in the top-down processing of pain and hence explain why chronic pain in PD is prevalent. Importantly the pain rating and sensitivity were not changed by Levodopa administration or D2R manipulation and supports the notion that dopamine is central to modulating top-down processing, rather than directly coding the intensity of pain. Therefore, together with further neuroimaging research conducted during the PhD, there is strong evidence of a disruption in the central processing of pain in PD and promotes the use of alternative therapies in the treatment of chronic pain in PD.
Lay Abstract

It is very common that people with Parkinson’s disease also suffer from long lasting pain, known as chronic pain. The pain can be caused by the symptoms of Parkinson’s disease such as; rigidity, bad posture and impaired movement. However, changes in pain perception have been seen very early in the disease before these symptoms have occurred. In addition, there is little evidence that the severity of the movement symptoms relates to the prevalence of chronic pain. Therefore, our research aimed to investigate how the changes within the brain could be involved in the increased susceptibility to chronic pain in Parkinson’s disease. During the anticipation of pain the activity of brain regions associated with pain have been shown to be different in people with chronic pain. Therefore, our experiments also recorded the brain activity during the anticipation of a painful stimulus.

Firstly, within people with Parkinson’s off their medication we recorded their brain activity whilst they anticipated pain. In comparison to a cohort of healthy participants, we discovered that the participants with Parkinson’s disease were more sensitive to the pain, plus a region of the brain known as the midcingulate cortex showed increased activity during anticipation. The midcingulate cortex is involved in pain sensitivity and therefore shows that the region is working differently in people with Parkinson’s. After the people with Parkinson’s had taken their normal medication, they remained more sensitive to the painful stimulus; however, the medication had stopped the increased brain activity within the midcingulate cortex during anticipation that was seen whilst they were off their medication.

The medication for Parkinson’s disease improves the activity of dopamine, a chemical within the brain. Therefore, our second investigation aimed to understand the role of dopamine in the anticipation and perception of pain within young healthy people by using drugs which increase and decrease the level of dopamine activity in the brain. The participants completed the same experiment which was
used in the Parkinson’s study. Our results showed that dopamine did not change an individual’s pain sensitivity; however it does alter the way we rate the pain depending on the information we provided during anticipation. This may indicate that long-term changes in dopamine in the brain within Parkinson’s disease could alter how an individual feels pain.

Our final aim was to investigate the difference in brain activity during the anticipation and perception of pain between young and old people. Our comparison showed that the young cohort showed a much higher level of brain activity during the experiment. This may have been due to a difference in pain processing and our relationship with pain changing over time between the two groups. The difference could also be due to changes in the quality of brain recordings in young and older brains.

In summary, the research highlighted an increased sensitivity to pain in people with Parkinson’s disease and some evidence for altered brain activity in Parkinson’s disease. With better understanding of the source of the pain, there will be better treatment for people with Parkinson’s disease and pain. Therefore, the evidence of altered brain activity during anticipation of pain indicates that treatments aimed at improving how the brain processes pain, such as meditation and cognitive behavioural therapy (CBT), could be beneficial for people with Parkinson’s disease.
Declaration

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I have been immensely lucky for the support that I have received throughout my PhD from my supervisors, colleagues, family and friends. Firstly I would like to extend huge thanks to my supervision team; Dr Monty Silverdale, Professor Anthony Jones, Dr Christopher Kobylecki, Dr Christopher Brown and Professor Wael El-Deredy, and my advisor Dr Joanna Neil. Together they have helped me to develop as a researcher and overcome challenges that I have faced.

I am hugely thankful for the teaching and guidance I received from my supervisors, including Anthony’s knowledge of all things pain related, Chris Brown’s EEG analysis training & support, and Chris Kobylecki’s Parkinson’s knowledge & recruitment.

Special thanks go to Monty, who is without a doubt, scarily intelligent, both in neuroscience and as a supervisor. His support has given me the confidence to be independent in my research and excitement to continue research in Parkinson’s disease.

Working in The Human Pain Research Group has been incredibly important to my enjoyment of my PhD. Anthony has created a supportive and team-focused lab where hard work and commitment are expected, yet a good work-life balance is expected in equal measure. An immense thank you to the irreplaceable Tim Rainey; I truly would have been lost without your EEG training, technical support, running and cycling commentary, and of course the amazing cups of teas. I also want to give huge thanks to Kate Lees, a fountain of knowledge and the greatest supplier of kitten and puppy photos! And finally to Grace Whitaker and Emily Hird who guided and trained me in my first couple of years. To be part of the group gave me an identity that I am proud of, and truly hope that the saying “You never leave The Human Pain Research Group” is true.

I am also indebted to the people at Salford Royal who have provided technical support from Medical Physics; Stuart Watson, Prawin Samraj, and Donald Allan and IT support from Adam Lounsbach.
It goes without saying that my family have provided fantastic support throughout my PhD – as they do in everything that I do. Special thanks go to my dad, Ian Martin, for his support. An extra thank you goes to my brother Paul Martin, for all of his help with coding throughout the early stages of my PhD.

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This research could not have happened without the funding from Parkinson’s UK, who I am incredibly grateful for. And finally, I am immensely thankful to my examiners, Dr Donna Lloyd and Dr Ellen Poliakoff, for taking the time to read and assess my thesis.

This thesis is dedicated to my mum, Carran Martin, a fun-loving, courageous and inspiring woman.
Preface

This thesis contains the research which stems from Monty Silverdale’s observations of Parkinson’s disease patients suffering from chronic pain which did not appear to correlate with the severity of their motor and peripheral symptoms. For better understanding and thus better treatment, Monty began a collaboration with The Human Pain Research Group at Salford Royal Hospital to further understand the brain’s role in pain in Parkinson’s disease. Together with Professor Anthony Jones, Dr Christopher Kobylecki, Dr Christopher Brown and Professor Wael El-Deredy, a series of studies were designed using EEG and laser-induced pain. The aim of the PhD was to investigate the processing of pain within the Parkinson’s disease in comparison to a healthy age-matched cohort, and to investigate the impact of the altered neurotransmitter levels within Parkinson’s disease could have on pain processing. I was lucky enough to be selected to undertake this research and knew the projects were very exciting for a PhD student to carry out.

Initially, two studies were outlined for the thesis, including a comparison of Parkinson’s patients with age-matched controls, and a second study investigating the role of Dopamine and Serotonin in pain perception within a young healthy cohort via the use of acute amino acid depletion. At the start of my PhD, an exciting collaboration was set up with Dr Grace Whitaker who would be recruiting participants for a pharmacological modulation of Dopamine. The research linked perfectly with our research aims and the study was carried out in my second year of the PhD.

Unfortunately, the building which the research took place, was being demolished throughout my second and third year, and caused significant delays to data collection of the amino acid depletion study due to the displacement, noise, vibration and dust. Therefore, for the submission of this thesis, the second study included in the thesis was switched to be the dopamine pharmacological modulation study carried out in collaboration with Dr Grace Whitaker, instead of the amino-acid depletion study. The theories behind the amino acid modulation
and dopamine modulation studies were both to investigate how altered neurotransmitters in Parkinson’s disease impacts pain perception, and hence, provide a similar narrative to the progression of this thesis.
Chapter 1

Introduction
1.1. PARKINSON’S DISEASE AND CHRONIC PAIN

In comparison to the general population, the prevalence of chronic pain in Parkinson’s disease (PD) has been reported to be significantly higher at between 40% and 85% (median = 57.78 %) (Valkovic et al., 2015; Allen et al., 2015; Beiske et al., 2009; Lee et al., 2006; Goetz et al., 1986; Tinazzi et al., 2006; Nègre-Pagès et al., 2008a; Hanagasi et al., 2011; Rana et al., 2013; Defazio et al., 2008; Silva, Viana and Quagliato, 2008; Silverdale et al., 2018) whilst the prevalence within the general population has been reported to be 22.75% [averaged from (Blyth et al., 2001b; Johannes et al., 2010; Breivik et al., 2006)]. There is clearly a higher prevalence of chronic pain reported in the PD population compared to the general population which indicates that the development of PD may increase an individual’s susceptibility to developing chronic pain.

The development and persistence of chronic pain is thought to be associated with dysfunctional bottom-up and top-down mechanisms of pain modulation. The term bottom-up refers to stimulus-driven modulation (i.e. stimulus intensity, stimulus novelty etc) and modulation of ascending pain signals in the dorsal horn of the spinal cord. In contrast, top-down modulation refers to subject-driven manipulation of the perception of pain (i.e. emotional state, cognition, attention orientation, memories etc). Both processes can either facilitate or inhibit pain perception and abnormalities within these mechanisms are associated with chronic pain conditions and pain hypersensitivity.

The presence of chronic pain in PD patients has often been considered to be a consequence of the musculoskeletal symptoms, and to be primarily driven by excessive nociceptive input due to bottom-up processes. Although musculoskeletal symptoms are likely to contribute to chronic pain, the fact that pain can precede the musculoskeletal symptoms and that the pain can be idiopathic, demonstrates that heightened pain may be a syndrome caused by the pathological changes within the brain associated with PD. There is a lack of neuroimaging research which
investigates the role of impaired top-down processing. However, the degeneration within the PD brain provides a scientific rationale to suggest that altered top-down processing, rather than merely atypical peripheral nociceptive transmission, may be the cause of the increased likelihood of chronic pain in PD.

To dispel theories that the pain is solely a result of peripheral musculoskeletal symptoms a large scale investigation of 1957 PD patients reported that there was no correlation between pain and motor impairments during early stage PD (Silverdale et al., 2018). In addition, Valkovic et al., (2015) reported that a high proportion of pain in PD is deemed to be central neuropathic pain which was widespread and not associated with the musculoskeletal or dystonic symptoms. Additionally, pain has been reported to be located in areas that are unlikely to be affected by the PD muscular symptoms such as oral, genital, (Ford et al., 1996) and abdominal pain (Nègre-Pagès et al., 2008a).

Finally, pain perception has been shown to be abnormal in PD patients irrespective of whether they have chronic pain or not. For instance, PD patients have an increased pain-induced cortical activation as measured by positron emission tomography (PET) (Brefel-Courbon et al., 2005), reduced pain thresholds and tolerance (Zambito Marsala et al., 2011), and reduced electrical and muscle withdrawal reflex to pain (Mylius et al., 2008). Furthermore, numerous studies which selectively investigate PD patients suffering from chronic pain have reported reduced pain thresholds and an inability to habituate to stimuli compared to healthy controls (Schestatsky et al., 2007a; Djaldetti et al., 2004a).

Chronic pain negatively impacts on daily life and has been considered to be more of an impairment than the motor symptoms (Silverdale et al., 2018). Therefore, there is clear evidence that further research is required to understand the cause of chronic pain in people with PD to provide an improvement in treatment. Here, I briefly review the pathology of PD, pain processing and the impairments within shared central process which shed light on a potential link between PD and chronic pain.
1.2. PARKINSON’S DISEASE: AN OVERVIEW

PD is a neurodegenerative disease affecting 127,000 people in the UK and 1 million in the US and is progressively more common with increasing age (Dorsey et al., 2007). It is the second biggest neurological disease after Alzheimer’s disease, affecting approximately 100/100,000 (Seidel et al., 2015b). PD is classically regarded to be due to the loss of dopaminergic neurons located in the basal ganglia, specifically the substantia nigra pars compacta (SNpc), however, there is a multitude of additional pathological changes. The basal ganglia are the main processors of voluntary motor movements and hence the main symptoms of PD are regarded to be motor related. The characteristic features of PD are the presence of a tremor, bradykinesia, and rigidity with symptoms including, shuffling gait, bad posture and reduced facial expression. Nevertheless, PD is not solely a movement disorder, and non-motor symptoms are seen to precede the motor aspects. The widespread degeneration throughout the brain also leads to non-motor symptoms including; cognitive impairments, dementia, pain, sleep disturbances, depression and anxiety (Barone, 2010).

The three main motor symptoms of PD are tremor, bradykinesia and rigidity and are normally the first point at which the disease can be clinically recognised. Below Table 1.1 defines the motor related symptoms seen in PD. The severity of each symptom varies between patients. The non-motor symptoms associated with PD are outlined in Table 1.2. It is important to state that not all PD patients experience all of the symptoms discussed, and the severity of the condition varies between each person. The presence of cognitive decline has been related to the altered neurotransmitter activity in the PD brain.
Table 1.1: Motor symptoms of Parkinson’s disease.

<table>
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<th>Symptom</th>
<th>Definition</th>
<th>Location</th>
<th>Reference</th>
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<tr>
<td>Tremor</td>
<td>Uncontrolled, somewhat rhythmic movements. The amplitude of the tremor can be as large as more than 10cm, as well as being as low as below 1cm.</td>
<td>Upper and lower limbs, Jaw/Lips</td>
<td>(Geraghty, Jankovic and Zetusky, 1985; Jankovic, Schwartz and Ondo, 1999)</td>
</tr>
<tr>
<td>Bradykinesia</td>
<td>Slow movement and freezing</td>
<td>n/a</td>
<td>(Berardelli et al., 2001)</td>
</tr>
<tr>
<td>Rigidity</td>
<td>Impaired movement of the joints</td>
<td>Joints</td>
<td>(Berardelli, Sabra and Hallett, 1983; Prochazka et al., 1997)</td>
</tr>
<tr>
<td>Akinesia</td>
<td>Impaired control of voluntary movement</td>
<td>n/a</td>
<td>(Pascual-Leone et al., 1994a; b)</td>
</tr>
<tr>
<td>Dystonia</td>
<td>Muscle spasms and abnormal muscle tone causing uncontrolled movements</td>
<td>Widespread</td>
<td>(Tolosa and Compta, 2006; Poewe, Lees and Stern, 1988)</td>
</tr>
<tr>
<td>Abnormal gait</td>
<td>Gait is shorter than normal, and can develop to become shuffling.</td>
<td>n/a</td>
<td>(Bloem et al., 2004; Rogers, 1996; Olson, Lockhart and Lieberman, 2019; Morris et al., 2019)</td>
</tr>
<tr>
<td>Impaired posture</td>
<td>A consequence of the rigidity, dystonia and akinesia</td>
<td>n/a</td>
<td>(Benatu, Vaugoyeau and Azulay, 2008; Dietz, Berger and Horstmann, 1988)</td>
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Table 1.2: Non-motor symptoms of Parkinson’s disease.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Definition</th>
<th>Reference</th>
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<tr>
<td>Dementia</td>
<td>Cognitive decline including memory and attention, and increased confusion. Reduced independence.</td>
<td>(Aarsland, Zaccai and Brayne, 2005; Gomperts et al., 2016)</td>
</tr>
<tr>
<td>Speech and communication problems</td>
<td>Their speech may become slurred and lack of expression within their speech. Handwriting may also become smaller and less-defined.</td>
<td>(Ho et al., 1999; Canter, 1963; Drótár et al., 2016)</td>
</tr>
<tr>
<td>Chronic pain</td>
<td>Chronic pain is persistent pain for more than 3 months. Pain can be caused by musculoskeletal problems and can also be idiopathic.</td>
<td>(Silverdale et al., 2018; Nègre-Pagès et al., 2008b; Beiske et al., 2009)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Prolonged tiredness</td>
<td>(Lou et al., 2001; Friedman and Friedman, 1993; Friedman et al., 2007)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>Excessive and idiopathic sense of dread, worry and with physical symptoms of breathlessness and heart palpitations</td>
<td>(Richard, Schiffer and Kurlan, 1996; Broen et al., 2016)</td>
</tr>
<tr>
<td>Depression</td>
<td>Prolonged intense feeling of sadness and lack of optimism.</td>
<td>(Thobois et al., 2017; Maillet et al., 2016; Hu et al., 2015)</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>Hallucinations can be visual, auditory, tactile and illusions.</td>
<td>(Fenelon et al., 2000; Sanchez-Ramos, Orttoll and Paulson, 1996; Inzelberg, Kipervasser and Korczyn, 1998; Barnes and David, 2001)</td>
</tr>
<tr>
<td>Delusions</td>
<td>Delusions include paranoia, jealousy and extravagance</td>
<td>(Schrag, 2004)</td>
</tr>
<tr>
<td>Memory problems</td>
<td>Impaired storage of new memories or retrieval of old memories</td>
<td>(Morris et al., 1988; Harrington et al., 1990; Lehrner et al., 2015)</td>
</tr>
<tr>
<td>Sleep problems</td>
<td>Impaired sleep patterns. Can include insomnia, nightmares, nocturia and sleep-apnoea.</td>
<td>(Faludi et al., 2015; Albers, Chand and Anch, 2017; Lin et al., 2017)</td>
</tr>
</tbody>
</table>
The development of PD is typically characterised by the loss of dopaminergic neurons and in some cases, the presence of Lewy body pathology within the brain. Although PD is considered a dopamine disorder, there is also prominent dysfunction of other neurotransmitters such as serotonin, noradrenaline, acetylcholine and glutamate (Xu et al., 2012; Mann and Yates, 1983; Reisine et al., 1977; Hornykiewicz, 1981). They are discussed in further detail in regards to the effect on pain perception in section 1.4.

In addition to the loss of dopamine and other neurotransmitters, a pathological characteristic in PD is the presence of Lewy body pathology throughout the brain, which are a type of protein aggregate and results in the reduction of neurotransmitter release or neuronal death (Sulzer and Surmeier, 2013). The presence of Lewy body pathology increases over time and has been classified by Braak et al., (2004) to be in six stages which is outlined in Table 1.3.

Therefore, the pathological and neurotransmitter changes in the Parkinsonian brain result in the diverse list of symptoms. Whilst the motor symptoms have been researched extensively, the non-motor aspect of PD has been investigated less. One of the least researched non-motor symptoms in PD is pain, yet the impact of chronic pain on quality of life is substantial and contributes to the development of depression, sleep disturbances and anxiety. The widespread pathophysiology and diverse range of symptoms in PD therefore may predispose patients to dysfunctional central processes including pain perception. First, section 1.3 will briefly summarise the brain’s involvement in pain processing, and section 1.4 will highlight how PD pathology could predispose patients to the development of chronic pain.
Table 1.3: Pathological findings of Lewy body Pathology in the Parkinsonian brain outlined by Braak et al., (2004)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Brain location (if applicable)</th>
<th>Sub-region</th>
<th>Lewy body Pathology</th>
<th>More details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Brain stem</td>
<td>Medulla</td>
<td>Dorsal motor nucleus of the vagal nerve Intermediate reticular zone (adjoins the dorsal motor nucleus) Lower raphe nuclei</td>
<td>Long unmyelinated preganglionic fibers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reticular formation</td>
<td>Magnocellular portions of the reticular portion (especially the gigantocellular reticular nucleus)</td>
<td>Responsible for the synthesis of serotonin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pons</td>
<td>Coeruleus-subcoeruleus complex</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>Brain stem</td>
<td>Descending tracts</td>
<td>‘Gain-setting’ system nuclei</td>
<td>Sparingly myelinated tracts. Involved in the pain control system involved in the inhibition of somatosensory and visceral sensory input.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Basal ganglia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temporal lobe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>Basal forebrain</td>
<td>Substantia Nigra pars compacta</td>
<td>Postero-lateral subnucleus</td>
<td>Eventually 70% neurons lost. Lose almost all melanoneurons LNs extends throughout the central subnucleus of the amygdala and isolates it from the neighboring structures</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amygdala</td>
<td>Central subnucleus</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pedunculopontine nucleus</td>
<td>Basolateral nuclei Cholinergic tegmental nucleus</td>
<td>Connections to the SN</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oral raphe nuclei Cholinergic magnocellular nuclei</td>
<td>Use serotonin and projects to the striatum</td>
</tr>
<tr>
<td>Stage 4</td>
<td>Cerebral cortex Hippocampus</td>
<td>The bridge between the allocortex and the neocortex.</td>
<td>Anteromedial temporal mesocortex Second sector</td>
<td>Thick layer of LNs appear. Sparingly myelinated fibers. Can be a marker of PD that a clinician can use for diagnosis.</td>
</tr>
<tr>
<td>Stage 5</td>
<td>Cerebral cortex</td>
<td>Neocortex</td>
<td>High-order sensory association regions Premotor region</td>
<td></td>
</tr>
<tr>
<td>Stage 6</td>
<td>Cerebral cortex</td>
<td>Neocortex</td>
<td>First order sensory association regions Primary fields (In some cases)</td>
<td></td>
</tr>
</tbody>
</table>
1.3. BRAIN REGIONS INVOLVED IN PAIN PROCESSING

There are multiple regions of the brain involved in pain processing and which constitute the pain network (see Figure 1.1). The main regions are regarded to be the insula, anterior cingulate cortex (ACC), thalamus, somatosensory cortex (S1/S2), prefrontal cortex (PFC) and the amygdala. The network integrates the somatosensory, emotional and contextual components of the pain input. It is important to note that these regions have multiple roles and are not unique to nociceptive processing. The network is considered to be important for the detection of salient stimuli within the environment (Legrain et al., 2011).

One theory of the organisation of the pain network within the brain is of two defined pathways; the medial and lateral systems. Firstly, the medial pathway ascends via the spinoreticulothalamic pathway and terminates in the amygdala, hypothalamus, insula and the ACC, and is responsible for the affective and emotional aspect of pain perception (Vogt and Sikes, 2000; Treede et al., 2000; Scherder et al., 2005). The medial pain pathway also has projections to the PFC, basal ganglia, and periaqueductal grey (PAG), which allows integration of the nociceptive transmission and a link to the descending pain pathway. Secondly, the lateral pathway ascends via the spinothalamic tract and terminates in the S1 and S2, parietal operculum and the posterior insula. The lateral pathway reports the intensity, duration and location of the pain stimuli (Sewards and Sewards, 2002; Treede et al., 2000; Vogt and Sikes, 2000). However, the division of the pain network into medial and lateral systems which have distinct roles in the perception of pain is an oversimplification, and both systems have been shown to be involved in the processing both sensory and affective aspects of pain.

In addition, there are descending modulatory tracts which originate in the brainstem and can be modulated by higher cortical regions (Millan, 2002). The descending modulation of pain is considered to be a type of top-down processing (Pertovaara, 2013; Donaldson and Lumb, 2017). The descending tracts begin from
the brainstem within the structures such as PAG and rostral ventromedial medulla (RVM), with descending targets including the dorsoreticular nucleus, parabrachial nucleus and the rostraventral medulla (Millan, 2002). The end target of the descending pathways is the dorsal horn of the spinal cord, a site for modulation of ascending pain transmission. The descending tracts are not independent of the higher brain areas and receive modulatory input from the cortex, hypothalamus and the amygdala and are therefore modulated by central processes (ie. Top-down modulation).

Figure 1.1: Regions of the pain network taken from Bushnell, Ceko and Low, 2013. Abbreviations: PFC; Prefrontal Cortex, ACC; Anterior Cingulate cortex, S1/S2; Primary/Secondary Somatosensory cortex, BG; Basal Ganglia, AMY; Amygdala, PAG; Periaqueductal grey, PB; Parabrachial nucleus. The ascending tracts from the spinal cord travel via the brainstem and terminate in regions of the midbrain, which connect to cortical regions for the integration of pain processing.
1.3.a. Top-Down modulation of pain

The focus of this thesis is regarding the top-down processing of pain in people with PD. Here I will briefly summarise the different types of top-down processing and their potential impact on the perception of pain and involvement in the development of chronic pain.

The processing of pain is often divided into bottom-up and top-down aspects. Bottom-up perception is stimulus-driven, whereby the stimulus influences our perception. For example, the salience of a stimulus or the novelty of a noxious stimulus can modulate the perceived pain intensity (Hauck et al., 2015a). In contrast, top-down processing is subject-driven where prior knowledge influences perception. An example of top-down processing in regards to pain perception is attentional orientation or emotional state, or prior experience of pain. Top-down pain processes are considered to be central to the development of chronic pain, yet are also believed to be the key to treating chronic pain. For instance, whilst negative expectation can cause an increase in pain (Bjørkedal and Flaten, 2012), positive expectation such as the placebo effect (Dobrila-Dintinjana and Nacinović-Duletić, 2011), and improving control by meditation (Zeidan et al., 2012; Brown and Jones, 2010) can modulate pain perception via top-down processes.

For instance, the activity of the dorsolateral prefrontal cortex has been linked to the ability to cope with chronic pain, and a reduced activity within the region has been associated with chronic pain conditions such as FM and osteoarthritis (Brown, Elderedy and Jones, 2014). Abnormalities within this region are thought to contribute to depression, Schizophrenia and other neurological mood disorders, which also have an impact on pain management (Narasimhan and Campbell, 2010).

One of the main top-down processes which can modulate pain perception is the orientation of attention (Miron, Duncan and Bushnell, 1989a). For instance, a simple distraction can result in reduced pain, and focused attention can cause an increase in pain intensity (Ruscheweyh et al., 2011). In addition, an unpleasant
distraction has shown to alter the activity within the ACC during painful stimuli (Phillips et al., 2003; Roy et al., 2009; Berna et al., 2010). Attention orientation also modulates the descending pain pathways which can change the perception of pain. The descending pain pathways receive input from regions associated with attention processing such as the amygdala, PFC and ACC, and modulate the descending pain systems, either facilitating or inhibiting pain transmission (Ong, Stohler and Herr, 2019)(Ossipov and Limitado, 2012). Therefore, dysfunction within the cortical and subcortical regions, influences the descending modulatory system and can alter pain perception (Ossipov and Limitado, 2012).

An example of how attention can alter the perception of pain is via the study of anticipation of a painful stimulus. Anticipation results in the pain network to be activated for the forthcoming pain as attention is oriented towards the stimulus, and can modulate the resultant perception of the pain (Koyama et al., 2005; Wang et al., 2008). For instance, when expectation of intensity is greater than what is delivered, the pain perceived is closer to the expected rather than the stimulus intensity. In contrast, a decreased pain expectation can reduce the pain intensity perceived, thereby emphasising the influence of a positive outlook may have on reducing pain (Schmid et al., 2013). The specific regions that are activated during anticipation include the ACC, insula, S1/S2, PFC and the PAG (Koyama et al., 2005). Within chronic pain conditions, such as FM and osteoarthritis, heightened anticipation is common and correlated to increased pain sensitivity (Brown, El-Deredy and Jones, 2014).

The mechanism by which expectation or anticipation of painful stimuli can alter the perception of pain is yet to be fully understood. A functional magnetic resonance imaging (fMRI) study has highlighted that the pain network regions including the ACC, insula, thalamus, PFC were increased relative to the level of pain intensity expected (Koyama et al., 2005). The study reported that the expectation of a lower pain than administered resulted in a decreased level of activation of the insula, ACC and S1, which is similar to that of a placebo response. The anticipation of a painful
stimulus is a technique used to investigate central processing of pain, and has highlighted abnormalities within chronic pain cohorts, and is therefore used in the research conducted within this thesis.

1.3.b. Chronic pain

Finally, I will briefly discuss the central processing associated with chronic pain conditions. Chronic pain is defined as persistent pain for at least 3 months and can be linked to a primary condition such as osteoarthritis, or can have an idiopathic cause. Living with chronic pain can be debilitating, with severity ranging from mild to excruciating, and with persistent or relapsing states (Von Korff et al., 1992). The development of chronic pain has been associated with atypical ascending and descending pathways, along with abnormalities in top-down processing of nociceptive information. For instance, the anti-nociceptive descending pathway of pain is altered in many chronic pain states (Porreca, 2002; Banic et al., 2004; Gebhart, 2004) and may be important in the persistence of the chronic pain (Gebhart, 2004).

Numerous experiments have investigated pain processing within different pain conditions and it is well-established that patients with chronic pain conditions such as Fibromyalgia (FM), arthritis and back pain, have reduced pain thresholds compared to controls (Wolfe et al., 1995; Huskisson and Hart, 1972; Imamura et al., 2013). The establishment of reduced pain thresholds in chronic pain conditions, and the persistence of the pain, is indicative of aberrant top-down processing. For instance, FM, which is characterised by widespread pain with no pathophysiological explanation, is considered to be a partly due to central sensitisation and abnormal top-down processing (Guymer and Littlejohn, 2007). FM is the most prominently researched chronic pain condition in regards to the role of top-down processing in its development – therefore, it is important to review the current research for comparison with pain processing in PD. The top-down processes include an imbalance of neurotransmitters and neuroendocrine factors, and dysfunction of the
hypothalamic-pituitary-axis (HPA) (Guymer and Littlejohn, 2007). FM patients have also shown augmented brain activity within regions associated with pain perception during the anticipation and perception of pain (Brown, El-Deredy and Jones, 2014). Further evidence of central dysfunction includes; reduced dopamine release within the basal ganglia (Wood et al., 2007b), reduced activation of the ACC and descending inhibitory pain pathway (Jensen et al., 2009), and increased rates of pain catastrophising within the FM population which correlates with altered brain activity (Gracely et al., 2004). The treatment of FM is limited and an increasing focus is being placed on the positive effects of alternative, non-pharmacological, treatments such as meditation and cognitive behavioural therapy due to their ability to improve top-down processes (Kaplan, Goldenberg and Galvin-Nadeau, 1993; Pleman et al., 2019; Montero-Marín et al., 2018; Aman et al., 2018; Amutio et al., 2018). This emphasises the role of the brain in the manifestation of persistent pain and why treatments for chronic pain which are targeted at central processing are promising.

To summarise, the baseline state of the brain, be it healthy or negatively affected by a condition such as depression or catastrophizing pain, affects the perception of pain. Additional to the baseline state, the expectation and level of attention can alter the pain perception, as does the context of the pain. Chronic pain states can alter the brain’s structure and neurotransmitter levels which further impact on the processing of pain, producing a negative cycle that can progress to worsening of the chronic pain state. Therefore, irrespective of whether the central dysfunction is the cause or consequence of the chronic pain state, the top-down modulation of pain is central to deterioration, and yet also very important in the treatment of pain.

1.4. PATHOLOGICAL PREDISPOSITION OF PAIN IN PD

The pathophysiology of PD has widespread affects throughout the brain and includes regions of the pain network and alteration in the level of neurotransmitters which are also evident in chronic pain conditions. This section
will highlight how the abnormalities seen in the PD brain can cause alteration in pain processing and hence contribute to the development of central sensitisation and chronic pain.

1.4.a. Pain network and Parkinson’s disease

Firstly, the regions associated with pain perception outlined in Figure 1.1 have been shown to be abnormal in PD patients. For instance, the activation of the insula and the ACC, regions of the medial pain system, are increased in PD patients following a painful stimulus compared to healthy participants (Brefel-Courbon et al., 2005). The augmented activation was shown to normalise following the administration of Levodopa, a common drug used to treat PD, which increases the level of dopamine and highlights the potential role of dopamine. The lateral pain pathway involves the somatosensory cortex and has been associated with abnormal somatosensory processing in PD patients, and affects their sensorimotor integration (Conte et al., 2013; Lewis and Byblow, 2002; Rossini, Filippi and Vernieri, 1998).

Furthermore, the impaired function of the basal ganglia is central to PD motor symptoms, however, there is extensive evidence of the basal ganglia’s involvement in pain processing and chronic pain states (see review: Borsook et al., 2010). The basal ganglia receive input from the ascending pain spinothalamic tract and have diverse connections with regions associated with pain processing; and is important for the integration of motor, cognitive, emotional and autonomic aspects of pain processing (Borsook et al., 2010). Additionally, there is an abundance of opioids and their receptors, which are responsible for endogenous analgesia, within the basal ganglia (Barceló, Filippini and Pazo, 2012). The basal ganglia also have roles in attention (Van Schouwenburg, Den Ouden and Cools, 2010), emotion (Lanciego, Luquin and Obeso, 2012), and avoidance behaviours (Hormigo, Vega-Flores and Castro-Alamancos, 2016), all of which play a part in the perception of pain. Aberrant function of the basal ganglia has been associated with chronic pain conditions such as FM (Shokouhi et al., 2016), chronic regional pain syndrome.
(CRPS) (Azqueta-Gavaldon et al., 2017) and burning mouth syndrome (BMS) (Jääskeläinen, 2012).

This section will summarise studies which have investigated the role of the basal ganglia nuclei in pain processing, and hence highlight possible explanations of the high prevalence of chronic pain in PD.

Firstly, the degeneration of the dopaminergic neurons in the substantia nigra pars compacta (SNpc) within PD patients is the most significant change within the basal ganglia and an early investigation reported that the SN consisted of approximately 50% nociceptive neurons (Pay and Barasi, 1982). The SN is connected to regions of the pain network and is shown in Figure 1.2. The degeneration of the SNpc is therefore likely to affect pain processing

![Figure 1.2: The SN and its connections to the regions of the pain network. Figure taken from (Wasner and Deuschl, 2012). S1: Primary Somatosensory Cortex, S2: Secondary Somatosensory Cortex. The Substantia nigra is connected, either directly (black line) or indirectly (red lines), to regions of the pain matrix. The red and yellow boxes highlight the pain-processing regions.](image-url)
The SN has also been associated with the descending inhibition of pain. The stimulation of the SN has been shown to increase the descending inhibition, as did the selective stimulation of PAG and the raphe nuclei (Barnes, Fung and Adams, 1979). The study also investigated the role of dopamine and serotonin on the inhibitory effect and highlighted that it relied on dopaminergic neurons of the SN innervating the serotonergic neurons within the raphe nuclei. Therefore, the substantial loss of dopamine neurons within the SN in people with PD indicates a possible impaired function of the descending inhibition of pain.

Within the basal ganglia, the striatum, made up of the putamen and caudate nucleus, has been linked to pain transmission. The striatum is involved in cortical and subcortical loops which are involved in both bottom-up and top-down processing. A study using anaesthetised rats showed that the activation of the peripheral nociceptors caused an increase in the activity of the nociceptive neurons in the caudate nucleus and the putamen (Chudler, Sugiyama and Dong, 1993). The caudate nucleus and the putamen of the rat have been analysed to show that there are different types of nociceptors ranging from; wide-dynamic range, nociceptive specific, and inhibited neurons (Chudler, Sugiyama and Dong, 1993). The blood flow in the striatum has also been shown to increase when the contralateral hand receives a painful stimulus (Barceló, Filippini and Pazo, 2012).

As previously mentioned, the basal ganglia is home to a high level of opioid activity; this being especially high in the striatum. This leads to a strong belief that the striatum is required for the endogenous analgesia generated by opioid activity. Patients with arthritis have been shown to have a high abundance of opioid receptors and a low binding availability within the striatum, which is thought to be an adaptive mechanism (Brown et al., 2015).
A high degree of dopaminergic transmission occurs within the striatum, yet is impaired within people with PD. Further detail of how this impacts on pain processing is discussed in section 1.4.

A study by Starr et al., 2011, highlights the putamen’s role in pain processing and provides evidence that the putamen is involved in sensory processing related to pain. The study reported that stroke patients with selective lesions of the putamen had a reduced activation of the pain matrix and alterations of pain thresholds depending on the type of stimulus. Within the healthy controls, it was shown that the putamen was involved in the cortical-basal-ganglia-thalamic-cortical loop and connections to the ACC, insula and the thalamus during painful stimulation. These connections proposed the theory that the putamen was involved in the memory, attention and emotional aspects of pain transmission (Starr et al., 2011). Therefore, the putamen appears to play an important role in pain processing within the brain, including the pain threshold levels and the cognitive aspects of pain perception. Hence, the atypical striatal activity in PD may impact on the role of the putamen in pain regulation.

The second region within striatum is the caudate nucleus and there is evidence that the caudate nucleus is involved in both acute and chronic pain. Firstly, the conscious act of suppressing electrically induced acute pain was shown to trigger a consistent bilateral activation of the caudate along with contralateral activation of the insula (Wunderlich et al., 2011). In contrast, heat pain did not show this strong activation pattern (Wunderlich et al., 2011). The caudate nucleus’ role in pain modulation is further supported by a study reporting its role in pain-avoidance (Koyama, Kato and Mikami, 2000). The study involved macaque monkeys and focused on the ACC, a well-known region of the pain network, and the caudate nucleus (Koyama, Kato and Mikami, 2000). Both regions were activated during the task, resulting in the idea that the caudate nucleus is functionally related to the ACC during pain transmission. Hence, the caudate nucleus might be associated with the ACC role of processing the affective component of pain (Besson, Guilbaud and Ollat,
1995). Furthermore, there was early research within rats that demonstrate that the caudate nucleus is involved in avoidance processing (Kirkby and Kimble, 1968; White and Rebec, 1993).

Finally, the globus pallidus, a region of the striatum affected by PD, is involved primarily in the control of voluntary movement and has been shown to be involved in pain transmission (Braz et al., 2005; Chudler, 1998). By using a tracing agent, the globus pallidus was shown to be a target for peripheral non-peptide nociceptors, whereas the peptide nociceptors did not terminate within the globus pallidus, indicating two separate ascending pathways depending on nociceptor type. There is evidence that the globus pallidus within the PD brain responds to thermal and mechanical noxious stimuli (Belasen et al., 2016). The deep brain stimulation of the globus pallidus (Loher et al., 2002), or the bilateral removal of the region in PD patients results in pain relief (Favre et al., 2000).

1.4.b. Basal ganglia connections to regions of the pain matrix

The basal ganglia have numerous connections to other regions which include those that are part of the pain network such as; the thalamus, insula, ACC and the S1/S2. There are also regions that are involved in the descending modulation of pain transmission which the basal ganglia is connected with. The following section discusses the important connections that the basal ganglia make with these regions of the pain network, and how they can be disrupted in PD.

The thalamus, a main output of the basal ganglia, is dysfunctional in PD and hence important to discuss its role in pain processing (Halliday, 2009; Pifl, Kish and Hornykiewicz, 2012). The thalamus receives input from the globus pallidus and substantia nigra pars reticulatar (SNpr), and is a major output of the basal ganglia. The basal ganglia afferents innervate the ventral nuclear and the intralaminar group (centromedian) of the thalamus. The ventral nuclear group projects to the motor, pre-motor and supplementary motor area (SMA), whereas the intralaminar group projects to the motor cortex and the striatum within the basal ganglia. The
thalamus receives input from the ascending nociceptive tract such as the spinothalamic tract which terminates in the lateral nuclei of the thalamus, and is deemed to be part of both the medial and lateral pain pathways within the brain. The thalamus innervates higher cortical regions including the insula, ACC and the S1/S2, along with the striatum of the basal ganglia and descending afferents to the brainstem. Thus, due to the thalamus being a major output of the basal ganglia and the altered activity of the thalamus in PD patients (Halliday, 2009) may impact on the thalamic role in nociception.

The basal ganglia is also connected to the insula which is central pain perception (Flynn, 1999). The insula projects to the basal ganglia for the integration of motor, somatosensory and vestibular processing (Flynn, 1999). The insula specifically targets the striatum and the globus pallidus, and the afferents which terminate in the dorsolateral striatum are deemed to be for somatosensory integration which is involved in pain processing (Starr et al., 2009; Kim et al., 2017). Hence, the reduced connectivity between the substantia nigra pars compacta (SNC), a region of the basal ganglia, and the insula in PD patients is likely to have an impact on the ability to integrate information during pain processing (Flynn, 1999; Wu et al., 2012). In addition, the pain processing region of the ACC innervates the ventral striatum (Alexander, DeLong and Strick, 1986) and has shown to be functionally correlated with the basal ganglia (Margulies et al., 2007).

The basal ganglia is also connected to the periaqueductal gray, a region of the midbrain and the main control centre for descending pain modulation (Northoff, 2013; Behbehani, 1995). Within a rat model of PD, a defective pathway between the Substantia nigra, PAG and rostral medulla oblongata, has been shown (Lima et al., 2018) and indicates that the disruption of the basal ganglia afferents may lead to altered nociceptive modulation via the descending pain pathway.
1.4.c. The Brainstem changes in PD and pain processing

The brainstem is one of the earliest regions to be affected by Lewy body pathology in PD (Braak et al., 2004), and as pain has been reported to precede motor symptoms, the brainstem could be involved in the development of chronic pain in PD.

Firstly, descending tracts are affected by Lewy body pathology during Braak stage 2 which has been linked with pain hypersensitivity and indicates that the descending pain modulation could be altered in PD patients (Latremoliere and Woolf, 2009). The pons, medulla (Gebhart, 2004) and reticular formation (Casey, 1980; Bowsher, 1976) are also involved in the descending modulation pain system and thus the Lewy body pathology in these regions may contribute to atypical pain processing.

Additionally, the nucleus raphe magnus (NRM) is highly populated with serotonergic neurons and is involved in both movement and endogenous analgesia within the healthy brain. However, in PD, the NRM has been shown to degenerate and thus likely to inhibit the endogenous analgesic response to nociceptive input (Gai, Blessing and Blumbergs, 1995). For instance, a lesion within the regions of the brainstem can alter pain sensitivity and rodent studies have shown how lesions impact the action of opiate related analgesia (Abbott and Melzack, 1982; Basbaum and Fields, 1984). For instance, the lesion of the median raphe nucleus or central tegmental nucleus induced a potentiation of the morphine response; whereas the loss of the nucleus raphe magnus lead to an attenuation of the morphine response (Abbott and Melzack, 1982).

Additionally, an interesting link between pain and PD is how the activation of the motor cortex is believed to disinhibit the PAG by inhibiting the GABAergic interneurons and thus stopping their inhibition on the descending anti-nociception pathway (Pagano et al., 2012). Therefore, this interesting finding implies that the reduced motor cortex activity in PD patients may lead to a reduced disinhibition of the PAG, and thus a reduced activation of the descending anti-nociception
pathway. This would also help to explain the reason why Levodopa improves pain states in PD as it increases the motor cortex output.

1.4.d. Cortical changes and pain processing

The final stages of PD involves the deterioration of the cerebral cortex leading to cognitive decline and damage to the hippocampus contributing to loss of memory and the development of PD dementia (Braak et al., 2003). The changes in the higher cortical regions will impact on the complex emotional and attention related aspects of nociception processing. As discussed in section 1.3, the top-down processes involved in pain have a significant impact on the development of chronic pain.

1.4.e. Neurotransmitter changes in Parkinson’s disease linked with pain

There are prominent changes in the levels of neurotransmitters in PD and abnormal neurotransmitter levels are related to chronic pain states such as Fibromyalgia (FM), Burning Mouth Syndrome (BMS) and Chronic Regional Pain Syndrome (CRPS) (Stahl, 2009). As such, this section will look at the role of neurotransmitters in pain processing and how abnormalities in neurotransmitter levels within PD may impact pain transmission.

1.4.e.i. Dopamine

PD is characterised by the degeneration of the SNpc, a highly dopamine abundant region, and the resulting decrease in dopamine produces both the motor and non-motor symptoms. In addition to dopamine’s role in regulating movement, there are theories of dopamine being central to the precision of prediction and the salience value of stimuli, and is considered to be involved in the integration of bottom-up and top-down processing (Friston et al., 2012; FitzGerald, Dolan and Friston, 2015; Shiner et al., 2015). Dopamine is therefore important in the top-down modulation of pain perception and dysfunctional transmission in PD may lead to impaired pain processing. Figure 1.3 summarises the importance of dopamine in pain perception.
1. Introduction

Dopamine is a modulatory neurotransmitter due to its role in both excitation and inhibition of neural activity. There are two main post-synaptic dopaminergic receptors, the D1 (subtypes D1 and D5) (excitatory) and D2 (subtypes D2, D3 and D4) (inhibitory) receptors, plus pre-synaptic D2 receptors which regulate the release of dopamine into the synapse. Dopamine receptors are found widespread, however, there are most abundant in the basal ganglia. Within a human study, the D1Rs were shown to be more widespread than the D2Rs and more abundant within the dopaminergic pathways, especially in the cortex and limbic system (Hall et al., 1994). The D2Rs have a higher abundance in the thalamus in comparison to the D1Rs, whilst both receptors are co-localised and highly abundant in the striatum.

Figure 1.3: A summary of the various roles of dopamine and how they are involved in pain perception. DA: Dopamine, BG: Basal ganglia, SN: Substantia Nigra.
Furthermore, a rodent study revealed higher levels of D2Rs in the midbrain compared to D1Rs (Meador-Woodruff et al., 1991). There are four well defined dopaminergic pathways within the brain, which originate from the SN, ventral tegmental area (VTA) and hypothalamus (see Figure 1.4).

Figure 1.4: Dopaminergic pathways. [Anatomical locations not precise]. Blue: Mesocortical; Ventral Tegmental area (VTA) to Prefrontal cortex (PFC). Orange: Mesolimbic; Ventral Tegmental area (VTA) to Nucleus Accumbens (NAc). Pink: Nigrostriatal; Substantia Nigra pars compacta (SNpc) to the Striatum (STR). Purple: Tuberinfundibular; Arcuate nucleus (Arc) of the Hypothalamus (Hyp) to the Pituitary gland.

Within healthy subjects, a PET study detected an increased D2R dopaminergic activity in the basal ganglia and connecting pathways during pain perception (Scott et al., 2006). The study identified different roles of the nigrostriatal and mesolimbic pathways; the nigrostriatal pathway was associated with the pain intensity perceived, whilst the mesolimbic pathway was associated with the affective processing such as unpleasantness ratings and the emotional aspect of the pain (Scott et al., 2006). In addition, the binding capacity of D2R/D3Rs within the
putamen of the striatum have been shown to be inversely correlated to individual pain thresholds (Pertovaara et al., 2004). Therefore, dopamine has the potential to modulate the pain intensity perceived whilst also being susceptible to affective aspects on pain perception.

The dopaminergic nigrostriatal pathway has been regarded to be involved sensory gating of nociceptive stimuli and the development of chronic pain (Jääskeläinen et al., 2001). The nigrostriatal pathway originates in the substantia nigra and terminates in the dorsal striatum; both regions are within the basal ganglia. Altered dopaminergic transmission within the nigrostriatal pathway has been shown to contribute to burning mouth syndrome (BMS) (Hagelberg et al., 2003b) and atypical facial pain (Hagelberg et al., 2003a). BMS patients have also been reported to have a reduced level of presynaptic dopaminergic function within the right putamen, a nuclei of the striatum (Jääskeläinen et al., 2001).

Chemical lesions of the substantia nigra to reduce dopamine levels in rats has shown to lead to an increase in pain sensitivity (Saadé et al., 1997). Furthermore, lesions of the substantia nigra in rats was shown to induce allodynia following a mild stimulation to the orofacial region (Dieb et al., 2014), and subsequent administration of a D2R agonist (Bromocriptine) remediated the allodynia. The study also demonstrated how the loss of the dopaminergic nuclei of the substantia nigra caused a reduced level of neurons within the striatum and the VTA (Dieb et al., 2014). Therefore, the degeneration of the dopaminergic neurons of the substantia nigra pars compacta within PD has an impact on the abundance of neurons within other regions, and there is evidence that this will lead to pain sensitivity.

Furthermore, the dopaminergic mesolimbic and mesocortical pathways have been linked to nociceptive processing. The main focus of research of the mesolimbic and mesocortical pathways in PD is in regards to their role in regulating reward and gambling behaviours (Berridge and Robinson, 1998; Anselme and Robinson, 2013).
It is common that PD patients have impaired hedonistic regulation and suffer from pathological gambling due to dysfunction of the pathways (Torta and Castelli, 2008). However, chemical lesions of the VTA (the origin of both pathways) have also proven to increase the pain sensitivity in rats during standard pain tests (Saadé et al., 1997). Analgesics which increase pain thresholds, such as opioids and amphetamine, increase the abundance of dopamine in the nucleus accumbens (Altier and Stewart, 1999). Hence, a low endogenous level of dopamine in PD may result in the opposite and lower pain thresholds.

The mesolimbic pathway is also known as the ‘reward pathway’ and has also been reported to be involved in pain. The naming of the pathway as the ‘reward pathway’ is an oversimplification as the pathway is involved in the regulation of both aversive and pleasurable stimuli (Leknes and Tracey, 2008; Taylor et al., 2016). The mesolimbic pathway has been shown to activate during noxious thermal stimuli (Leknes and Tracey, 2008). An fMRI study highlighted that the activation of regions within the mesolimbic pathway preceded the somatosensory activation during thermal pain (Becerra et al., 2001). Furthermore, the mesolimbic pathway is involved in the placebo response as the dopaminergic reward system is involved in the successful placebo effect (Borsook et al., 2010) and dopaminergic activity within the ventral basal ganglia has been reported to increase during a successful placebo response of pain (Scott et al., 2008; Schweinhardt et al., 2009). There is also a positive correlation between the level of dopamine activity and effectiveness of the placebo (Scott et al., 2008).

The final dopaminergic pathway, the mesocortical pathway, has reduced dopamine levels in PD (Ford, 2010). The mesocortical dopaminergic pathway is associated with emotional, attention and motivational processing (Levy, 1991; Bertolucci-D’Angio, Serrano and Scatton, 1990; Bromberg-Martin, Matsumoto and Hikosaka, 2010). All three aspects are involved in pain processing and can manipulate the perception of pain. For instance, a rodent study concluded that the mesocortical
pathway increased the release of dopamine within the PFC via D2Rs mechanisms, and resulted in a reduction in pain (Sogabe et al., 2013).

Therefore, dopamine transmission within the four pathways has been associated with a negative effect on pain perception. Thus, the reduced production of dopamine in PD is likely to contribute to the dysfunctional pain processing and a susceptibility of chronic pain in the disease.

In addition to the altered activity within the dopaminergic pathways, the altered level of dopamine leads to an altered abundance and activation of the dopaminergic receptors within PD (Ryoo, Pierrotti and Joyce, 1998; Brooks et al., 1992) and further disrupts the role of dopamine in pain processing.

For instance, the D2 subtype of dopamine receptors has been associated with pain transmission and the administration of D2R agonists and antagonists have been shown to modulate pain perception. For example, pain was reduced in rats following an injection of a D2R agonist (Quinpirole) into the nucleus accumbens (Taylor, Joshi and Uppal, 2003), which was blocked if preceded by a selective D2 antagonist (Raclopride) (Taylor, Joshi and Uppal, 2003). In addition, the abundance of D2 receptors within the putamen and ratio of D1 and D2 receptors within the striatum have been suggested to have a relationship with atypical facial pain syndrome (Hagelberg et al., 2003a).

The abundance of D2-receptors is altered in PD, with studies reporting variability (Brooks et al., 1992), and a 15% increase in abundance (Ryoo, Pierrotti and Joyce, 1998). The level of D2 binding potential in the putamen and the right medial temporal cortex has shown an inverse correlation to cold pain thresholds (Hagelberg et al., 2002). Participants who had a low D2 binding potential reported a high pain threshold, whereas subjects with high D2 binding potential reported low pain thresholds. In contrast, when heat and cold induced pain was administered concurrently, low binding potential correlated with a low pain threshold and vice versa (Hagelberg et al., 2002). Although the study’s conclusions were speculative,
they suggested that low D2 binding potential was due to a high level of dopamine, and the high dopamine abundance correlated with a high pain threshold. Therefore, the study supports the fact that in PD, a low level of dopamine would be correlated with subsequent low pain threshold for cold stimuli.

Further evidence of how abnormal dopamine signalling may contribute to chronic pain is how patients with FM have atypical dopamine signalling. A PET study which recorded D2 and D3 receptor activity reported that the FM patients did not show dopamine release within the basal ganglia in response to pain, whilst the control subjects showed a release of dopamine which was positively correlated to the intensity of pain (Wood et al., 2007b). This study highlights how dopamine transmission within the basal ganglia is abnormal in FM patients, and this provides strong evidence that the low dopamine levels and dysfunctional basal ganglia activity in the PD basal ganglia contributes to the development of persistent pain.

1.4.e.ii. Serotonin

Similar to dopamine, serotonin is a modulatory neurotransmitter with effects seen in a diverse range of processes such as cognition, mood and stress (see review: Olivier, 2015). Serotonin dysfunction is associated with the manifestation of depressive and anxiety related disorders (Young et al., 1985; Cannon et al., 2006, 2007). It is well-known that low mood is correlated with chronic pain and exacerbates the intensity and unpleasantness of pain (Bair et al., 2003; Giesecke et al., 2005).

Serotonin dysfunction is present in PD and has been associated with the development of non-motor symptoms (Poewe and Luginger, 1999; Politis and Niccolini, 2015; Langston, 2006; Kish, 2003; Bernheimer, Birkmayer and Hornykiewicz, 1961). The raphe-nuclei which produces serotonin degenerates in PD and the neuronal loss contributes the low serotonin levels in the PD brain (Hornung, 2003; Braak et al., 2003; Halliday et al., 1990). A PET study has shown that serotonin dysfunction is widespread and amongst other regions, was present
in regions associated with the pain network, including the thalamus, ACC, PFC, insula and amygdala (Politis et al., 2010). PD patients also have a lower serotonin level within the cerebrospinal fluid which has been correlated to the presence of depression (Mayeux et al., 1988). Serotonin is involved in the modulation of pain via the descending inhibitory pain pathways (Ossipov, Morimura and Porreca, 2014; Millan, 2002; Bowker et al., 1983) and the development of chronic pain has been associated with reduced serotonergic activity within the descending pathways (Kwon et al., 2013; Ossipov, Morimura and Porreca, 2014). Serotonin has a modulatory role on pain processing, and depending on the receptor subtype, can induce either analgesia or algesia (Sommer, 2004). For instance, selective-serotonergic reuptake inhibitors (SSRIs), a common anti-depressant, increases serotonin transmission and can evoke an analgesic effect (Sansone and Sansone, 2008). Additionally, a lower level of serotonin has been linked with FM (Wolfe et al., 1997).

Therefore, the dysfunction of serotonergic transmission within PD is likely to impair the anti-nociceptive descending pain pathways, and negatively affect the contextual-affective aspect of pain processing. Importantly, there is a link between depression and the prevalence of chronic pain PD, however, it is difficult to differentiate between cause or consequence (Ehrt, Larsen and Aarsland, 2009).

1.4.e.iii. Noradrenaline

In addition to Dopamine and Serotonin, the level of noradrenaline is reduced in PD (Scatton et al., 1983) due to degeneration of the locus coeruleus, the main source of noradrenaline production (Reches and Meiner, 1992; Mavridis et al., 1991; Mann, 1983; Benarroch, 2009; Del Tredici and Braak, 2013). In addition, there is widespread Lewy body pathology in the brain stem nuclei including the medulla, pons and descending tracts which are involved in descending pain inhibition (Braak et al., 2003). A reduction in the abundance of noradrenaline due to the presence of Lewy body pathology in the locus coeruleus has been reported in the PD brain (Del Tredici and Braak, 2013).
Noradrenaline is involved in nociceptive processing within the spinal cord, brainstem nuclei and higher brain regions (see review: Pertovaara, 2006). Noradrenaline is present in the nociceptive processing nuclei within the brainstem which are important for descending pain pathways, and noradrenergic efferents to the amygdala are involved in the effect of emotion on pain perception (Strobel et al., 2014). Within the spinal cord, noradrenaline can suppress the nociceptive input via the descending pain pathways (Pertovaara, 2006).

Noradrenaline is theorised to mainly play a role in persistent pain, whereby sustained pain activates noradrenergic mechanisms to inhibit pain transmission. Research has also highlighted how chronic pain can induce atypical noradrenergic transmission (Alba-Delgado et al., 2013). Furthermore, the role of noradrenaline is involved with the modulation of behavioural states and as such is considered to be involved in top-down modulation of pain (Strobel et al., 2014; Pertovaara, 2006; Hirata, Aguilar and Castro-Alamancos, 2006; Onur et al., 2009). The combination of chronic pain and noradrenaline dysfunction can lead to negative emotional state such as anxiety and depression (Alba-Delgado et al., 2013), which also increases pain sensitivity and reduces the ability to cope with chronic pain (Geisser et al., 1994).

Hence, the abnormal noradrenergic transmission triggered by PD results in a disruption in its normal role in the pain processing and predisposes PD patients to be more susceptible to develop chronic pain.

**1.5. IMAGING STUDIES SHOWING ALTERED CENTRAL PAIN PROCESSING IN PARKINSON’S DISEASE**

The changes in the PD brain discussed in Section 1.4. demonstrate how PD patients may be predisposed to the development of chronic pain. Therefore, there has been a clear scientific rationale to research the mechanisms of pain in the Parkinsonian
brain via imaging techniques. Here I will review the current neuroimaging results of research into central pain processing in PD.

Firstly, PET imaging research has shown that PD patients had a higher activation within the prefrontal cortex, insula and ACC during thermal pain (Brefel-Courbon et al., 2005, 2013). PET has also highlighted reduced activity within the PFC and insula during the cold pressor test, and an increase within the ACC (Brefel-Courbon et al., 2013). Within both experiments, the differences in activation were normalised after taking L-DOPA.

fMRI has also been useful in understanding pain processing in PD. A recent fMRI study investigating the descending pain pathway in drug-naive PD patients and reported that the PD patients had dysfunctional activity and connectivity during anticipation of pain. Regions affected included the PFC, ACC and MCC which are associated with the top-down modulation of descending pain inhibition (Forkmann et al., 2017). The study by Forkmann supports the theory that the descending pain pathway is impaired in PD. Furthermore, the latest investigation by Tessitore et al., (2018) showed significant BOLD signal changes in the drug-naïve PD brain following heat stimulation. The study concluded that there was an increased activation within the cerebellum, somatosensory cortex and pons of the brainstem compared to healthy controls (Tessitore et al., 2018).

Finally, a study used EEG to assess the brain response to a range of nociceptive stimuli in PD patients, off and on their medication, in comparison to healthy controls (Priebe et al., 2016). Priebe showed that brain activations did not correlate with pain rating in the PD group, and concluded that amplitude and frequencies of brain responses were lower in PD. In contrast, the latency of brain activation was increased in the PD cohort and together with increased pain sensitivity, supports the theory that central pain processing is dysfunctional in the PD.
1.6. CONCLUSION

Although the general consensus is often that chronic pain is driven by peripheral nociceptive dysfunction, there is strong evidence that many chronic pain states are due to disruption to central mechanisms. The high prevalence of chronic pain in PD patients and the presence of idiopathic and widespread pain unrelated to the musculoskeletal symptoms present a foundation to investigate the central mechanisms involved in PD pain. There are numerous regions that are mutually affected by PD pathology and are involved in pain processing within the healthy brain. Furthermore, the development of pain in PD has been shown to precede the onset of motor symptoms which may be correlated to the early Lewy body pathology within the brainstem. Research has shown that the perception of pain is significantly modulated by central processing and disruption to the somatosensory, emotional and cognitive networks have shown to lead to hyperalgesia, catastrophizing states and hence, the development of chronic pain. The fact that neurotransmitters such as dopamine, serotonin and noradrenaline are involved in pain processing and are also irregular in PD, strengthens the theory that central mechanisms are likely to be involved in PD pain. Therefore, to conclude, the combination of Lewy body pathology and neurotransmitter changes within regions involved in pain processing are likely to increase the likelihood of a chronic pain state in PD patients.
Chapter 2

General Methods and Introduction to study aims
This section will discuss the rationale behind the choice of methodology and outline the studies which will be discussed in this thesis.

2.1. GENERAL METHODS

The research included in this thesis used electroencephalography (EEG) to record brain activity during the anticipation and perception of laser induced acute pain. The study design of the research was based on the protocol carried out in Brown et al., (2014) and Brown et al., (2008). Firstly, the Brown et al., (2014) investigated the brain activity during the anticipation and perception of pain in people with Fibromyalgia, Osteoarthritis and healthy age-matched participants. The research used EEG source localisation analysis to conclude regional differences in the patient groups during the anticipation of pain, and hence demonstrated that the protocol was sensitive to distinguishing differences in the processing of pain within patients groups. Secondly, the Brown et al., (2008) study demonstrated the effect of certainty on the rating of pain and neural activity. There is evidence that dopamine is involved in the processing of uncertainty and hence was included as an aspect of the protocol (Fiorillo, 2017).

The same experimental protocol was applied in each investigation, and as such, the rationale for the study design is introduced in the following sections, with further detail included in the individual papers of the thesis.

2.1.a. Experimental pain

The common techniques used to induce pain in research are via thermal, mechanical, chemical and electrical stimulation. The choice of stimulation is dependent on the experimental design and for the studies included in this thesis, thermal stimulation via a CO₂ laser was used (Brown, El-Deredy and Jones, 2014) due to its quick pulse duration, small stimulation area and selective activation of the nociceptive A and C-fibres. The laser induced pain has been used to evaluate pain thresholds in healthy and patient groups (Gibson et al., 2001; Brown, El-Deredy and Jones, 2014; Brown et al., 2008a; Derbyshire et al., 2002). In addition, laser
induced pain stimuli have been used in numerous pain experiments investigating pain anticipation (Brown, El-Deredy and Jones, 2014; Clark et al., 2008a; Wang et al., 2008; Brown and Jones, 2010, 2008; Forkmann et al., 2017). The fast onset and offset of the laser produces well-defined evoked responses which can be time-locked in neuroimaging techniques and are referred as laser evoked potentials (LEPs).

A limitation of the laser is the potential of skin sensitivity and damage due to the heating of the skin. To limit the potential, the laser was moved in a pattern within a grid on the forearm of the participant. The laser was also limited to a maximum voltage that could be delivered to avoid skin damage. Nevertheless, the skin sensitivity of the individual must be considered and the maximum voltage adjusted accordingly, or stimulation stopped altogether in rare instances.

Pain is a subjective experience and the rating of pain can therefore be variable between individuals. To limit the subjective interpretation of how to rate the laser stimuli, a pain rating scale was presented to the participants and was explained using a script by the researcher (see figure 2.1).

![Pain Rating Scale](image.png)

Figure 2.1: The pain rating scale that was used for the psychophysics and main experiments. The explanation of the rating scale was identical for all participants to limit subjective interpretation.

### 2.1.b. EEG

EEG is a method used to record the neural activity by monitoring the voltage oscillations generated by the brain. The post-synaptic activity of neighbouring neurons produces local dipoles which are recorded by electrodes placed on the
scalp (Baillet, Mosher and Leahy, 2001). The pyramidal neurons are the main
generators of the EEG signal due to their uniform orientation to the surface and
their long length allowing dipoles to form. EEG detects the synchronised activity of
neurons with a high temporal resolution that is significantly higher than other
imaging techniques.

As such, EEG has allowed for a deeper understanding of cognitive processing and
disease states, along with providing a diagnostic tool for epilepsy, sleep disorders
and many other diseases. The non-invasive nature of EEG allows researchers to
record the activity in a broad range of people, irrespective of their health or age.
EEG has also helped to compare the differences of electrical activity between
disease states and healthy volunteers.

2.1.b.i. The Forward and Inverse EEG Problems

EEG has two main confines, the forward and inverse problem. The forward problem
consists of how the activity within the brain is recorded on the scalp electrodes,
whilst the inverse problem is in regards to calculating the precise source of the
potential generated at the scalp. Over decades of research, more complex models
have been developed to address the forward problem, which take into
consideration; head shape, tissue heterogeneity and the difference in conductivity
(Hallez et al., 2007). There are also several techniques to resolve the inverse
problem [see review: (Grech et al., 2008)]. The LORETA (Low resolution
electromagnetic tomography) technique which is used within the research of this
thesis has been reviewed to be able to accurately identify sources, even of deep
regions (Grech et al., 2008).

The LORETA technique overcomes the inverse problem using a tomographic
solution whereby it calculates the current source density in a large cortical area to
produce a 3D volume image of neural activity. The solutions created by LORETA are
based on the assumption that neighbouring neurons respond similarly, such as the
same orientation, latency and activation. An advantage of using the LORETA
technique is that it does not require a priori estimates of sources, which are a
requirement in other inverse solutions such as the equivalent current dipole (ECD) technique. Therefore, LORETA is not restricted by a limited number of expected sources.

Research into the accuracy of the LORETA technique has shown that the source estimates are analogous to other imaging techniques which have a higher spatial resolution. Concurrent recording of EEG and fMRI has shown that LORETA successfully detects current source densities which are consistent and are on average 14.5/16 mm within the fMRI loci (Mulert et al., 2004; Vitacco et al., 2002). Similar validation has been shown using PET and LORETA (Zumsteg et al., 2005), as have intra-cerebral recordings (Seeck et al., 1998; Trébuchon-Da Fonseca et al., 2005). LORETA is also able to localise activity within deep brain structures such as the insula (Mulert et al., 2004).

2.1.b.ii. Anticipatory-evoked potentials

A cue indicating a forthcoming stimulus can evoke an anticipatory response which is detected in EEG as a negative waveform known as the stimulus-preceding negativity (SPN) (Figure 2.2). The SPN can be triggered by both innocuous and noxious stimuli. There is evidence to show that the SPN induced by a noxious stimulus may be generated within the cingulate cortex, especially the anterior- and mid- regions (Böcker et al., 2001). Therefore a more negative response during the anticipation of noxious stimuli indicates a higher anticipatory response. The characteristic topographic map of anticipation is a negative response over the dorsal regions and is shown in Figure 2.2.

For the research conducted within this thesis two baselining methods were applied to investigate the neural activity during the anticipation of pain. Initially, a single baseline of -3500 -3000 ms was used to compare with the Early (-2500 -2000ms), Mid (-1500ms -1000ms) and Late (-500 0ms) time windows of interest. However, as this time window used for the single baseline was between trials and no cue was presented yet, observations of participants highlighted that they were using this time period to readjust their position, relax or stretch. Furthermore, the tremor of
the participants with PD was more prominent during the inter-trial periods. Therefore, the single baseline of -3500 to -3000ms was potentially variable and affected by artefacts, and a secondary baselining method was applied.

Therefore, primary baselining method was adjusted to use individual baselines for each time window of interest set as -500ms prior to the auditory beeps. The baselines (BL) for each time window of interest (TWOI) were as follows: Early (BL: -3500 -3000ms, TWOI: -2500 -2000ms), Mid (BL: -2500 -2000, TWOI: -1500 -1000ms), Late (BL: -1500 -1000ms, TWOI: -500 0ms). An aim of baselining uniquely for each anticipation window was to reduce variability in the data as the anticipation phase progressed, such that the analysis of each phase of anticipation was unique to that phase and not subject to variability arising from neural activity occurring in the previous phase.

The analysis using single baseline of -3500 -3000ms was also conducted to enable comparison to previous studies (Brown et al., 2008a; Brown, El-Deredy and Jones, 2014) that used the same baselining method. All statistical analysis for the anticipation phase was adjusted for multiple comparisons.

Figure 2.2: (Left) An EEG waveform of an SPN generated by a visual anticipatory cue, auditory cues, and the LEP response following acute laser pain. Diagram from (Jones, Brown and El-Deredy, 2013). (Right) An example of a characteristic anticipatory response recorded via EEG displayed on a topographic map. Plot displays the grand mean response of a healthy cohort during the late SPN (-500 ms) preceding a noxious laser stimulus.
2.1.b.iii. Suitability of EEG for research aims

EEG was chosen as the method to record brain activity for the two studies due to its suitability in assessing pain perception which was used in Brown et al., (2014) and other pain research studies (Clark, Brown, Jones, et al., 2008; Shao et al., 2012; Jones et al., 2013; Hauck et al., 2015).

A benefit of EEG is that the setup is quick and does not require restricted movement compared to fMRI. Therefore, the technique was suitable for volunteers with PD as movement was allowed between trials and did not present possible complications of claustrophobia. The movement constraints of other imaging techniques meant that EEG was the optimal tool for recording brain activity in people with Parkinson’s disease. In addition the use of EEG for pain processing has been used extensively within pain research due to its good temporal resolution. The research conducted in The Human Pain Research Group has developed protocol and analysis methods using EEG to investigate the top-down modulation of pain via the anticipation signal.

The good temporal resolution of EEG is in contrast to its poor spatial resolution in comparison to fMRI and PET. Therefore, source localisation was a key focus for the research to establish the sources of the brain activity recorded that would allow a deeper interpretation of the EEG data. The LORETA source localisation technique is used to solve the inverse problem with EEG recording (Pascual-Marqui, 2002). The technique was selected due to its accuracy in localisation activity. In addition, due to the LORETA technique being used in previous research conducted by The Human Pain Research Group, the findings can be comparable to prior research.

2.2. INTRODUCTION TO STUDY OBJECTIVES

The research within this thesis was planned to investigate central pain processing in Parkinson’s disease with the aim to improve understanding of the cause of the higher rate of chronic pain in the disease. The first study explored pain processing in PD patients and age-matched healthy volunteers (Chapter 3 & 4). This study was
followed by an investigation into the role of dopamine in pain processing and was carried out in young healthy volunteers (Chapter 5). An additional analysis was carried out between young and old healthy volunteers to establish the age-related differences in the response to the protocol (Chapter 6).

2.2.a. Chapters 3 & 4: Study 1: PD vs Healthy Controls

The first study investigated pain processing in volunteers with PD and age-matched healthy volunteers. We wanted to investigate pain processing both on and off medication within the PD group. The benefit of investigating the ‘off’ state of PD was to remove any effect of the medication and to understand the ‘normal’ state of the brain in PD patients. The additional study of ‘on’ medication was to highlight whether the dopaminergic medication used for PD changed pain processing as L-DOPA had previously been shown to have analgesic qualities. The experimental paradigm explained below was carried out in the morning following the withdrawal of PD medication and repeated on the same day 1 hour after PD patients had taken their medication. The order of the two recordings could not be randomised due to the need of 12 hours withdrawal of the medication within the ‘off’ condition. The healthy controls also repeated the study in the afternoon after a 1 hour break. The decision to complete the two recordings on the same day was after discussions with potential PD volunteers and their limited willingness to complete two separate visits. Furthermore, although the order of the visits could have been randomised, there would have been no blinding to the study due to the use of personal medication and the researcher’s interaction with the participant. Therefore, the two groups completed the paradigm twice on the same day, and the healthy control group was a control for each time-point.

The limitations of this study design became apparent after data collection as a high proportion of the EEG datasets needed to be excluded due to high levels of noise in the data. The reason is likely to be due to the length of the EEG cap being connected, and the reduced quality of EEG data in the aged population. In addition, there was a clear difference in both the PD and HC groups’ tolerance to the laser due to the repetition of the paradigm. For this reason, the morning ‘off’ and
afternoon ‘on’ sessions were analysed and discussed individually, without comparison between time-points. Therefore, Chapter 3 includes the main report of PD ‘off’ state, and Chapter 4 summarises the results of the ‘on’ state.

The protocol was based on the previous research carried out in The Human Pain Research Group within chronic pain groups and healthy volunteers (Brown, Elderedy and Jones, 2014; Brown et al., 2008a). The original protocol used a CO2 laser to induce acute pain to the forearm. The laser stimuli were preceded by a visual cue of Low, Mid, High or Unknown which informed the participant of the forthcoming intensity of the laser. The Unknown visual cue indicated that either intensity would be given and allowed investigation into the effect of certainty on pain perception. The visual cue was also concurrently presented with auditory beeps for an accurate 3 second countdown to the stimulus. The participants completed the paradigm whilst EEG recording was taken, and both sensor level and source localisation was used to analyse the data.

The original laser paradigm was slightly adjusted to be suitable for the PD patient group and the protocol was consistent between studies to allow for comparison. The number of conditions was reduced to be Low, High and Unknown, by removing the Mid intensity condition, to reduce the time needed for the PD participants to be sat still. The reduced time was also required to allow time to repeat the study once the PD patients had taken their PD medication. The three main conditions were sufficient for analysis as the inclusion of the Low and High conditions allowed for analysis on for the effect of expectation on brain activity, and the Unknown condition was used to establish an effect of certainty on brain activity and pain rating. The protocol is outlined in full within the papers of the thesis.

2.2.b. Chapter 5: Study 2: D2 Dopamine Study

The second study investigated the manipulation of striatal dopamine due to its strong links with pain and PD (discussed in section 1.4). Therefore, an agonist and antagonist of dopamine D2Rs were selected to study both high and low levels of dopamine transmission. The D2R was selected as the target of modulation due to
the high abundance in the striatum in comparison to the more widespread
distribution of other dopamine receptors. The use of D2 modulation via drugs has
been shown to be successful in numerous studies (Nandam et al., 2013; Mehta et
al., 2005; Becker et al., 2013). A D2R agonist and antagonist were selected via Dr
Grace Whitaker for their selectivity, safety and efficacy. The agonist Cabergoline
and the antagonist Amisulpride were selected. The in depth comparison between
other drugs and the reason for the final choice is reported in her thesis (Whitaker,
2017).

*Cabergoline* is often used as a Parkinson’s treatment and has one of the highest
binding affinity to D2Rs compared with other agonists (Gerlach et al., 2003). Also,*
*Cabergoline* has shown to be highly tolerable and safe to administer in patient
groups and experimental studies (Vilar et al., 2018; Webster et al., 1993; Gibson et
al., 2012; Nomoto et al., 1998).

*Amisulpride* is a selective antagonist of D2 receptors (subtypes D2 and D3) and
prescribed as an anti-psychotic for conditions such as schizophrenia (Silveira da
Mota Neto, Soares and Silva de Lima, 2002). *Amisulpride* has a good tolerability and
safety profile (Rosenzweig et al., 2002; Juruena, de Sena and de Oliveira, 2010),
however, *Amisulpride* been associated with prolongation of the QTc interval in an
electrocardiogram (ECG) (Wenzel-Seifert, Wittmann and Haen, 2011; Isbister et al.,
2010; Isbister and Page, 2013). The risk of QTc prolongation is low and will only
occur in combination with additional risk factors such as a pre-existing heart
condition or concurrent administration with other drugs (Wenzel-Seifert, Wittmann
and Haen, 2011). Therefore, all participants recruited to the study underwent an
ECG assessment to ensure that no underlying heart condition was present.

The D2R is a G-coupled receptor and activation induces an inhibitory response to
the post-synaptic neuron. The D2R is mainly located on non-dopaminergic neurons,
however, is also present on dopaminergic neurons and acts as an autoreceptor
which regulate the activity of the dopaminergic neurons (see Figure 2.3). The
activation of the autoreceptors causes a reduction in dopamine transmission and
dopamine production, and previous studies have demonstrated that low doses of
D2R drugs favours the activation of the autoreceptors. Therefore, due to the possible conflicting outcome of the activation of the autoreceptors (reducing dopamine transmission), rather than the desired activation of the post-synaptic receptors, the two drugs were administered at a relatively high dose. On the basis of previous research, the agonist, *Cabergoline* was given at 1.25mg (Nandam et al., 2013; Frank and O’Reilly, 2006), and the antagonist, *Amisulpride*, was given at 400mg (Rosenzweig et al., 2002).

The modulation of the dopamine levels was assessed via eye-blink rate (EBR) which has previously been positively correlated to striatal dopamine activity and associated with the D2R (Taylor et al., 1999; KARSON, 1983). The method to quantify the blink rate was via the ICA function within the EEGLAB toolbox in MATLAB. The upward and downward deflection, shape and topography of the blink was searched for within the resting state data. The blink-rate has been shown to reduce in Parkinson’s disease and is hypothesised to be due to the reduced dopaminergic activity within the nigrostriatal pathway. Therefore, the blink rate of each participant was calculated using the EEG recording during a period of resting.

In addition to the selection of the drugs, the recruitment of participants and health-screening was completed by Dr Whitaker. The running of the study was carried out by Dr Whitaker and myself in equal measure. All data presented in this thesis related to the D2 Dopamine study were collected and analysed by myself. The collaboration was organised due to the central role of dopamine in PD and the thus a suitable follow-on to the Parkinson’s study.

### 2.2.c. Chapter 6: Age comparison

The same protocol was completed in Study 1 (*PD v age-matched healthy controls*) and Study 2 (*D2 dopamine modulation in young healthy volunteers*). Therefore, a comparison was made between the brain activity from the older healthy controls in Study 1, and the young volunteers in the control condition in Study 2. The rationale to compare the two age-groups was that there is limited research in the effect of age on pain processing, especially top-down modulation.
Dopamine

D2 Autoreceptor

D2 Autoreceptor activation decreases dopamine release and synthesis.

Agonist

Decrease in dopamine release and decrease in postsynaptic D2R activation.

Antagonist

Increase in dopamine release and increase in postsynaptic D2R activation.

Presynaptic neuron

Postsynaptic Neuron

D2R activation inhibits postsynaptic activation.

Antagonist

Decrease in postsynaptic D2R activation despite presynaptic dopamine up-regulation.

Figure 2.3: A schematic diagram of the action of the dopamine D2 receptor and how low and high doses of agonists and antagonists have different effects on postsynaptic D2 receptor activity.
2.3. RECRUITMENT

2.3.a. Recruitment: Study 1: PD vs Healthy Controls

The recruitment of the PD group was carried out by Dr Monty Silverdale and Dr Christopher Kobylecki at their clinics held at Salford Royal NHS Foundation Trust. The study was introduced to people who could be temporarily withdrawn from their PD medication and pain relief on the study visit. Patients presenting a severe tremor or dystonia were not recruited to the study due to the EEG recording being sensitive to movement. The contact details of those interested in taking part were passed over to me for a follow-up phone-call. Approximately 50% of the patients who showed interest took part in the research.

The recruitment for the HC group was via the Citizen Scientist website and adverts within local golf clubs. The HC group were age-matched to the PD group. The recruitment of the partners of PD patients was avoided due to previous research demonstrating an altered pain perception within partners who had a caring role (Romano et al., 2000).

The PD study recruited age-matched controls to act as the health control group for the study. The HC group carried out the experiment twice to replicate the experience of the PD group completing the study off and on their medication. The HC also repeated the study and acted as a control for the ‘on’ condition of the PD group.

2.3.b. Recruitment: Study 2: D2 Dopamine Study

The recruitment of the Dopamine D2 Study was via the email announcements at The University of Manchester, and advert posters circulated in the University buildings. The age range was selected to be 18-35 years old to limit age-related changes which may affect the results. In addition, the criteria for the study required a healthy cohort and as such a younger age range was appropriate. Prior to selection for the study, the participants completed the Barratt impulsiveness scale (BIS-11 (provided by Dr Grace Whitaker) to establish their baseline level of striatal
dopamine as the (BIS-11) has previously been correlated with individual dopamine levels, specifically striatal dopamine (Costa et al., 2013; Buckholtz et al., 2010; Korponay et al., 2017). Final recruitment to the experiment was restricted to the participants who scored in the mid-range on the questionnaire as this indicated that their dopamine levels were also mid-range and within the peak of the inverted-u relationship of dopamine and performance (see figure 2.4). The inclusion of this assessment was aimed to reduce the range of the variability in baseline dopamine levels in the participants which may have affected the outcome of the D2R manipulation. A study by Cools et al (2009) demonstrated the importance of baseline dopamine and D2R manipulation. The study assessed the relationship between reward- and punishment-based learning and baseline dopamine level and concluded using PET imaging that participants’ with high striatal baseline dopamine performed better in the paradigm than those with low dopamine levels. They further highlighted that the baseline dopamine level affected the result of a D2R agonist on punishment driven outcomes; such that performance was improved by the agonist in participants’ with low baseline dopamine, whilst impairing performance in those with high baseline dopamine (Cools et al., 2009).

![Figure 2.4](image_url)

**Figure 2.4: The inverted-U relationship between dopamine level and performance.** There is an optimal range of dopamine level at the peak of the curve, and a low or high level of dopamine has been shown to impair performance in cognitive responses. The BIS-11 score of impulsivity has been correlated with baseline dopamine levels and was used as a screening technique to recruit participants with dopamine levels within the optimal range. This recruitment criterion aimed to limit the variability in the effect of the D2R manipulation on performance output.
Furthermore, the Dopamine D2R antagonist *Amisulpride* required pre-health screening due to it being dangerous for people with extended QTc intervals (Täubel et al., 2017). Therefore, an electrocardiogram (ECG) was performed on all potential volunteers by either Dr Monty Silverdale or Dr Christopher Kobylecki. All ECGs were assessed prior to recruitment to the study and if a volunteer showed abnormalities they would have been withdrawn from the study and their GP informed.

The study was designed to be repeated-measures and all participants completed the experiment three times over a six week period. Each visit was separated by 10-days to allow for the skin the recover between each laser session. The study was pseudo-randomised and double-blinded. The randomisation was pseudo-randomised so that near equivalent numbers were in each of the six possible orders of the drug conditions.

The benefit of a repeated-measures design is that there is less variability within condition groups and the highly subjective and individuality of pain perception would have increased the variability within a between-subject design. Therefore, a between-subject design would require a much higher n number to account for the high degree of variability.

After each study visit the participants were given specially made business cards with information about the study. The participants were advised to keep it on their person as it provided information to medical personnel if an emergency arose (Figure 2.5).
2. General Methods and Introduction to Study Aims

Figure 2.5: Business cards given to participants of the D2 dopamine study. The participants were instructed to carry the cards on them for 24 hours in the unlikely case that they needed medical attention and were not able to explain their involvement in the study.
Chapter 3

A neurophysiological investigation of pain anticipation in Parkinson’s disease

Sarah Martin, Anthony Jones, Christopher Brown, Christopher Kobylecki, Wael El-Deredy, Monty Silverdale
3.1. ABSTRACT

Chronic pain is common in people with Parkinson’s disease, and is often considered to be caused by the motor impairments associated with the disease. Altered top-down processing of pain characterises several chronic pain conditions and occurs when the cortex modifies nociceptive processing in the brain and spinal cord. This contrasts with bottom-up modulation of pain whereby nociceptive processing is modified on its way up to the brain. Although several studies have demonstrated altered bottom-up pain processing in Parkinson’s, the contribution of enhanced pain anticipation and enhanced top-down processing of pain has not been fully explored.

In the present study, EEG was recorded whilst noxious stimuli were delivered by a carbon dioxide laser to the forearm. Participants with Parkinson’s disease were compared with a Healthy Control group.

EEG source localisation reported an increased activation in the mid-cingulate cortex and supplementary motor area in the Parkinson’s disease group compared to the healthy control group during mid [-1500 -1000 ms] and late anticipation [-500 0 ms], indicating enhanced cortical activity before noxious stimulation. The Parkinson’s disease group was also more sensitive to the laser and required a lower energy level to induce pain.

This study provides evidence supporting the hypothesis that enhanced top-down processing of pain may contribute to the development of chronic pain in Parkinson’s. With further research to confirm these findings, our results inform a scientific rationale for novel treatment strategies of pain in Parkinson’s disease, including mindfulness, cognitive therapies and other approaches targeted at reducing top down processing of pain.
3.2. INTRODUCTION

3.2.a. Pain in Parkinson’s disease

Chronic pain is a highly prominent symptom in people with Parkinson’s disease (PwPD), yet there is a limited understanding of whether the pain is primarily a consequence of motor impairment, including muscle rigidity, or whether Parkinson’s disease (PD) causes a centrally produced heightened sensitivity to pain. Whilst the percentage of the general population living with chronic pain is approximately 20% (van Hecke, Torrance and Smith, 2013; Breivik et al., 2006), there is a significantly higher prevalence within the PD population of approximately two-thirds (Ozturk et al., 2016; Nègre-Pagès et al., 2008a; Skogar and Lokk, 2016; Silverdale et al., 2018). There is evidence that PwPD have lower threshold and tolerance of pain compared to age-matched healthy cohorts (Brefel-Courbon et al., 2005; Chaudhuri and Schapira, 2009; Djaldetti et al., 2004a; Schestatsky et al., 2007a), and EEG and functional imaging studies have demonstrated an altered central response to pain in PD (Tinazzi et al., 2009; Schestatsky et al., 2007b; Brefel-Courbon et al., 2005). These abnormalities provide strong evidence that altered central pain processing contributes to the development of pain in PD (Silverdale et al., 2018). Therefore, an increased understanding of the pathophysiological mechanisms causing the chronic pain is imperative to make an informed improvement in the treatment of pain in Parkinson’s.

3.2.b. Top down alteration in pain processing

Research into chronic pain has largely focused on bottom-up mechanisms amplifying the pain signal on its way from the peripheries to the brain (Reicherts et al., 2017; Watson et al., 2009). However, there is a developing field in the role of top-down modulation of pain, whereby the cortical activity modulates the incoming pain signal as it reaches the brain.

Previous research within our group has used EEG source localisation to investigate the anticipatory processing of painful stimuli. Our previous research has shown that the anticipation of pain leads to changes in the activity of the same brain regions
that subsequently respond to nociception (Brown and Jones, 2008; Brown, El-Deredy and Jones, 2014; Clark et al., 2008b; Brown et al., 2008a). Examples of regions which have been seen to activate during anticipation include the cingulate cortex, insula, primary and secondary somatosensory cortex (SI/SII), prefrontal cortex (PFC) and the periaqueductal gray (PAG) (Koyama et al., 2005; Babiloni et al., 2004). The degree of anticipation has been shown to be correlated with subsequent pain perception and the EEG laser-evoked potential (LEP) (Brown et al., 2008a). Hence, we have previously demonstrated that the cortex can modulate the perception of pain of the incoming pain signal via top-down modulation. Within chronic pain conditions, such as fibromyalgia and osteoarthritis, heightened anticipation within the insula cortex is correlated with the extent and severity of chronic pain symptoms (Brown, El-Deredy and Jones, 2014).

Unlike fMRI and PET, the higher temporal resolution of EEG allows for a more accurate recording of the anticipatory processes. Prior to a noxious stimulus, during anticipation, there is cortical activity from a network of regions including the cingulate cortex, basal ganglia and thalamic structures (Brunia and van Boxtel, 2001; Vogt, Finch and Olson, 1992). In this study we used EEG with source localisation analysis because of the temporal advantage over neuroimaging techniques reliant on slow haemodynamic responses. The accuracy and reliability of EEG source localisation has been verified by research which shows high similarity to other neuroimaging techniques such as PET (Christoph M. Michel, 2004; Lantz et al., 2003), fMRI (Mulert et al., 2004; Vitacco et al., 2002) and intracerebral recordings (Seeck et al., 1998; Trébuchon-Da Fonseca et al., 2005). EEG source localisation has reliably reported activations within the pain network during anticipation (Brown and Jones, 2012; Brown et al., 2008a; b). For instance, impaired processing within the parietal and frontal regions during anticipation has been reported using source localisation in patients with the chronic pain associated with Fibromyalgia and Osteoarthritis (Brown, El-Deredy and Jones, 2014).
3.2.c. Aim of study

Here we investigated anticipatory processing in PwPD in the “off” medication state in comparison to age-matched healthy volunteers. We used a CO\textsubscript{2} laser to induce acute noxious stimuli and monitored brain activity throughout via EEG. We hypothesised that the anticipatory phase would be abnormal in the PD group and would provide evidence that top-down mechanisms are key to explaining the mechanisms of chronic pain in PD.

3.3. METHODS

The study was approved by the local ethics committee and all participants gave informed consent according to the Declaration of Helsinki to participate in the study.

3.3.a. Participants

Twenty-four participants with Parkinson’s were recruited (sixteen males). PD patients were recruited via correspondence with their neurologist (MS or CK). Participants were screened to ensure safe withdrawal of medication for the study, and to exclude cases of severe tremors which might interfere with EEG recording. Clinically significant peripheral neuropathy was excluded by clinical examination and neuropathy scale. Symptom duration ranged from 1-month to 16 years, with a mean duration of 5.13 years. One PD participant was unable to complete the study due to severe symptoms after medication withdrawal. Twenty-three participants with PD were included for analysis of behavioural measures. Twenty PD participants were included for EEG analysis due to two datasets being removed because of noisy data and one for a low MoCA (Montreal Cognitive Assessment) score (12/30).

Twenty-five age-matched healthy controls (HCs) were recruited (fourteen males). One HC participant was unable to complete the study due to the laser failing to induce a sufficient pain level, and one HC dataset was removed due to noisy data. Twenty-four HCs were included in the behavioural measures and twenty-three HC
participants were included for EEG data analysis. The participants with PD [age range; 63.3 ± 8.27] were age matched to the HCs [age range; 65.5 ± 8.59].

3.3.b. Medication

The PD group omitted their evening medication and were studied in the practically-defined OFF state (after 12 hours withdrawal of anti-Parkinsonian medication). The motor section of the Movement Disorder Society Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) was completed to report the current severity of their parkinsonian disability off their medication.

3.3.c. Assessments

The participants were assessed for; PD motor severity, pain, mood and cognitive state. PD motor severity: The motor section (III) of the MDS-UPDRS was completed to evaluate the motor disability due to PD (Goetz et al., 2008). The assessment in the PD group was completed off their medication. Pain: All participants rated their current pain state via a number rating scale (NRS) prior to starting the laser protocol. The PD participants with chronic pain reported their minimum and maximum degree of pain over the last 6 months via a visual analogue scale (VAS). All participants completed the pain catastrophising scale (PCS) (Sullivan et al., 1995) to report their psychological coping ability when experiencing pain. Mood: All participants completed the Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983) to dissociate results from anxiety or depression. Cognitive state: The Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005) test was carried out to assess the participants’ cognitive ability. Participants with low scores (<25) were removed from the study to avoid cognitive decline affecting the EEG signal.

3.3.d. Experimental design

3.3.d.i. Pain stimuli

A CO₂ laser [50W Synrad 48-5 J-series (J-48-5(S)W) Wavelength: 10600nm] was used to deliver acute pain to the dorsal surface of the right forearm. The CO₂ laser
delivered a beam with a diameter of 15 mm and 150 ms duration. The voltage (V) of the laser is linearly related to the laser energy delivered to the forearm. For each test, the stimuli were delivered in an area measuring 4 x 5cm and was delivered in a predetermined randomised path (Brown et al., 2008a). This was to avoid habituation, sensitization, or skin damage.

3.3.d.ii. Psychophysics

Before starting the experimental protocol, psychophysics was used to calibrate the laser to the individual’s pain sensitivity. An ascending method of limits procedure was used, starting from 0.6 V with 0.06 increments each time. The participant used an eleven point NRS (0 - 10) to rate the intensity of the pain perceived for each stimulus and the following description was provided; 0=no sensation, 4=pain threshold, 7=moderately painful, 10=unbearably painful. The rating scale was introduced to the participant via these standardised descriptives to ensure that no explanation altered their interpretation of the scale. The procedure was repeated three times to allow participants to get used to the laser and was used to calculate the average voltage to induce level 4 (low) and level 7 (moderate) pain. These two levels provided ‘low’ and ‘high’ stimuli intensity for the main laser experiment protocol.

3.3.d.iii. Main experiment

The participants received 120 laser stimuli at the two intensities (low and high) separated into four conditions; Low (level 4), High (level 7), Unknown Low (level 4) and Unknown High (level 7). To investigate the anticipation of a painful stimulus, a 3 second auditory countdown preceded the laser stimuli (see Figure 3.1). The first auditory cue was presented concurrently with an anticipatory cue to indicate the forthcoming laser stimuli and to maintain attention. The participant was either shown ‘Low’, ‘High’ or ‘Unknown’. The presentation of the word ‘Unknown’ indicated that the laser stimulus has an equal chance of being low or high. This was to investigate the importance of certainty in the anticipation of the laser stimuli. The image was also used as a visual fixation cue to discourage eye movements. After the laser stimuli, the 0-10 numerical rating scale was shown on the screen and
the participant rated the intensity of the pain. The order of the stimuli was randomised and separated into three blocks with short breaks in-between.

![Figure 3.1: A schematic diagram of a single trial of the experimental paradigm. A computer monitor showed the participant a visual cue of: Low, High or Unknown from -3s to +2s. A three second countdown of beeps at -3, -2 and -1 allowed accurate anticipation of the laser stimuli at time 0s (red bar). The presentation of the visual cue at -3s was concurrent with the first auditory cue. The visual cue was consistent throughout the anticipation and laser stimulus. At +2s, an eleven-point number rating scale (NRS) was presented for the participant to rate the laser stimulus. For each condition (Low, High, Unknown Low and Unknown High) there were thirty trials. The trials were presented in a randomised order and divided into three blocks of forty trials.](image)

3.3.d.iv. EEG recording:

A BrainVision MR EEG cap was used to record from 63 scalp electrodes using a BrainVision-cap system [Standard BrainCap-MR with Multitrodes]. The arrangement of the electrodes was modelled on the extended 10-20 system. Recording parameters were set at: Filter (DC to 70 Hz), Sampling rate (1000 Hz), Gain (500). To reduce electrical interference, a 50Hz notch filter was applied. Prior to starting the laser protocol, resting states were recorded with eyes open and closed for two minutes in all participants. This ensured that the experience prior to the experiment was identical. The three experimental blocks were recorded separately to allow for better artefact rejection of the EEG data.
3.3.e. Analysis Methods

3.3.e.i. Statistical analysis of behavioural data

Statistical analyses of the behavioural measures were carried out using IBM SPSS Statistics 22 software. The questionnaires were all investigated for significant group differences. Prior to using statistical tests, the data was assessed for normality using a combination of Q-Q plots, histograms, and the values of skew and kurtosis. Normally distributed data was analysed using independent t-tests and ANOVA tests, whilst data reported to be not normally distributed, the appropriate non-parametric test was utilised. Specific statistical tests for each analysis step are reported in the results section.

3.3.e.ii. EEG analysis method

EEG pre-processing was carried out using EEGLAB toolbox (Delorme and Makeig, 2004) in MATLAB version R2015a (The Mathworks Inc) whilst statistical analysis was carried out using SPM12 toolbox (Wellcome Department of Imaging Neuroscience, Institute of Neurology, UCL, London, United Kingdom) running in MATLAB. The EEG data was pre-processed for scalp and source localisation analysis. The main motivation of the analysis was to establish the anatomical origin of the brain activity using (Low Resolution Electromagnetic Tomography) LORETA source localisation.

3.3.e.iii. EEGLAB Pre-processing

Pre-processing consisted of; removal and interpolation of bad channels, down-sample to 500, low-pass filter of 20Hz and re-reference to the common average. The four conditions were separated and -3500ms to 2000ms epochs extracted and Linear detrend applied. Independent Component Analysis (ICA) was carried out on all datasets using the SemiAutomatic Selection of Independent Components for Artifact correction (SASICA) toolbox to select components to remove via pre-determined thresholds. The thresholds were set to; Autocorrelation (threshold = 0.35 r, lag = 20 ms), Focal (threshold = 3.5 z), Focal trial (threshold 5.5 z), Signal to noise (period of interest (POI) = [0 Inf], baseline (BL) [-Inf 0], threshold ratio = 0.5),
and Adjust Selection enabled. The thresholds were sufficient to remove artefacts from the majority of the datasets; however, a number of datasets required further manual removal of eye-blink components where not picked up by SASICA.

### 3.3.e.iv. SPM EEG Analysis

The pre-processed datasets were converted to Statistical Parametric Mapping (SPM) compatible files. Statistical analysis was carried out to investigate the anticipation evoked potentials and the post-stimulus LEPs using scalp-level and source localisation analysis techniques available in the SPM toolbox.

SPM scripts for batch processing were used to analyse the EEG data at the scalp level and source localisation. Two baselining methods were applied to the data analysis for the anticipation phase and were applied for scalp-level and source localisation analysis (see 2.1.b.ii). Primary analysis applied distinct baselines (BLs) of 500 ms, occurring prior to each of the three auditory cues respectively, to analyse each of the three anticipation phases. The BLs and time window of interest (TWOI) were as follows; Early [BL: -3500 ms -3000 ms: TWOI: -2500 ms -2000 ms], Mid [BL: -2500 ms -2000 ms: TWOI: -1500 -1000 ms] and Late [BL: -1500 -1000 ms: TWOI: -500 0 ms] anticipation phases. The secondary analysis method applied a single baseline of 500 ms prior to the first auditory cue [BL: -3500 ms -3000 ms] that was common to every TWOI anticipation phase (early, mid and late). This second analysis was conducted to enable comparison to previous studies (Brown et al., 2008a; Brown, El-Deredy and Jones, 2014). All statistical analysis for the anticipation phase was adjusted for multiple comparisons.

The SPM whole-head analysis for the post-stimulus phase TWOI [200 ms 600 ms], centred on the LEP, was baseline corrected to -500 ms prior to the laser stimulus [BL: -500 ms 0 ms].

Two further analysis steps were carried out to investigate the LEP further. Firstly, the N2 and P2 components of the LEP were calculated for the Cz electrode at scalp level (chosen for showing highest amplitude during LEP). The time window was restricted to 0 600 ms and the amplitude and latency of the most negative (N2-
peak) and positive (P2-peak) peaks were extracted for statistical analysis. All laser conditions were averaged. The values extracted for the N2-peak was restricted to be prior to the P2-peak due to the N2 peak occurring prior to the P2 peak.

Secondly, a focussed analysis of the P2 peak using source localisation and individual time windows was carried out. A time window of 80ms centred on each participant’s P2 peak latency was used to calculate source estimates, and was assessed for group differences.

We also calculated the SD (Standard Deviation) of the EEG potential over trials for every time sample across the whole-epoch [-3500 1500 ms] and compared the results between the two groups to evaluate for possible differences in variability of the data over trials. This was to test whether any group differences found in ERP amplitudes and sources from the main analyses might have resulted from differences in data variability; such variability can arise from noise in the EEG signal (including motion and other artefact) rather than from neural signals. Such noise was expected to be greater in the PD group and therefore required assessing in order to interpret the results.

3.3.e.v. Source Localisation analysis parameters

SPM12 EEG and MATLAB scripts were used to estimate the sources of the anticipation- and laser-evoked potentials using LORETA. The forward model was created using an 8196 vertex template cortical mesh coregistered to the electrode positions of the standard 10-20 EEG system. A three-shell boundary element model (BEM) EEG head model available in SPM12 was used to compute the forward-model. The images were smoothed with a 12mm full-width-at-half-maximum (FWHM).

3.3.e.vi. EEG Analysis Statistical analysis

For the analysis of the anticipatory TWOIs, a three way repeated measures ANOVA was applied, with one between-subject factor of Group (HC vs PD) and two within-subject factors of Certainty [Known (Low/High) v Unknown] and Expectation [Low v High (Known)]. For the analysis of the post-stimuli TWOI, a three way repeated
measures ANOVA was applied, with one between-subject factor of Group (HC v PD) and two within-subject factors of Certainty [Known (Low/High) v Unknown] and Intensity [Low v High (Known and Unknown)].

Source localisation results were reported as follows. To control for multiple comparisons, a cluster-forming threshold of p<0.001 was used and resulting clusters were considered significant at FWE (p<0.05). Significant clusters were also restricted to >100 voxels in size and regions labelled using the Anatomical Automatic Labelling (AAL2) toolbox in SPM. We also extracted the eigenvariate data from source estimates to investigate possible correlations with behavioural measures including MDS-UPDRS (PD group only), HADS, PCS, chronic pain maximum VAS score and laser voltage for high pain (V). The p value was adjusted for multiple comparisons, p<0.01.
3.4. RESULTS

3.4.a. Behavioural Results

3.4.a.i. Questionnaires

Group comparisons were carried out using an independent t-test between questionnaire scores and are reported in Table 3.1. There was a significant difference reported in the PCS, such that the PD group reported a higher level of catastrophising behaviours towards pain compared to the HC group. In contrast, there was no significant difference seen in the HADS, although a trend is seen towards higher scores in the PD group compared to the HC group. The OFF MDS-UPDRS-III motor score recorded in the PD group ranged from 15 to 78, with a mean of 38.3±16.56. For reference, a high MDS-UPDRS-III score indicates more severe movement impairments.

Table 3.1: The table displays the reported measures for the assessments for the PD and HC groups. *Levene’s Test for Equality of variance p<0.05 hence equal variances not assumed and significance adjusted. * = Significant result p<0.05. PD: Parkinson’s disease, HC: Healthy Control, PCS: Pain Catastrophising Scale, HADS: Hospital Anxiety and Depression Scale, SD: Standard deviation.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Significance (2-tailed T-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCS</td>
<td>PD</td>
<td>23</td>
<td>11.59</td>
<td>10.85</td>
<td>0.019*</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>24</td>
<td>5.29</td>
<td>5.67</td>
<td></td>
</tr>
<tr>
<td>HADS</td>
<td>PD</td>
<td>23</td>
<td>13.83</td>
<td>9.89</td>
<td>0.070</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>24</td>
<td>9.21</td>
<td>6.76</td>
<td></td>
</tr>
</tbody>
</table>

3.4.a.ii. Laser behavioural results

The psychophysics ramping procedure was used to calculate the participants’ individual energy level required to induce a High pain score. A Welch t-test was run to determine if there were differences between the HC and PD groups in the energy required to induce a High pain score due to the assumption of homogeneity of variances being violated, as assessed by Levene’s test for equality of variances (p =
There was a statistically significant difference in energy required to induce a High pain between HC and PD (Figure 3.2), with PD requiring a lower energy level (1.99±0.44 V) compared to HC (2.20±0.25 V), (95% CI, 0.003 to 0.432), t(34.75) = 2.060, p = 0.047.

Figure 3.2: The energy of the laser required to induce high (level 7) pain. The PD group required a lower energy level compared to the HC group to induce the equivalent pain. The blue and red boxes show the median (bold horizontal line) (HC: 2.32V, PD: 2.24V), lower and upper quartiles. The vertical lines show the minimum (HC: 1.68V, PD: 1.30V) and maximum (HC: 2.50V, PD: 2.48V) values. Laser voltage was delivered from 0.8V to a maximum of 2.5V in increments of 0.06V. * = p<0.05. HC: Healthy Control, PD: Parkinson’s disease.

The mean and standard error (SE) of the pain rating scores for all conditions were calculated; Low (PD: 2.54±0.23, HC: 2.0±0.18), High (PD: 4.94±0.30, HC: 4.7±0.26), Unknown Low (PD: 3.06±0.25, HC: 2.4±0.22) and Unknown High (PD 4.53±0.33, HC: 4.1±0.26). The pain rating scores for the laser stimuli during the main experiment were investigated using a three-way mixed ANOVA with a between-subject factor of group, and within-subject factors of certainty (Known v Unknown) and Intensity.
3. A neurophysiological investigation of pain anticipation in Parkinson’s disease

(Low v High). The data met homogeneity threshold (p>0.05) calculated by the Levene’s Test of Equality of Variances. There was no effect of group F(1, 43)=2.347, p = 0.133, η² = 0.52. There was an effect of intensity F(1, 43)=290.992, p=0.000, η² = 0.871, yet no effect of certainty F(1, 43)=0.006, p= 0.937, η² = 0.000. However, there was a significant two-way interaction between certainty and intensity F(1, 43)=59.188, p=0.000, η² = 0.579. A follow-up paired t-test comparing the difference between known and unknown for Low and High pain rating reported a significant result; T(1, 43)= -7.820, p<0.000, d=42, such that the pain ratings of unknown low were higher (+0.52) compared to known low, whilst ratings of unknown high were lower (-0.46) compared to known high. This result acts as a manipulation check that demonstrates that the experiment was successful in inducing expectations (via anticipation cues) that modulated pain in the expected direction as seen in previous research (Brown et al., 2008b). There was no difference in the effect of intensity or certainty on pain ratings between the two groups (HC v PD), nor was there any interaction between intensity, certainty and group.

3.4.b. EEG Results

3.4.b.i. LEP component analysis

The N2 and P2 components of the LEP at the Cz electrode were assessed for differences between the two groups using independent t-tests. Outliers were assessed via boxplot function in SPSS and one data point was excluded from the statistical analysis due to being more than 1.5 box-lengths away from the box edge. The statistical analysis concluded that there were no significant differences between the HC and PD groups in the amplitude or latency of the N2 and P2 peaks (see Figure 3.3).
Figure 3.3: Graphs showing the information of the N2 and P2 components of the laser-evoked potential (LEP). The N2 peak was deemed to be the most negative amplitude prior to the P2 peak latency and within the post-stimulus time-window. The P2 peak was the most positive amplitude within the time window of [0 600ms]. A) and B) show the amplitude of the N2 and P2 peaks respectively, whilst C) and D) show the latency of the N2 and P2 peaks respectively. All data is presented as mean and error bars of standard deviation. Statistical analysis to assess group differences between HC and PD, reported no significant differences.

3.4.b.ii. Scalp-level Whole head analysis

SPM whole-head statistical analysis of the early [-2500 -2000 ms], mid [-1500 -1000 ms] and late [-500 0 ms] anticipatory phases, reported no significant difference of group, certainty or expectation. This was true for both baseline methods (see Methods section). Similarly, the post-stimulus TWIOIs [200 600 ms] reported no significant group or certainty differences. The intensity factor in the post-stimulus
TWOI reported increased activity in high pain condition compared to low pain (SPM results reported in Supplementary materials Table 3.3).

The anticipatory response over time was calculated for each group and is displayed as topography plots in Figure 3.4. The difference between HC and PD group was also determined by subtracting the HC amplitudes from the PD data. Despite the SPM scalp-level analysis not reporting a significant difference, an anticipation response is seen in the central scalp region which is more prominent in PD than HC. Because scalp responses are a summation of deeper brain events from different sources, the scalp-level analysis was hence followed up by source localisation analysis.

Figure 3.4: Topography plots showing the average response for the mid- and late-anticipation time windows for HC and PD. Mid-anticipation is baseline corrected to [-2500 -2000 ms] and late-anticipation of [-1500 -1000]. The difference between the PD and the HC group was calculated via subtracting the HC data from the PD data. The same scale has been used for all topography plots. In the group difference plot, the red highlights a more positive response in the PD participants, whilst blue indicates a more negative amplitude in PD.
3.4.b.iii. Source EEG analysis

Source localisation analysis using the distinct baselines prior to the auditory cues revealed that the PD group showed a higher degree of activity compared to the HC group during mid and late anticipation phases (see Table 3.2). In addition, a trend was also seen in the early anticipation phase. Cluster-level statistics which showed a trend within regions previously associated with pain anticipation including the hippocampus (Reicherts et al., 2017), the postcentral gyrus (Yang, Jackson and Huang, 2016), and the cingulate cortex (Shackman et al., 2011) and so these were also reported at the uncorrected p value, 0.001. Figure 3.5, shows the significant clusters which are significant F-contrasts after FWE and adjustment of the p value (p<0.025) due to multiple analysis techniques. The clusters are denoted with ‘◊’ in Table 3.2 and were used for correlation analysis with behavioural parameters.
Table 3.2: Group effect on sources of early, mid and late anticipation-evoked responses. A threshold of clusters >100 voxels was set and results were restricted to FWE correction and the p value adjusted for multiple comparisons (p<0.025). Results labelled with ‘◊’ are used for figure 3.5 and correlation analysis. Results showing trend are reported and signified with †, and the uncorrected value (0.001) reported below. The Anatomical Automatic Labelling (AAL2) atlas was used to report the regions within the significant cluster for the group effect. The region with the highest percentage overlap is shown, unless an equivalent share of percentage overlap was seen. A label of ‘Unknown’ was not reported. The full report of all of the percentage overlaps can be seen in the Supplementary Materials Table 3.5.

<table>
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<tr>
<th>Group Difference</th>
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<th>Peak-Level</th>
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Figure 3.5: Source estimates for significant F-contrasts at FWE correction using MRICroN software. Red represents the clusters of increased activity in PD compared to HC. A) Shows the increased activation in the PD group compared to the HC group within the mid-anticipation phase: i) Clusters are within the Right Supplementary Motor Area and Right Mid Cingulate Cortex. ii) Clusters are within the Left Mid Cingulate Cortex and Left Supplementary Motor Area. B) Shows the increased activation within the PD group compared to the HC group during the late anticipatory phase: i) Clusters are within the bilateral Mid Cingulate and Precuneus. The image is aligned by the peak-voxel at peak-level and reported as x y z (mm).

The certainty (Known v Unknown) contrast reported a trend within the early anticipatory TWOI, [(F-contrast: p = 0.053, F=19.87, $k_E = 520$), (T-contrast: Unknown>Known: p = 0.013, T= 4.32, $k_E = 967$)], with peak voxel at x:48 y:16 z:-16 (mm). The T-contrast indicated an increased activity within the cluster in the unknown condition in contrast to the known condition. The cluster is within the right superior temporal pole (37.5%), right mid temporal pole (18.3%), right superior temporal (14.6%) and the right insula (3.7%). Contrasts for expectation
(Low v High) during anticipation, and certainty (Known v Unknown) within the post-stimulus TWOI, showed no differences in source estimates.

During the post-stimuli TWOI there was a significant difference in the intensity, such that a greater response was seen in the High intensity condition compared to Low, (see supplementary materials Table 3.4). The additional source localisation analysis of the P2 component which applied individual time-windows centred on the P2-peak latency, reported no group differences.

3.4.b.iv. Source correlations with behavioural measures

The eigenvariate data from the source estimates (denoted with ‘◊’ in Table 3.2) for the mid and late anticipation group effects were extracted for correlation analysis. Spearman rank correlation of mid-anticipation clusters (A: i & ii) and the late anticipation cluster (B: i) were investigated for correlation with MDS-UPDRS motor score (PD only), HADS, PCS, maximum chronic VAS score and the energy of the laser. The P value was adjusted to account for multiple comparison and no significant correlations were highlighted.

3.4.b.v. Standard Deviation analysis

The analysis of the SD-over-trials across the whole epoch at sensor-level EEG revealed no significant differences between the HC and PD groups, nor within-subject contrasts, certainty or expectation.
3.5. DISCUSSION

In the present study, we used EEG source localisation techniques to investigate the pain processing in PwPD in the practically-defined OFF state (after 12 hours withdrawal of anti-Parkinsonian medication). There were three main findings. Firstly, the PD group were more sensitive to the laser and required a lower energy level to evoke painful stimuli. Secondly, EEG source localisation showed that the PD group had a heightened anticipatory signal during the anticipation phase prior to acute heat pain. And finally, the heightened anticipatory response was independent of PD motor severity (MDS-UPDRS), mood (HADS), pain coping mechanisms (PCS) and severity of chronic pain (max chronic VAS score). This present study provides evidence to support the hypothesis that there is an augmented top-down processing during the anticipation of pain in PwPD which may help us to understand the high prevalence of pain in PwPD.

3.5.a. Altered Top-Down control

The amplified anticipatory signal seen in the PD group is evidence of altered top-down modulation within the pain network prior to the pain signal reaching the brain. This opens the possibility that the heightened pain sensitivity in PD is not solely due to impaired peripheral, bottom-up, factors, but may also be due to the modulation within the pain network of the brain.

The significance of altered top-down processing is well established in the pain field: different attentional states can directly and substantially alter pain intensity and unpleasantness (Miron, Duncan and Bushnell, 1989b); anxious anticipation of aversive stimuli activates regions of the pain network, resulting in altered perception of the stimuli (Reicherts et al., 2017). Furthermore, the degree of anticipation of a noxious stimulus has been shown to directly correlate with the perceived stimuli intensity (Pfingsten et al., 2001; Fairhurst et al., 2007), and abnormal anticipation of a painful stimulus has been shown in a number of chronic pain conditions such as Fibromyalgia (Burgmer et al., 2011; González-Roldán et al., 2016; Brown, El-Deredy and Jones, 2014).
Here we have shown increased activation in the PD group within the pain network, including, the Mid-cingulate cortex (MCC), Supplementary Motor Area (SMA) and Precuneus. Importantly, the group effects seen during anticipation were independent of the MDS-UPDRS, PCS and HADS emphasising that it is likely that the underlying PD pathological process is causing the greater anticipatory activity rather than the consequence of motor impairment, coping mechanisms and mood state. The fact that the abnormal processing is independent of the MDS-UPDRS supports the hypothesis reported in Silverdale et al., (2018) that the high prevalence of chronic pain in PD is independent of the severity of the motor impairments and could be due to top-down modulation of the pain signal.

Our results are consistent with a single previous fMRI study, using a very different protocol which demonstrated that PwPD showed a significantly reduced activation within the inhibitory descending pain pathway during the anticipation of pain and an increased activation within the MCC during pain perception (Forkmann et al., 2017). In combination with the results presented in this current study, we reason that PwPD display maladaptive top-down modulation during pain processing which may help to explain the high prevalence of pain within the PD community.

3.5.b. Regions activated during the anticipatory response

The location of the amplified anticipatory signal within the PD group during mid and late anticipation was mainly, but not limited to, the SMA and the MCC. The SMA and MCC have a role in the selection and planning of movements (Russo et al., 2002; Morecraft and Van Hoesen, 1998; Rushworth et al., 2004), and are indicated to be involved in the prediction of aversive events and the movement response required (Morrison, Peelen and Downing, 2007). The SMA and the MCC have shown overlapping functional connectivity during pain-processing and motor control (Misra and Coombes, 2015), and enhanced activation within the SMA has been associated with patients with phantom limb pain (Dettmers et al., 2001).

The MCC is a region involved in cognitive processing and is associated with the fear response, pain processing and specific motor outcomes to painful stimuli (Vogt,
A neurophysiological investigation of pain anticipation in Parkinson’s disease

Berger and Derbyshire, 2003; Shackman et al., 2011; Böcker et al., 2001). The MCC has previously been shown by Böcker et al., (2001) to be activated during anticipation of threats and suggested to be the source of generating the slow-wave anticipatory response (see figure 3.6.

Figure 3.6: Diagram from Böcker et al., (2001) which reports the grand average of the stimulus preceding negativity associated with anticipating threatening stimuli. The data is representative of eleven participants and show conclusions drawn from a 1-dipole source model. Böcker et al., (2001) concluded that the dipoles clustered within the posterior of the medial frontal cortex, and show high similarity with loci of the LORETA source localisation results shown in this study. Thereby validates the role of the MCC in the anticipation of noxious stimuli.

Abnormal connectivity within the MCC is associated with patients with migraines (Hubbard et al., 2014) and a heightened response within the MCC has been reported in PwPD during pain perception (Forkmann et al., 2017). In addition, in an extensive rodent study, the MCC showed to be central to the development of pain hypersensitivity in the absence of peripheral noxious stimuli (Tan et al., 2017). The study also demonstrated that silencing the MCC via optogenetic techniques, partially reversed inflammatory hypersensitivity, thus highlighting the role of MCC in development of chronic pain conditions (Tan et al., 2017).
Additionally, the MCC is also associated with reward processing (Hayden et al., 2008; Pearson et al., 2009; Shackman et al., 2011), a process highly dependent on dopaminergic signalling and hence may be important due to the depletion of dopamine seen in PD. The reward pathway is linked to pain perception as it encodes the inverse of a reward signal and is closely correlated to the opioid system. Abnormal reward processing is a possible predictor of the development of increased pain sensitivity (Nees and Becker, 2017; Nees et al., 2017), and provides a potential explanation of why abnormalities in the MCC during pain perception are present in PwPD.

Thus, the MCC and SMA are important for the processing of the pain signal and provide a putative anatomical substrate for enhanced top-down modulation of incoming pain signals.

3.5.c. Laser Evoked Potential

The LEP was not significantly different between the two groups. The subjective experience of pain was standardised in both groups, such that participants experienced what they subjectively considered to be a low and high pain. We therefore did not expect a group difference in the LEP.

Although research has previously shown that an altered degree of anticipation can modulate the LEP amplitude and pain unpleasantness, there is research by Clark et al., (2008) which reported that the characteristics of the LEP, specifically the P2 peak, was not affected by the duration of the anticipation period, nor correlated to the behavioural pain ratings. They concluded that the anticipatory response is more closely aligned with attentional processing rather than intensity coding of the stimulus. Hence the research is a potential explanation of how a difference during the anticipatory period was reported, yet not the LEP.

3.5.d. Considerations of interpretation

The present study has used source localisation to successfully quantify the anatomical origin of the activity recorded at scalp electrodes. These differences were not directly correlated to significant changes at scalp-level EEG and thus needs
to be considered when interpreting these results. It is known that there is no simple relationship between scalp and source activity, as a single electrode summates the potentials generated within neural sources and is dependent on the combination of the orientation, strengths and location of these potentials. Hence, as scalp topographies are the product of the addition of multiple brain sources, it is difficult to interpret them directly in relation to source localisation estimates. The comparison of SD-over-trials between the groups did not report any differences, and indicates that group differences in data variability (e.g. due to noise) is unlikely to be a factor driving group effects at the source level. Hence we are confident that despite not seeing scalp-level group differences, the significant differences reported via source localisation are valid.

3.5.e. Clinical impact

The current perception of pain in PwPD is often considered to be a consequence of peripheral symptoms such as rigidity and stooped posture. However, our findings suggest the possibility that the treatment for chronic pain in PD could beneficially incorporate alternative treatments such as mindfulness and cognitive behavioural therapy (CBT). These treatments have been shown to reduce pain anticipation (Brown and Jones, 2010), improve cognitive control of pain over time and reduce the severity of chronic pain (Kabat-Zinn, Lipworth and Burney, 1985; Kabat-Zinn, 1982). Nevertheless, further investigations are essential to establish whether cognitive therapies are a suitable treatment for pain in Parkinson’s disease.

3.6. CONCLUSION

In conclusion, Parkinson’s patients demonstrated enhanced anticipatory activity in the pain network before an acute pain stimulus. Thus we have provided evidence for enhanced top-down processing of pain in Parkinson’s disease, increasing the evidence for abnormal central processing in this and other chronic pain conditions. Our results contribute to the building knowledge of the relationship between chronic pain and Parkinson’s disease; and inform a possible scientific rational for novel treatment strategies in Parkinson’s pain, including mindfulness, cognitive therapies and other treatments targeted at reducing top down processing of pain.
### 3.7. SUPPLEMENTARY MATERIALS

Table 3.3: Scalp-level SPM results for the F-contrast of Intensity (Low v High) for post-stimulus TWOI [200 600]. Significant results were restricted to clusters of >100 voxels and significant following FWE-correction. During the TWOI centred on the laser evoked potential (LEP) the data shows an increased activation in the High condition compared to the low condition. Results are presented at cluster- and peak-level, plus the coordinates of the peak-voxel within the cluster.

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Table 3.4: The source localisation results for the significant within-subject contrast of Intensity (Low v High) during the post-stimulus time window [200 600 ms]. A threshold of clusters >100 voxels was set and results were restricted to FWE correction. The Anatomical Automatic Labelling (AAL2) atlas was used to report the regions within the significant cluster for the group effect. All regions are reported including ‘Unknown’. The T-contrast shows that High intensity evoked a higher activation in comparison to Low intensity.

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### Table 3.5: A detailed version of Table 3.2 to include all regions highlighted by the AAL2 toolbox.

The table displays the group effect on sources of early, mid and late anticipation-evoked responses. A threshold of clusters >100 voxels was set and results were restricted to FWE correction and the p value adjusted for multiple comparisons (p<0.025). Results labelled with ‘◊’ are used for figure 3.5 and correlation analysis. Results showing a trend are reported and signified with †, and the uncorrected value (0.001) reported below. The Anatomical Automatic Labelling (AAL2) atlas was used to report the regions within the significant cluster for the group effect. All regions are reported including ‘Unknown’. L = Left, R = Right, Sup = Superior, Inf = Inferior.

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Chapter 4

Central pain processing in people with Parkinson’s disease whilst on medication

Sarah Martin, Anthony Jones, Christopher Brown, Christopher Kobylecki, Wael El-Deredy, Monty Silverdale
4. Central pain processing in people with Parkinson’s disease whilst on medication

4.1. ABSTRACT

The administration of dopaminergic medication for Parkinson’s disease has previously been shown to have analgesic qualities. Here we investigated the pain processing of people with Parkinson’s disease whilst on their medication via EEG recording and receiving acute noxious stimuli. We have previously shown that in the same participants, whilst off their medication, there was a significant amplification of the anticipatory processing within the mid-cingulate cortex compared to healthy age-matched volunteers. This study reported that following medication administration, the people with Parkinson’s disease remained significantly more sensitive to the noxious stimulus in comparison to a healthy cohort. However, no difference was seen in the brain activations between the PD and healthy groups post medication administration. Therefore, in the PD medication normalised the augmented anticipatory processing that was reported in the patient cohort whilst off their medication.

4.2. INTRODUCTION

There is a higher prevalence of chronic pain in Parkinson’s disease (PD) than the general population (Nègre-Pagès et al., 2008; Ozturk et al., 2016; Skogar & Lokk, 2016; Silverdale et al., 2018). Pain is often considered to be secondary to muscle stiffness and reduced movement characteristic to PD, however, there is growing evidence that altered brain activity during pain processing contributes to the development of chronic pain.

The severity of pain in Parkinson’s has been shown to be worse in the ‘off’ state, when medication is wearing off, and improved after taking medication (Skogar and Lokk, 2016). Hence, the stabilisation of the dopamine levels following Parkinson’s medication may regulate the dopamine-dependent processes involved in pain processing. L-DOPA, a commonly used drug in PD, has been associated with analgesic qualities (Shimizu et al., 2004a; Kernbaum and Hauchecorne, 1981; Ertas et al., 1998; Nixon, 1975; Minton, n.d.; Sandyk et al., 1987; Cotzias et al., 1970). The
role of dopamine in the brain is widespread and contributes to the affective and attentional processing in pain (de Wied and Verbaten, 2001; Tiemann et al., 2014). Dopamine has also shown to have analgesic properties (Wood, 2014) and is considered to be a potential treatment of chronic pain conditions (Lawson, 2016; Haddad et al., 2018).

Hence, we aim to investigate the processing of acute pain in people with Parkinson’s disease (PwPD) whilst on their medication in comparison to healthy age-matched controls. This investigation follows the report that PwPD whilst off their medication were more sensitive to the noxious stimuli and showed augmented anticipatory processing prior to acute pain (see Chapter 3). On the basis that dopamine has shown analgesic qualities, we hypothesised that the Parkinson’s medication, which increases dopamine, would reduce pain sensitivity and normalise the amplified central processing seen whilst off their medication.

4.3. METHODS

4.3.i. Participants

Nineteen PD participants and twenty-one HC participants repeated the experiment outlined in Chapter 3. However, within the PD cohort, five datasets were removed due to noisy EEG data, and within the HC cohort, two datasets were removed due to habituation to the laser, and eight datasets removed due to noisy EEG data. The high degree of noise seen in the EEG was likely to be due to the EEG cap being kept on for a long time. Therefore, fourteen PD participants and eleven HC participants were included in the final analysis.

4.3.ii. Medication

The PD group were studied in their ON state after taking their medication at least 1 hour prior to experimental assessment. All participants were prescribed their usual levodopa medication which increases dopamine activity.
4.3.ii. Assessments

The motor section (III) of the Movement Disorder Society Unified Parkinson’s Rating Scale (MDS-UPDRS) was completed to assess the PD participants’ symptom severity whilst on their medication (Goetz et al., 2008). Other assessments also carried out were the Hospital Anxiety and Depression HADS, Pain Catastrophizing Scale (PCS), Montreal Cognitive Assessment (MoCA) and PD participants used a visual analogue scale (VAS) to report their minimum and maximum intensity of chronic pain over the last 6 months.

4.3.iii. Experimental design

4.3.iii.a. Pain stimuli

A CO₂ laser [50W Synrad 48-5 J-series (J-48-5(S)W) Wavelength: 10600nm] was used to deliver acute pain to the dorsal surface of the right forearm. The CO₂ laser delivered a beam with a diameter of 15 mm and 150 ms duration. The voltage (V) of the laser is linearly related to the laser energy delivered to the forearm. For each test, the stimuli were delivered in an area measuring 4 x 5cm and were delivered in a predetermined randomised path (Brown et al., 2008a). This was to avoid habituation, sensitization, or skin damage.

4.3.iii.b. Psychophysics

Before starting the experimental protocol, psychophysics was used to calibrate the laser to the individual’s pain sensitivity. The procedure was carried out 1 hour post medication administration. An ascending method of limits procedure was used, starting from 0.6 V with 0.06 increments each time the laser was delivered. The participant used an eleven point NRS (0 -10) to rate the intensity of the pain perceived for each stimuli and the following description was provided; 0=no sensation, 4=pain threshold, 7=moderately painful, 10=unbearably painful. The rating scale was introduced to the participant via these standardised descriptives to ensure that no explanation altered their interpretation of the scale. The procedure was repeated three times to allow participants to get used to the laser and was
used to calculate the average voltage to induce level 4 (just painful) and level 7 (moderate pain). These two levels provided low and high stimuli intensity for the main laser experimental protocol.

4.3.iii.c. Main experiment

The participants received 120 laser stimuli at the two intensities (low and high) separated into four conditions; Low (level 4), High (level 7), Unknown Low (level 4) and Unknown High (level 7). The unknown condition was to investigate the effect of certain or uncertain anticipatory cues on neural activity and pain perception. To investigate the anticipation of a painful stimulus, a 3 second auditory countdown preceded the laser stimuli (see Figure 1). The first auditory cue was presented concurrently with an anticipatory cue to indicate the forthcoming laser intensity and to maintain focused attention. The participant was either shown ‘Low’, ‘High’ or ‘Unknown’. The presentation of the word ‘Unknown’ indicated that the laser stimulus has an equal chance of being low or high. This was to investigate the importance of certainty in the anticipation of the laser stimuli. The image was also used as a visual fixation cue to discourage eye movements. After the laser stimuli, the 0-10 numerical rating scale was shown on the screen and the participant rated the intensity of the pain. The order of the stimuli was randomised and separated into three blocks with short breaks in-between.

4.3.iii.d. EEG recording:

A BrainVision MR EEG cap was used to record from 63 scalp electrodes using a BrainVision-cap system [Standard BrainCap-MR with Multitrodes]. The arrangement of the electrodes was modelled on the extended 10-20 system. Recording parameters were set at: Filter (DC to 70 Hz), Sampling rate (1000 Hz), Gain (500). To reduce electrical interference, a 50Hz notch filter was applied. Prior to starting the laser protocol, resting states were recorded with eyes open and closed for two minutes in all participants. This ensured that the experience prior to the experiment was identical. The three experimental blocks were recorded separately to allow for better artefact rejection of the EEG data.
4.3.iv. Analysis Methods

4.3.iv.a. Statistical analysis of behavioural data

Statistical analyses of the behavioural measures were carried out using IBM SPSS Statistics 22 software. The questionnaires were all investigated for significant group differences. Prior to using statistical tests, the data was assessed for normality using a combination of Q-Q plots, histograms, and the values of skew and kurtosis. Normally distributed data was analysed using independent t-tests and ANOVA tests, whilst data reported to be not normally distributed, the appropriate non-parametric test was utilised. Specific statistical tests for each analysis step are reported in the results section.

4.3.iv.b. EEG analysis method

EEG pre-processing was carried out using EEGLAB toolbox (Delorme and Makeig, 2004) in MATLAB version R2015a (The Mathworks Inc) whilst statistical analysis was carried out using SPM12 toolbox (Wellcome Department of Imaging Neuroscience, Institute of Neurology, UCL, London, United Kingdom) running in MATLAB. The EEG data was pre-processed for scalp and source localisation analysis. The main motivation of the analysis was to establish the anatomical origin of the brain activity using LORETA (Low Resolution Electromagnetic Tomography) source localisation.

4.3.iv.c. EEGLAB Pre-processing

Pre-processing consisted of; removal and interpolation of bad channels, down-sample to 500, low-pass filter of 20Hz and re-reference to the common average. The four conditions were separated and -3500ms to 2000ms epochs extracted and Linear detrend applied. Independent Component Analysis (ICA) was carried out on all datasets using the SemiAutomatic Selection of Independent Components for Artifact correction (SASICA) toolbox to select components to remove via pre-determined thresholds. The thresholds were set to; Autocorrelation (threshold = 0.35 r, lag = 20 ms), Focal (threshold = 3.5 z), Focal trial (threshold 5.5 z), Signal to
noise (period of interest (POI) = [0 Inf], baseline (BL) [-Inf 0], threshold ratio = 0.5), and Adjust Selection enabled. The thresholds were sufficient to remove artefacts from the majority of the datasets; however, a number of datasets required further manual removal of eye-blink components where not picked up by SASICA.

4.3.iv.d. SPM EEG Analysis:

The pre-processed datasets were converted to Statistical Parametric Mapping (SPM) compatible files. Statistical analysis was carried out to investigate the anticipation evoked potentials and the post-stimulus LEPs using scalp-level and source localisation analysis techniques available in the SPM toolbox.

SPM scripts for batch processing were used to analyse the EEG data at the scalp level and source localisation. Two baselining methods were applied to the data analysis for the anticipation phase and were applied for scalp-level and source localisation analysis (see 2.1.b.ii). Primary analysis applied distinct baselines (BLs) of 500 ms, occurring prior to each of the three auditory cues respectively, to analyse each of the three anticipation phases. The BLs and time window of interest (TWOI) were as follows; Early [BL: -3500 ms -3000 ms: TWOI: -2500 ms -2000 ms], Mid [BL: -2500 ms -2000 ms: TWOI: -1500 -1000 ms] and Late [BL: -1500 -1000 ms: TWOI: -500 0 ms] anticipation phases. The secondary analysis method applied a single baseline of 500 ms prior to the first auditory cue [BL: -3500 ms -3000 ms] that was common to every TWOI anticipation phase (early, mid and late). This second analysis was conducted to enable comparison to previous studies (Brown et al., 2008a; Brown, El-Deredy and Jones, 2014). All statistical analysis for the anticipation phase was adjusted for multiple comparisons.

All analysis for the post-stimulus phase TWOI [200 ms 600 ms], centred on the LEP, was baseline corrected to -500 ms prior to the laser stimulus [BL: -500 ms 0 ms].

4.3.iv.e. Source Localisation analysis parameters

SPM12 EEG and MATLAB scripts were used to estimate the sources of the anticipation- and laser-evoked potentials using LORETA. The forward model was
created using an 8196 vertex template cortical mesh co-registered to the electrode positions of the standard 10-20 EEG system. A three-shell boundary element model (BEM) EEG head model available in SPM12 was used to compute the forward-model. The images were smoothed with a 12mm full-width-at-half-maximum (FWHM).

4.3.iv.f. EEG Analysis Statistical analysis

For the analysis of the anticipatory TWOIs, a three way repeated measures mixed ANOVA was applied, with one between-subject factor of Group (HC vs PD) and two within-subject factors of Certainty [Known (Low/High) v Unknown] and Expectation [Low v High (Known)]. For the analysis of the post-stimuli TWOI, a three way repeated measures ANOVA was applied, with one between-subject factor of Group (HC v PD) and two within-subject factors of Certainty [Known (Low/High) v Unknown] and Intensity [Low v High (Known and Unknown)].

Source localisation results were reported as follows. To control for multiple comparisons, a cluster-forming threshold of p<0.001 was used and resulting clusters were considered significant at FWE (p<0.05). Significant clusters were also restricted to >100 voxels in size and regions labelled using the Anatomical Automatic Labelling (AAL2) toolbox in SPM.
4.4. RESULTS

4.4.i. Behavioural Results

4.4.i.a. Questionnaires

The PCS was significantly higher in the PD group (11.18±10.66, mean±SD) compared to the HC group (4.36±4.11), a statistically significant difference of 6.81 (95% CI, 13.35 to 0.28), t(23)=−2.194, p=0.042. Hence, indicating a higher level of pain catastrophizing behaviours in PD. In contrast, there were no statistically significant differences in the scores of the HADS or MoCA between the HC and PD groups.

The MDS-UPDRS Motor score (III) was recorded 1 hour after medication administration within the PD group to be 20.75±7.70 (mean±SD). The score had reduced from the score recorded during the PD participants’ ‘off’ state (36.42±15.84) demonstrating symptom improvement.

Within the PD group, the level of current pain recorded via a VAS was compared to their baseline current pain level whilst they were off their medication. A 2-tailed paired t-test showed that within those with chronic pain n=8, there was a significant reduction in their current pain levels (Mean±SD: 15.85±23.15 to 8.75±19.63) T(1, 7)=2.486, p=0.041. 

4.4.i.b. Laser Sensitivity

The voltage of the laser was calibrated to the participants’ subjective perception of the intensity. An independent-samples t-test was carried out on the two pain intensities to determine any differences in sensitivity between the HC and PD group (Figure 4.1). The voltage to induce low pain was lower in PD (1.48±0.36; mean±SD) than the HC group (1.75±0.24), a statistically significant difference of 0.27 (95% CI, 0.01 to 0.52), t(23)=2.13, p=0.045. The voltage to induce high pain was assessed via a Welch test due to equal variances not assumed assessed by Levene’s Test for Equal Variances (p=0.033). The result showed that the voltage required to induce high pain was also lower in PD (mean±SE: 1.95±0.44) compared to HC (2.25±0.25).
The comparison was a statistically significant difference of 0.14 (95% CI, 0.01 to 0.59), t(21.32)=2.12, p=0.046.

Figure 4.1: The laser voltage required to induce a low and high pain intensity. The PD group were more sensitive to the laser stimuli as they required a significantly lower voltage level of the laser to induce a high pain. The data is separated into HC (blue) and PD (red), and for Low and High pain. The boxes show the median (bold horizontal line), and lower and upper quartiles. The vertical lines show the minimum and maximum values. * denotes significance p<0.05.

4.4.i.c. Pain rating

The rating of the laser stimuli (Table 4.1) was assessed using a mixed-ANOVA to determine any effect of the certainty of the visual cues and the intensity of the laser, and any differences between the PD and HC group. Firstly, there was no difference in pain rating between the PD and HC groups [F(1, 23)=0.738, p=0.992], nor an effect of certainty on pain ratings. As expected, the rating of the high intensity laser stimuli was significantly higher than low intensity [F(1, 23)= 108.7, p<0.000].

There was a significant effect of Certainty (Known v Unknown) on Intensity (Low v High), [F(1, 23)=34.91, p<0.000], such that the “Unknown” visual cue resulted in a higher rating of the Low intensity, yet a lower rating of the high intensity of the
4. Central pain processing in people with Parkinson’s disease whilst on medication

laser (Figure 4.2). This manipulation of pain rating has previously been shown (Chapter 3, (Brown et al., 2008a)) and is thus a good validation of the experimental design. There was no difference seen between the two groups in the effect of Certainty and Intensity.

<table>
<thead>
<tr>
<th>Laser Condition</th>
<th>HC Mean</th>
<th>HC SD</th>
<th>PD Mean</th>
<th>PD SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>2.34</td>
<td>0.55</td>
<td>2.17</td>
<td>0.99</td>
</tr>
<tr>
<td>High</td>
<td>4.92</td>
<td>1.09</td>
<td>4.90</td>
<td>1.87</td>
</tr>
<tr>
<td>Unknown Low</td>
<td>2.76</td>
<td>0.81</td>
<td>2.56</td>
<td>1.16</td>
</tr>
<tr>
<td>Unknown High</td>
<td>4.25</td>
<td>1.03</td>
<td>4.63</td>
<td>1.65</td>
</tr>
</tbody>
</table>

**The contrasting effect of certainty on low and high intensity**

Figure 4.2: The effect of certainty on the pain rating scores. The graph shows the averaged pain ratings for both HC and PD groups. The effect of the uncertain visual cue ‘Unknown’ resulted in a higher rating of the low intensity compared to the known ‘Low’ visual cue. In contrast, the rating of the high laser intensity was lower when preceded by the ‘Unknown’ cue rather than the certain ‘High’ visual cue. This manipulation of pain ratings by certainty has been reported previously and is a good validation of experiment design. The boxes show the median (bold horizontal line), and lower and upper quartiles. The vertical lines show the minimum and maximum values. The patterned boxes denote the Unknown cues which denote uncertain anticipation. UnLow = Unknown Low, UnHigh = Unknown High. *** denotes significance p<0.001.
4.4.ii. EEG Results

The EEG recording was analysed to determine whether there were any differences between the HC and PD whilst anticipating and perceiving the laser stimuli. During anticipation, scalp-level analysis reported no difference in Group (HC v PD), Certainty (Known v Unknown) or Expectation (Known Low v Known High). During the post-stimulus time window centred on the LEP, there was no difference between Group (HC v PD), or an effect of Certainty (Known v Unknown). However, there was a significant effect of Intensity (Low v High), such that the higher laser intensity evoked a higher degree of activity \( k_e=5101, p(FWE)=0.000, t=5.99, [-4 -52 \text{ mm}], 518\text{ms} \). The cluster was located on the top of the head over dorsal regions.

The source localisation analysis showed no group differences in brain activity between the PD and HC groups during both the anticipation and perception of the laser stimuli. An expectation effect was seen in both PD and HC groups, such that a higher degree of activity was seen during anticipation of high stimuli compared to low stimuli. The increased activity during expectation of high stimuli was seen within the left superior parietal lobe \( k_e=656, p(FWE)=0.042, t=4.63 \ [-26 -54 64\text{mm}] \) during early anticipation, and higher activity within the left postcentral gyrus during mid expectation \( k_e=1620, p(FWE)=0.002, t=4.24 \ [-36 -22 36\text{mm}] \) (baselined to -2500-2000 ms).

4.5. DISCUSSION

The results presented in this report contribute further to the findings described in the Chapter 3. Here we have shown that the PD patients whilst on their medication did not show any significant differences in central pain processing compared to healthy controls, yet were more sensitive to the laser stimuli than controls.

The results in this study indicate that the Parkinson’s disease medication could normalise the amplified anticipatory response seen following withdrawal of their medication (see Chapter 3). The reduction in their current pain levels demonstrate a potential analgesic quality and supports the theory that the lack of dopamine
results in heightened pain sensitivity. Nevertheless, the direct comparison of behavioural and neural responses whilst PD participants were off and on medication was not completed due to the confounding effect of protocol repetition alongside medication administration. Thereby caution must be taken when suggesting normalisation of the PD response to the HC response. The result was in agreement with our prediction that the Parkinson’s medication, which increases dopamine, would normalise the amplified central processing seen in the off state. This finding suggests that the amplification of the anticipatory processing seen in the mid-cingulate cortex whilst PD patients are off their medication is due to the reduced level of dopamine.

Interestingly, we have shown that the PD participants were significantly more sensitive to the laser stimuli as they required a lower laser voltage to induce both low and high pain. This further supports the same finding in Chapter 3, and contributes to the evidence of increased pain sensitivity in Parkinson’s and that the increased pain sensitivity in PwPD is due to the dysfunction of other neurotransmitter systems (Silverdale et al., 2018; Tinazzi et al., 2009). In contrast, previous research on the effect of PD medication on pain tolerance of experimentally induced pain has shown a reduction of sensitivity. Assessments of cold pain tolerance and the nociceptive flexion reflex was shown to be lower in PD patients off their medication, yet equal to healthy controls following taking their medication (Brefel-Courbon et al., 2005; Gerdelat-Mas et al., 2007).

Dopamine’s role in pain processing is not considered to be solely specific to intensity coding, instead it is known to be fundamental to the precision of prediction coding of changing sensory inputs (Schwartenbeck et al., 2015; Friston et al., 2014, 2012), which includes cognitive processing (Nieoullon, 2002) and salience assignment (Borsook et al., 2013; Shiner et al., 2015; Mitsi and Zachariou, 2016). The role of dopamine in the development of chronic pain has been shown to be due to dysfunctional dopaminergic pathways affecting these processes (Wood, 2004; Jarcho et al., 2012; Bushnell, Ceko and Low, 2013; Hagelberg et al., 2004). The anticipatory response to the pain is highly likely to be dependent on dopaminergic
transmission due to the role of attention, cognition and salience assignment processes occurring prior to pain perception. Therefore, the repletion of the dopamine via PD medication has shown to be sufficient to normalise the anticipatory response seen in the ‘off-state’ (Chapter 3).

The high prevalence of chronic pain in the PD population, and the consistent increase in pain sensitivity that we have seen in this study, demonstrates that PD medication is not sufficient to prevent or reverse chronic pain in PD. It could be argued that the fluctuating dopamine levels caused by PD physiology and the on-off states caused by the PD medication causes long-term changes within key brain regions involved in pain processing, predisposing and maintaining chronic pain.

Nevertheless, a confident conclusion is not possible when interpreting the results from this study due to the limitations of the numerous uncontrolled variables which may have resulted in a lack of a significant difference between the PD and HC groups. Firstly, the type of PD medication was variable within the PD participants, and no placebo medication was administered to the HC group to account for a placebo effect of medication administration. In addition, the current level of pain within the PD cohort was reduced following medication and may have had an effect on neural activation. Furthermore, the number of participants who successfully completed the study on their medication was lower than the cohort within the off-medication study (Chapter 3). One reason of not taking part of the on-medication aspect of the study was tiredness due to being off their medication. Hence, the final cohort for the on-medication aspect of the study may have been biased to patients with less severe symptoms as they did not tire as much as those with severe off-state symptoms. This will have resulted in an imperfect representation of the disease within the PD group.

4.6. CONCLUSION

The anticipation of pain is regarded to be a useful technique to investigate top-down modulation of pain and has been shown to be dysfunctional in chronic pain
conditions. The processes associated with top-down modulation of pain include processes such as salience assignment, the orientation of attention, and cognitive processing, which are all modulated by dopaminergic transmission. Therefore, it would be expected that PD patients show altered anticipatory neural activity to pain whilst off their medication, which is normalised following dopaminergic medication. This study provides potential evidence to support this theory and further highlights the role of dopamine dysfunction in the development of persistent pain in PD. This study also concludes that the PD patients had a reduced tolerance to the pain stimuli, even with being on their medication, and contributes to the prior knowledge of increased sensitivity in the PD population.
Chapter 5

An investigation of the role of the dopamine D2 receptor in pain perception

Sarah Martin, Anthony Jones, Christopher Brown, Christopher Kobylecki, Monty Silverdale
5.1. ABSTRACT

Striatal dopamine dysfunction has been shown in a number of chronic pain conditions and is associated with altered top-down modulation of pain processing. Unlike the widespread abundance of the dopamine D1 receptor, the dopamine D2 receptor is confined primarily to the striatum and therefore, a focus within our research. Here we investigated the effect of modulating striatal dopamine D2 receptor activity on the anticipation and perception of acute pain stimuli whilst brain activity was recorded by EEG. Participants completed the experiment under three conditions; control (Sodium Chloride, 20 mg), D2 receptor agonist (Cabergoline, 1.25 mg) and D2 receptor antagonist (Amisulpride, 400 mg) in a repeated-measures, double-blind study. The main experiment included the presentation of cues which allowed for certain or uncertain prediction of two pain intensity levels. Our results demonstrate that the agonist and antagonist did not affect pain sensitivity. However, the antagonist amplified the effect of certainty on pain rating. The EEG recording showed that the agonist and antagonist induced a significant reduction in brain activation within the right temporal and parietal regions during the anticipation of the pain. In addition, during the perception of pain, the antagonist evoked a reduction in activity within the right insula and inferior temporal lobe in comparison to the control and agonist conditions. This study highlights that the D2 receptors are involved in the anticipatory processing which occurs prior to the receipt of pain stimuli, and supports the notion that dopamine has a role in the processing of uncertain predictions.
5.2. INTRODUCTION

5.2.a. Dopamine and pain

There is evidence to show that there is a relationship between pain processing and dopaminergic transmission. However, the role of dopamine in coding the intensity of acute pain is unlikely to be its main role in pain perception. The majority of dopaminergic neurons do not respond directly to aversive stimuli, with only a small percentage (5-15%) reportedly activated during pain perception (Taylor et al., 2016). Instead, the role of dopamine has been theorised to be involved in processing salient stimuli within the environment (Cooper and Knutson, 2008; Wulff et al., 2019; Gentry, Schuweiler and Roesch, 2018; Roughley and Killcross, 2019; Nieoullon, 2002), assignment of emotional valence (Jensen et al., 2003), and to modulate sensorimotor and motivation-related processes (Horvitz, 2002; Bromberg-Martin, Matsumoto and Hikosaka, 2010).

There is evidence that dopamine has endogenous analgesic qualities (Bhagyashree et al., 2017; Wood, 2014; Esposito et al., 1986). However, although drugs which increase dopamine have been demonstrated to have analgesic properties (Shimizu et al., 2004b; Nixon, 1975; Cobacho, de la Calle and Paino, 2014), they are rarely prescribed to treat pain, especially not for acute pain. On the basis of dopamine’s widespread modulatory role in higher cortical processing, it is likely that the analgesic qualities of dopamine are related to the improvement in the cognitive and affective aspects of pain processing, rather than directly reducing pain intensity.

Central processing (i.e. attention, emotional state, and motivational state) can manipulate pain perception and are regarded as top-down processes, whereby subjective factors can modulate pain perception. The top-down modulation (subject-driven) occurs in parallel with the bottom-up factors of pain processing (stimulus-driven), including stimulus novelty and physical saliency. A prominent theory of dopamine function outside of pain processing, is that it acts within a Bayesian framework which codes the precision in predicting desired or expected outcomes based on prior beliefs, thereby serving a top-down function (Friston et
5. An investigation of the role of the dopamine D2 receptor in pain processing

al., 2014; FitzGerald, Dolan and Friston, 2015). Altered top-down processing has been associated with chronic pain conditions such as Fibromyalgia (Miron, Duncan and Bushnell, 1989a; Burgmer et al., 2011; Brown, El-Deredy and Jones, 2014; González-Roldán et al., 2016; Reicherts et al., 2017). Dopamine plays a central role in modulating a wide variety of processes such as attention flexibility (van Holstein et al., 2011; Klanker, Feenstra and Denys, 2013; Whitaker, 2017), salience assignment (Nieoullon, 2002; Kapur, Mizrahi and Li, 2005; Howes and Nour, 2016; Shiner et al., 2015; Parr and Friston, 2017), motivational states (Pultorak et al., 2018; Elliot and Covington, 2001) and emotional processing (Salimpoor et al., 2011; Baixauli, 2017; Peciña et al., 2013), and thus demonstrates the potential detrimental affect dopamine dysfunction would have on top-down modulation of pain. For example, impaired dopaminergic transmission has been associated with chronic pain conditions (Jääskeläinen et al., 2001; Wood et al., 2007b; a) such as Fibromyalgia (Häuser, 2016; Albrecht et al., 2016) and atypical facial pain (Hagelberg et al., 2003a), plus it is likely to contribute to pain in Parkinson’s disease (Blanchet and Brefel-Courbon, 2018; Silverdale et al., 2018).

5.2.b. Dopamine Receptors & Pain Perception

The dopaminergic activity within the striatum has been associated with modulation of pain perception, especially the activity of the D2 dopamine receptor (D2R) (Barceló, Filippini and Pazo, 2012; Hagelberg et al., 2002). For instance, direct injection of the D2R agonists apomorphine or quinpirole into the striatum has shown to reduce pain related behaviours within rodents, whilst a D1R agonist did not induce any change in response (Barceló, Filippini and Pazo, 2012; Cobacho, de la Calle and Paíno, 2014). Within humans, PET imaging has shown that striatal D2R binding potential is inversely correlated with an individuals’ pain tolerance of the cold pressor test (CPT) (Hagelberg et al., 2002) and heat thermode (Martikainen et al., 2005) and their ability to suppress pain (Hagelberg et al., 2002).

The research into D2R’s role in pain has primarily been focused on pain tolerance and brain activity during the receipt of noxious stimuli. Therefore, the role of the D2R in the top-down modulation of pain processing is yet to be investigated. While
the role of the D2Rs during the anticipation of noxious stimuli has not been investigated, their role in anticipating rewarding stimuli has been conducted. Research by Ye et al., (2011), targeted the striatal presynaptic D2/D3 autoreceptors to reduce dopamine release via the agonist Pramopexole\(^1\), and highlighted an increase in striatal activity during the anticipation of rewarding stimuli. They also reported a reduction in the connection between the striatum and prefrontal cortex, and concluded that manipulation of dopamine induced a reduction in top-down control of impulsive behaviours.

5.2.c. Aim of study

In summary, the purpose of this study was to establish whether the D2R had a role in top-down modulation of pain perception. To investigate this, a D2R agonist (Cabergoline) and antagonist (Amisulpride) was chosen for their selectivity for the D2R. Using EEG source localisation analysis we aimed to investigate changes in brain activity during the anticipation and perception of a noxious stimuli following D2R manipulation. Due to the role of dopamine in salience assignment, predicting outcomes and attentional flexibility, the experimental paradigm was configured to evaluate the effect of expectation of both low and high pain stimuli, and certain and uncertain anticipation of the forthcoming stimuli intensity.

\(^1\) Pramopexole was given at a dose which activated the presynaptic auto-receptors and resulted in a reduction of dopamine synthesis and release from the presynaptic neuron. In turn, there was a reduction in the activation of the post-synaptic dopamine receptors.
5.3. METHODS

5.3.a. Ethical approval

The study was approved by the University of Manchester Ethics Committee and was the UK Health Research Authority for the use of a National Health Service (NHS) site (Salford Royal NHS Foundation Trust Hospital).

5.3.b. Drug selection

To target the striatum, we selected a D2R agonist, *Cabergoline*, and a D2R antagonist, *Amisulpride*, which both have a high affinity for D2Rs. Previous studies have shown that at low doses of D2R drugs, the binding of the presynaptic D2 autoreceptor is favoured and produces the opposite of the desired results (Maruya et al., 2003; Ford, 2014). Therefore, a sufficiently high dose was selected to modulate the postsynaptic D2R despite the action of the autoreceptors.

5.3.c. Participant recruitment

A total of twenty-nine healthy participants (16 females) were recruited for the study (mean age 22.4 years, SD 3.2 years). All subjects gave written informed consent. The exclusion criteria of the study was as follows; history of significant head injury or seizures, diagnosed or taking medication for any neurological or psychiatric condition, history of drug or alcohol dependence, use of psychotropic medication within the past 6 months, use of dopaminergic drug within the past month or lifetime use exceeding 3 months, pregnant or breastfeeding or attempting to conceive (females only), suffering from chronic pain.

All participants also completed the Barratt Impulsivity Scale (BIS-11, Patton et al., 1995) subscale of ‘cognitive instability’. The degree of impulsivity has shown to be related to dopaminergic transmission and receptor abundance within the striatum (Bucker and Theeuwes, 2014; Costa et al., 2013; Buckholtz et al., 2010; Dalley and Robbins, 2017). The participants’ were only recruited if their score was within 1.5 standard deviations of the mean reported from a large control sample (1,577 healthy adults) (Stanford et al., 2009).
We used a repeated-measures, double-blind and triple-crossover design such that all participants were recruited to attend three visits and complete the experimental protocol following ingestion of the agonist, antagonist and control substance. Two participants did not complete all three visits of the study, and one participant was removed due to an adverse reaction to Amisulpride. Therefore, twenty-six datasets were included for behavioural and EEG analyses (mean age 22.2 years, SD 3.7 years, 14 females).

5.3.d. Health Screening

Before the first experimental visit, the participants attended a health screening to deem them safe to take part in the study. A medical doctor assessed the participants’ heart rate, blood pressure, temperature and recorded an electrocardiogram (ECG). Due to the potential risk of Amisulpride causing arrhythmias, all participants were assessed for QTc abnormalities in the ECG prior to inclusion in the study. No participant presented with any abnormalities resulting in the exclusion from the study. A letter to the participants’ general practitioner (GP) was also organised to inform them of the participants’ involvement in the study.

5.3.e. Prior to experimental visits

Participants were instructed to not consume alcohol for 24 hrs prior to the visit, to only drink their normal intake of coffee or tea on the morning of each visit, and to refrain from consuming other caffeinated drinks 2 hours prior to each visit. Participants were also informed to not consume any psychoactive substances for the duration of the study, or any over-the-counter medicine 48 hrs prior to each visit.

5.3.f. Experimental Visit Protocol

5.3.f.i. Drug administration

One of three substances was administered to the participant at each experimental visit; Cabergoline (Agonist) (1.25 mg), Amisulpride (Antagonist) (400 mg), or Sodium
Chloride (Control) (20mg). All drugs hold a full product licence (EU). The administration of the drug\(^2\) was double-blinded and each participant had each drug condition once over the three visits in a randomised order. The three visits were separated by at least ten days. The administration of the drug was recorded as time 0 hr and experimental testing commenced at 3 hr 30 mins post drug administration.

5.3.f.ii. Resting state

Previous research has indicated a potential correlation between the level of striatal dopamine and the rate of blinks per minute (KARSON, 1983; Elsworth et al., 1991). The blink rates of the participants were recorded using frontal EEG electrodes during a resting state of nine minutes at +2 hours after taking the drug and prior to any experimental testing. The participants were not informed that their blink rate was being assessed to avoid affecting their spontaneous blink rate.

5.3.f.iii. Pain stimuli

A CO\(_2\) laser [50W Synrad 48-5 J-series (J-48-5(S)W) Wavelength: 10600nm] was used to deliver acute pain to the dorsal forearm surface (Brown, El-Deredy and Jones, 2014). The CO\(_2\) laser delivered a beam with a diameter of 15 mm and 150 ms duration. For each test, the stimuli were delivered in an area measuring 4 x 5cm and were delivered in a predetermined randomised path (Brown et al., 2008a). This was to avoid habituation, sensitization, or skin damage.

5.3.f.iv. Psychophysics

Before starting the experimental protocol, psychophysics was used to calibrate the laser to the individual’s pain sensitivity. An ascending method of limits procedure was used, starting from 0.6 V with 0.06 increments each time. The participant used an eleven point number rating scale (NRS) (0 - 10) to rate the intensity of the pain perceived for each laser stimuli (0=no sensation, 4=pain threshold, 7=moderately painful, 10=unbearably painful). The rating scale was introduced to the participant

\(^2\) The term drug or drug condition is in reference to all three substances; Control, Agonist and Antagonist, unless specified.
via these standardised descriptives to ensure that no explanation altered their interpretation of the scale. The procedure was repeated three times to allow participants to get used to the laser and was used to calculate the average voltage to induce level 4 (low) and level 7 (moderate) pain. These two levels provided ‘low’ and ‘high’ pain stimuli for the laser protocol.

5.3.f.v. Main experiment

The participants received 120 laser stimuli at the two intensities (low and high) separated into four conditions; Low (level 4), High (level 7), Unknown Low (level 4) and Unknown High (level 7). To investigate the anticipation of a painful stimulus, a 3 second auditory countdown preceded the laser stimuli (see Figure 5.1). The first auditory cue was presented concurrently with a visual anticipatory cue to indicate the forthcoming laser stimuli. The participant was either shown ‘Low’, ‘High’ or ‘Unknown’. The presentation of the word ‘Unknown’ indicated that the laser stimulus has an equal chance of being low or high. This was to investigate the importance of certainty in the anticipation of the laser stimuli. The image was also used as a visual fixation cue to discourage eye movements. After the laser stimuli, the 0-10 numerical rating scale was shown on the screen and the participant rated the intensity of the pain via a numerical keypad. The order of the stimuli was randomised and separated into three blocks with short breaks in-between. Following each block, the participants rated the unpleasantness on average for each laser condition. Unpleasantness was scored using an 11-point NRS whereby 0 is not unpleasant and 10 is most unpleasant sensation.
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Figure 5.1: A schematic diagram of a single trial of the experimental paradigm. A computer monitor showed the participant a visual cue of; Low, High or Unknown from -3s to +2s. A three second countdown of beeps at -3, -2 and -1 allowed accurate anticipation of the laser stimuli at time 0s (red bar). The presentation of the visual cue at -3s was concurrent with the first auditory cue. The visual cue was consistent throughout the anticipation and laser stimulus. At +2s, an eleven-point number rating scale (NRS) was presented for the participant to rate the laser stimulus. For each condition (Low, High, Unknown Low and Unknown High) there were thirty trials. The trials were presented in a randomised order and divided into three blocks of forty trials.

5.3.f.vi. EEG recording

A BrainVision MR EEG cap was used to record from 63 scalp electrodes using a BrainVision-cap system [Standard BrainCap-MR with Multitrodes]. The arrangement of the electrodes was modelled on the extended 10-20 system. Recording parameters were set at: Filter (DC to 70 Hz), Sampling rate (1000 Hz), Gain (500). To reduce electrical interference, a 50Hz notch filter was applied. Prior to starting the laser protocol, resting states were recorded with eyes open and closed for two minutes in all participants. This ensured that the experience prior to the experiment was identical. The three experimental blocks were recorded separately to allow for better artefact rejection of the EEG data.

5.3.g. Analysis Methods

5.3.g.i. Statistical analysis of behavioural data

Statistical analyses of the behavioural measures were carried out using IBM SPSS Statistics 22 software. Prior to using statistical tests, the data was assessed for normality using a combination of Q-Q plots, histograms, and the values of skew and kurtosis. Normally distributed data was analysed using independent t-tests and ANOVA tests, whilst for data reported to be not normally distributed, the
appropriate non-parametric test was utilised. Specific statistical tests for each analysis step are reported in the results section.

5.3.g.ii. EEG analysis method

EEG pre-processing was carried out using EEGLAB toolbox (Delorme and Makeig, 2004) in MATLAB version R2015a (The Mathworks Inc) whilst statistical analysis was carried out using SPM12 toolbox (Wellcome Department of Imaging Neuroscience, Institute of Neurology, UCL, London, United Kingdom) running in MATLAB.

5.3.g.iii. Blink rate

For each participant and visit, the number of blinks per minute was calculated using a MATLAB script. The shape of waveform and topography was used to detect blinks within the resting state data. A script which used the ICA function within the EEGLAB toolbox was applied to count the blinks. The blinks per minute were reported as the blink rate and were assessed for differences via repeated measures ANOVA.

5.3.g.iv. EEGLAB Pre-processing

Pre-processing consisted of: removal and interpolation of bad channels, down-sample to 500, low-pass filter of 20Hz and re-reference to the common average. The four conditions were separated and -3500ms to 2000ms epochs extracted and Linear detrend applied. Independent Component Analysis (ICA) was carried out on all datasets using the SemiAutomatic Selection of Independent Components for Artifact correction (SASICA) toolbox to select components to remove via pre-determined thresholds. The thresholds were set to; Autocorrelation (threshold = 0.35 r, lag = 20 ms), Focal (threshold = 3.5 z), Focal trial (threshold 5.5 z), Signal to noise (period of interest (POI) = [0 Inf], baseline (BL) [-Inf 0], threshold ratio = 0.5), and Adjust Selection enabled. The thresholds were sufficient to remove artefacts.

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3 The MATLAB script was written and executed by Dr Grace Whitaker and Professor Wael El-Deredy. All participants within this experiment were part of a wider study in collaboration with Dr Whitaker. The resting state data and assessment of the blink rate was a validation step in the all elements of the study and hence the data shared.
from the majority of the datasets; however, a number of datasets required further manual removal of eye-blink components where not picked up by SASICA.

5.3.g.v. SPM EEG Analysis

The pre-processed datasets were converted to Statistical Parametric Mapping (SPM) compatible files. Statistical analysis was carried out to investigate the anticipation-evoked potentials and the post-stimulus LEPs using scalp-level and source localisation analysis techniques available in the SPM toolbox.

SPM scripts for batch processing were used to analyse the EEG data at the scalp level and source localisation. Two baselining methods were applied to the data analysis for the anticipation phase and were applied for scalp-level and source localisation analysis (see 2.1.b.ii). Primary analysis applied distinct baselines (BLs) of 500 ms, occurring prior to each of the three auditory cues respectively, to analyse each of the three anticipation phases. The BLs and time window of interest (TWOI) were as follows; Early [BL: -3500 ms -3000 ms: TWOI: -2500 ms -2000 ms], Mid [BL: -2500 ms -2000 ms: TWOI: -1500 -1000 ms] and Late [BL: -1500 -1000 ms: TWOI: -500 0 ms] anticipation phases. The secondary analysis method applied a single baseline of 500 ms prior to the first auditory cue [BL: -3500 ms -3000 ms] that was common to every TWOI anticipation phase (early, mid and late). This second analysis was conducted to enable comparison to previous studies (Brown et al., 2008a; Brown, El-Deredy and Jones, 2014). All statistical analysis for the anticipation phase was adjusted for multiple comparisons.

All analysis for the post-stimulus phase TWOI [200 ms 600 ms], centred on the LEP, was baseline corrected to -500 ms prior to the laser stimulus [BL: -500 ms 0 ms].

5.3.g.vi. Source Localisation analysis parameters

SPM12 EEG and MATLAB scripts were used to estimate the sources of the anticipation- and laser-evoked potentials using Low Resolution Electromagnetic Tomography (LORETA). The forward model was created using an 8196 vertex template cortical mesh coregistered to the electrode positions of the standard 10-
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20 EEG system. A three-shell boundary element (BEM) EEG head model available in SPM12 was used to compute the forward-model. The images were smoothed with a 12mm full-width-at-half-maximum (FWHM).

Source localisation results were reported as follows; to control for multiple comparisons, a cluster-forming threshold of p<0.001 was used and resulting clusters were considered significant at FWE (p<0.05). Significant clusters were also restricted to >100 voxels in size and regions labelled using the Anatomical Automatic Labelling (AAL2) toolbox in SPM. Small volume correction (SVC) was applied using a 25 mm sphere to further investigate effects within regions of the pain matrix, specifically the Insula, Anterior Cingulate Cortex (ACC), Thalamus and Amygdala, using coordinates from the Brede Database (Årup Nielsen, 2003).

5.3.g.vii. EEG Analysis Statistical analysis

The anticipatory time windows of interest (TWOIs) were analysed using a repeated-measure three-way ANOVA, with within subject factors of Drug (Control v Agonist v Antagonist), Certainty (Known v Unknown), and Expectation (Known Low v Known High). The analysis of the post-stimuli TWOI was conducted using a repeated measure three-way ANOVA, with within subject factors of Drug (Control v Agonist v Antagonist), Certainty (Known v Unknown), and Intensity (Low v High).

5.4. RESULTS

5.4.a. Behavioural results

5.4.a.i. Laser Sensitivity

To assess whether dopamine modulation evoked changes in sensitivity to the laser stimuli, the participants’ tolerance to the laser was compared between each drug condition. The voltage (V) required to induce a high (level 7) pain rating that was determined by the psychophysics procedure was analysed for within-subject differences using a repeated measures one-way ANOVA. The mean ±SD laser energy (V) was calculated for control (1.93±0.43 V), agonist (1.98±0.49 V) and
antagonist (1.97±0.41 V). There was no significant effect reported between drug conditions $F(2, 50) = 0.465, p=0.631$, signifying no drug-induced effect on sensitivity to the nociception.

### 5.4.a.ii. Pain rating

Throughout the experiment, the participants rated the pain of the laser stimuli to establish whether dopaminergic manipulation and anticipatory cues affected the rating of the stimuli (see Table 5.1). The mean pain rating score for each drug condition was calculated and analysed using a repeated measures three-way ANOVA with within-subject factors of drug \( (control, \text{ agonist and antagonist}) \), certainty \( (\text{known and unknown}) \), and intensity \( (\text{low and high}) \). The pain rating scores were normally distributed, as assessed by Shapiro-Wilk's test of normality \( (p > .05) \).

The within-subject comparison of the three Drug Conditions \( (\text{Control, Agonist & Antagonist}) \) reported no effect on pain rating $F(2, 50) = 0.60, p=0.552$. This outcome was expected as the laser intensity was calibrated to the participants' individual pain sensitivity on each visit to induce a score of 7 on the pain NRS. The calibration was completed +3hr30 mins post drug administration and thus pain rating was not expected to differ between drug conditions.

<table>
<thead>
<tr>
<th>Drug condition</th>
<th>Low</th>
<th>High</th>
<th>Unknown Low</th>
<th>Unknown High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.97</td>
<td>6.30</td>
<td>3.28</td>
<td>5.86</td>
</tr>
<tr>
<td>Agonist</td>
<td>3.05</td>
<td>6.28</td>
<td>3.50</td>
<td>5.75</td>
</tr>
<tr>
<td>Antagonist</td>
<td>2.83</td>
<td>6.36</td>
<td>3.31</td>
<td>5.58</td>
</tr>
</tbody>
</table>

Table 5.1: The pain rating scores for the experimental paradigm. The rating is separated into laser conditions and reported as mean and SD.

As we expected, there was a significant effect of intensity \( [F(1, 25)=262.98, p<0.001]\), such that high intensity laser stimuli were rated more painful than low intensity. Although the assessment of the effect of Certainty \( (\text{Known v Unknown}) \) on
the pain rating did not show a significant result $F(1, 25)= 3.33, p=0.80$, a two-way interaction between Certainty and Intensity was shown [$F(2, 50)=47.82, p<0.001$]. Pain ratings increased from Low $(2.95\pm0.94) < $ Unknown Low $(6.31\pm0.70) < $ Unknown High $(5.73\pm0.70) < $ to High $(6.31\pm0.70)$. This shows that when participants received a certain cue of Low, they rated the pain less than when they received the Unknown cue and received a low intensity stimulus. In contrast, when the participants were presented with a certain cue of High, they rated the pain higher than when they received the Unknown cue and a high intensity stimulus was delivered. This demonstrates how the rating of the low and high intensity stimuli was affected differently by the certainty during the anticipation period. This manipulation of pain perception by anticipatory cues replicates previous findings (Brown et al., 2008b) and acts to validate the experimental paradigm.

Interestingly, there was a three-way interaction between drug, certainty and intensity [$F(2, 50)=5.60, p=0.006$]. This indicates that the modulation of the D2 dopamine receptors via the agonist and antagonist affected the interaction between certainty and intensity. Follow up two-way ANOVAs established how the drug condition affected the interaction between certainty and intensity. Firstly, the comparison of control and agonist revealed no three way interaction between drug, certainty and intensity [$F(1,25)=2.658, p=0.116$]. However, the comparison of control versus the antagonist did reveal a significant three way interaction, [$F(1, 25)=9.535, p<0.005$], such that the effect of uncertainty was increased within the antagonist condition. The significant interaction was followed-up with paired t-tests to establish the differences observed between the placebo and antagonist condition. To investigate the degree of modulation via certainty, the Unknown pain rating was deducted from the Known pain rating for low and high laser intensity. In contrast to the placebo condition, the Antagonist showed a significantly higher degree of certainty modulation for the high laser stimuli $T(1,25)=3.525, p=0.002$, and did not show evidence of a difference within the low laser condition $T(1,25)=1.009, p=0.322$. The degree of effect that the certainty condition had on the pain rating for each Drug Condition, is shown in Figure 5.2.
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The effect of certainty on Low and High laser intensity within each drug condition.

Figure 5.2: The effect of certainty on each laser intensity was calculated and plotted for each drug. The difference between Known and Unknown pain rating of each laser intensity was calculated by subtracting the Known rating from the Unknown rating. A positive value is indicative of a higher rating in the Unknown condition, whereas a negative value is indicative of a lower rating in the Unknown condition. The bigger the number, the bigger the effect of certainty had on pain rating. Therefore, the graph shows that for Low intensity, there was an increase in pain rating following the Unknown visual cue within all drugs. In contrast, for High intensity, there was a decrease in pain rating following the Unknown visual cue within all drugs. Both the agonist and antagonist caused a bigger effect of certainty on pain rating compared to the control condition, with the antagonist showing a significantly bigger difference in comparison to the Control condition. **=p<0.005.

5.4.a.iii. Unpleasantness rating

The rating of unpleasantness was recorded for each laser condition (Low, High, Unknown Low & Unknown High) and documented at three time points during the laser experiment. The mean unpleasantness rating for each drug condition (Control, Agonist & Antagonist) (Table 5.2) was compared and reported no significant difference [F(2, 30) = 2.766, p=0.079]. In contrast, there was an expected effect of Intensity, such that high intensity stimuli were rated more unpleasant than low intensity stimuli [F(1, 15) = 197.75, p=0.000]. There was also an effect of Certainty
(Known v Unknown) [F(1, 15) = 11.924, p=0.004] on unpleasantness ratings which did not differ between the three drug conditions. The effect demonstrated that the presentation of the Unknown cue caused a higher rating in unpleasantness compared to the Known cues (‘Low’ and ‘High’).

Table 5.2: The unpleasantness rating for each laser condition for each Drug Condition. There was a significant difference between low and high laser intensity, and the unknown conditions were rated significantly more unpleasant than the known conditions. There was no significant difference between the three drug conditions. Unpleasantness score is reported as mean and SD.

<table>
<thead>
<tr>
<th>Drug Condition</th>
<th>Unpleasantness Rating (VAS score /10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Agonist</td>
<td></td>
</tr>
<tr>
<td>Antagonist</td>
<td></td>
</tr>
</tbody>
</table>

5.4.b. EEG results

5.4.b.i. Blink rate analysis

Blink rate has been shown to have a potential relationship with striatal dopamine activity. As such, we analysed blink rate in order to test whether the agonist and antagonist had modulated striatal dopamine. The analysis of the blink rate during the resting state recording highlighted a linear relationship between the eye blink rate and the expected dopamine D2R activation (Figure 5.3). The eye blink rate (mean blinks per minute ±SD) was lowest in the Antagonist condition (23.58±13.13) and highest in the Agonist condition (28.70±12.27), with the control eye blink rate (26.77±13.35) in the middle of the Agonist and Antagonist. This monotonic relationship was not significant, F(2, 46)=1.806, p=0.176. Nevertheless, the monotonic relationship between the dopamine level and eye blink rate is consistent with previous reports (Karson et al., 1984; Shukla, 1985; Taylor et al., 1999; Kleven
and Koek, 1996) and demonstrated that the dosage of the agonist and antagonist was sufficient to induce an increase and decrease of striatal dopamine respectively.

![Dopamine Manipulation Graph](image)

Figure 5.3: The eye-blink rate (EBR) calculated for each dopamine manipulation condition highlighted a monotonic relationship between dopamine D2R activation and EBR. The D2 receptor agonist increased the EBR and the antagonist reduced the EBR in comparison to the control condition. Data is presented as mean and 95% confidence-intervals (cf. Cousineau, 2005).

5.4.b.ii. Scalp-level EEG analysis

The analysis of the EEG recording via whole head scalp-level analysis of the anticipation TWOIs (early, mid and late) reported no main effect of Drug, Expectation or Certainty for both baselining methods. The mean activity at each electrode during anticipation is plotted as topographies for all drug conditions in Figure 5.4.
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Figure 5.4: Topography plots showing the average response for early-, mid- and late-anticipation for all conditions. Data is presented as µV. All plot baselined to a common baseline of [-3500 –3000 ms] pre laser stimulus.

In addition, the analysis of the post-stimulus TWOI [200 600 ms] at scalp-level, also reported no significant effects of drug or certainty. A main effect of intensity produced two clusters (p<0.000, $k_E = 20418$, x:-13mm y:-36mm, 464ms, and p<0.000, $k_E = 3510$, x:55mm y:-68mm, 450ms) with the T-contrast indicating an enhanced activation during high pain in contrast to low pain (p<0.000, $k_E = 17732$, x: -13mm y: -41mm, 460ms).

5.4.b.iii. Source EEG analysis

The source localisation analysis reported novel results which we did not predict. There were significant contrasts within the anticipatory and post-stimulus time
5. An investigation of the role of the dopamine D2 receptor in pain processing

window only when using pre-auditory cue baselines. Firstly, the mid-anticipation TWOI [-1500 -1000 ms] reported significant clusters within the main contrasts of Drug and Expectation (see Table 5.2). The subsequent T-contrasts of the drug effect during mid-anticipation reported a lower degree of activity within both the agonist and antagonist in comparison to the control condition. The clusters in both the agonist and antagonist condition were located within the right hemisphere. The lower activity in the agonist condition was reported in the right mid-temporal region and angular gyrus, whilst the antagonist condition induced reduced activity within the postcentral, mid temporal and inferior parietal regions (see Figure 5.5).

In addition to the main effect of drug condition, all drug conditions showed an effect of Expectation (Known Low v Known High) during mid-anticipation. The significant differences in activity were located in the left hemisphere, contralateral to the pain, and showed augmented activity when participants were presented with the visual cue of High in comparison to the visual cue of Low. The clusters were located in the contralateral mid-temporal, mid-occipital and insula regions and are shown in Figure 5.6.

The post-stimulus TWOI [200 600 ms] produced a main effect of intensity (Low v High) and drug condition (control v agonist v antagonist) (see Table 5.3). The effect of intensity during the post-stimuli time window was as expected such that the high condition induced a higher degree of activation in contrast to the low condition (T-contrast: High>Low: p = 0.000 F=8.44, $k\varepsilon=98792$, x: 8, y: -14, z: -16). The main effect of the drug condition highlighted a difference within the right insula, a main region of pain perception. Subsequent T-contrast tests specified that the differences were between the control and antagonist conditions, and the agonist and antagonist conditions. Firstly, the antagonist was reported to cause a reduced level of activation within the insula in comparison to the control condition (see Figure 5.7). The agonist condition showed a significantly higher activation than the antagonist within multiple regions; right hippocampus, mid- and inferior temporal region, insula and the Heschl (auditory processing) (see Figure 5.7).
Table 5.3: Source localisation significant results within mid-anticipation and the post stimulus TWOI. The mid-anticipation TWOI is baseline corrected to the distinct pre-auditory cue time window. The significant clusters were restricted to >100 voxels, reported at FWE correction with a threshold of p<0.025 to account for multiple comparison. Results are divided into main F-contrast and post-hoc T-contrasts. The MNI coordinates are reported as the peak-voxel response within the cluster. The brain regions were labelled using the Anatomical Automatic Labelling (AAL2) atlas. The percentage overlap of the significant cluster with the brain region is reported. The region with the highest percentage overlap is shown, unless an equivalent share of percentage overlap was observed. A label of ‘Unknown’ was not reported. FWE=Family Wise Error, K=number of voxels, F/T=F-contrast/T-contrast, Z=Z-score, Expectation=Known Low v Known High visual cues, Antag = Antagonist, R = Right, L = Left.

<table>
<thead>
<tr>
<th>Cluster-Level</th>
<th>Peak-Level</th>
<th>MNI coordinates</th>
<th>Brain Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain Region</td>
<td>P(FWE)</td>
<td>K</td>
<td>F/T</td>
</tr>
</tbody>
</table>

**Mid Anticipation**

<table>
<thead>
<tr>
<th>F-contrast</th>
<th>Drug</th>
<th>0.004</th>
<th>909</th>
<th>11.62</th>
<th>4.20</th>
<th>32</th>
<th>-44</th>
<th>34</th>
<th>R</th>
<th>Inferior Parietal (48.0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.013</td>
<td>700</td>
<td>9.79</td>
<td>3.79</td>
<td>34</td>
<td>-54</td>
<td>14</td>
<td>R</td>
<td>Mid Temporal (56.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>4098</td>
<td>25.90</td>
<td>4.85</td>
<td>-44</td>
<td>-48</td>
<td>6</td>
<td>L</td>
<td>Mid Temporal (35.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L</td>
<td>Mid Occipital (23.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.013</td>
<td>783</td>
<td>18.64</td>
<td>4.09</td>
<td>-56</td>
<td>2</td>
<td>-16</td>
<td>L</td>
<td>Mid Temporal (69.1%)</td>
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</tr>
<tr>
<td></td>
<td>0.007</td>
<td>931</td>
<td>15.28</td>
<td>3.69</td>
<td>-14</td>
<td>-86</td>
<td>2</td>
<td>L</td>
<td>Calcarine (52.8%)</td>
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<tr>
<td>T-contrast</td>
<td>Drug:</td>
<td>0.031</td>
<td>693</td>
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<td>10</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Angular gyrus (29.4%)</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>4184</td>
<td>4.60</td>
<td>4.52</td>
<td>34</td>
<td>-40</td>
<td>40</td>
<td>R</td>
<td>Postcentral (17.2%)</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R</td>
<td>Mid Temporal (16.9%)</td>
<td></td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R</td>
<td>Inferior Parietal (15.0%)</td>
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</tr>
<tr>
<td>Expectation:</td>
<td></td>
<td>0.000</td>
<td>8555</td>
<td>5.09</td>
<td>4.98</td>
<td>-44</td>
<td>-48</td>
<td>6</td>
<td>L</td>
<td>Mid Temporal (30.4%)</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L</td>
<td>Mid Occipital (17.9%)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.003</td>
<td>641</td>
<td>3.96</td>
<td>3.90</td>
<td>-30</td>
<td>-18</td>
<td>8</td>
<td>L</td>
<td>Insula (58.8%)</td>
</tr>
</tbody>
</table>

**Post Stimulus**

<table>
<thead>
<tr>
<th>F-Contrast</th>
<th>Drug</th>
<th>0.022&lt;sup&gt;◊&lt;/sup&gt;</th>
<th>164</th>
<th>10.82</th>
<th>4.02</th>
<th>28</th>
<th>-26</th>
<th>14</th>
<th>R</th>
<th>Insula (40.9%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-Contrast</td>
<td>Drug:</td>
<td>0.033&lt;sup&gt;◊&lt;/sup&gt;</td>
<td>131</td>
<td>3.73</td>
<td>3.68</td>
<td>28</td>
<td>-26</td>
<td>14</td>
<td>R</td>
<td>Insula (45.0%)</td>
</tr>
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<td>Hippocampus (54.3%)</td>
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<td>0.022</td>
<td>748</td>
<td>3.93</td>
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<td>-20</td>
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<td>R</td>
<td>Mid Temporal (52.0%)</td>
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<td>Inferior Temporal (38.7%)</td>
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<td>0.010</td>
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<td>Inferior Temporal (38.7%)</td>
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<td>0.008&lt;sup&gt;◊&lt;/sup&gt;</td>
<td>347</td>
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<td>28</td>
<td>-26</td>
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<td>Insula (32.6%)</td>
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<td>R</td>
<td>Heschl (18.9%)</td>
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<sup>◊</sup> Small volume correction (SVC) has been applied using a sphere of 25 mm radius

<sup>></sup> Symbolises that the left-sided condition showed a greater response in comparison to the right-sided condition.
5. An investigation of the role of the dopamine D2 receptor in pain processing

Figure 5.5: Source estimates during mid-anticipation for T-contrast of Control versus agonist (Cabergoline) and antagonist (Amisulpride). The time window of mid anticipation [-1500 -1000 ms] was baseline corrected to [-2500 -2000 ms]. Clusters are shown at FWE correction using MRICron software and regions labelled using the AAL2 atlas. The agonist (blue) reported reduced activity within the right mid-temporal and angular regions in contrast to the control. The antagonist (pink) reported reduced activity within the right postcentral, mid-temporal and inferior parietal regions. The image is aligned via the peak-voxel at peak-level and reported as x y z (mm).
Figure 5.6: Source estimates during mid-anticipation for the Expectation T-contrast of known High versus known Low. The time window of mid anticipation [-1500–1000 ms] was baseline corrected to [-2500–2000 ms]. Clusters are shown at FWE correction using MRicroN software and regions labelled using the AAL2 atlas. Two clusters of enhanced activity were seen in the high condition versus the low condition and were contralateral to the noxious stimuli. The regions included the left mid temporal and the occipital lobe, and the left insula. The image is aligned via the peak-voxel at peak-level and reported as x y z (mm).
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Figure 5.7: The drug effect reported using source localisation for post-stimulus TWOI [200 600 ms]. The TWOI was baseline corrected to -500ms prior to the noxious stimuli. There was a significant difference between the control and antagonist conditions, showing a lower degree of activity within the right insula in the antagonist condition. The antagonist condition also reported a lower activity in comparison to the agonist within the right hippocampus, mid/inferior temporal lobe, insula and Heschl. The alignment of the images is fixated on the peak-voxel MNI coordinates from the peak-level results.
5.5. DISCUSSION

In this study, we aimed to address the role of D2 dopaminergic receptors during pain anticipation and pain perception by using EEG source localisation. The D2 receptor agonist *Cabergoline* and the antagonist *Amisulpride* were used to modulate striatal dopaminergic activity within healthy volunteers. The agonist and antagonist amplified the effect of anticipation on pain ratings, with the antagonist inducing a significantly larger effect in comparison to the control condition. Using EEG source localisation, we have shown that both an increase and decrease in striatal dopamine D2R activity results in a reduced degree of activity during mid-anticipation within regions of the right mid-temporal and parietal lobes in comparison to the control condition. In addition, EEG source localisation highlighted that the modulation of D2 receptor activity induced altered processing during the perception of the laser stimuli, yet did not change behavioural outcomes of pain sensitivity or pain rating. This latter result is comparable to the research by Becker et al., (2013), whereby dopamine manipulation did not change the rating of acute pain.

Importantly, the manipulation of striatal dopaminergic activity via the Agonist (*Cabergoline*) and Antagonist (*Amisulpride*) was shown via the calculation of the eye blink rate during resting state. Although the differences between the drug conditions were not significant, the eye blink rate showed a monotonic relationship with the level of striatal dopamine D2R activity and indicates that the dose of the agonist and antagonist were sufficient to increase and decrease striatal dopamine respectively (Karson et al., 1984; Shukla, 1985; Taylor et al., 1999; Kleven and Koek, 1996). Nevertheless, caution should be taken when interpreting eye blink rate as there is recent evidence that eye blink rate does not correlate with dopamine synthesis capacity or D2R availability, or that it is an inverse relationship (Sescousse et al., 2018; Dang et al., 2017).
5.5.a. Behavioural results

5.5.a.i. Pain sensitivity

Subjective pain intensity within this study was deliberately maintained at similar levels across all drug conditions in order to maintain similar levels of anticipation. Adjusting the laser intensity to each condition allowed us to identify that the dopamine manipulation did not affect the participants’ sensitivity to the laser, consistent with a previous study by Becker et al., (2013).

5.5.a.ii. Pain unpleasantness

The unpleasantness rating was not affected by dopamine manipulation. This result is in contrast to previous research which has shown that acute reduction in global dopamine results in the increase in unpleasantness ratings (Tiemann et al., 2014). Furthermore, an investigation of dopamine neurotransmission in chronic back pain has shown that a low abundance of D2/D3 receptors within the striatum is correlated with the unpleasantness rating. Therefore, we would have expected that the agonism and antagonism of the D2Rs would modulate the rating of unpleasantness. Several reasons may explain the difference reported in this study. Firstly the method of recording the rating of unpleasantness may not have been sufficiently accurate due to it being recorded as a mean after each experimental block. Also, the acute action of the D2R agonist and antagonist within this study could have been insufficient to evoke changes in the affective processing and a more persistent change in dopamine may be required to alter affective processing. Another explanation could be that the feeling of unpleasantness is not a D2R-dependent process.

5.5.a.iii. Pain rating

The rating of the pain intensity within this study was also not altered by dopamine manipulation. As mentioned, this was expected as the stimulus intensity was calibrated to the individuals’ subjective perception after drug administration. However, the dopamine manipulation did cause a difference on the effect of
Certainty (known v unknown) on Intensity (low v high), such that the effect of uncertainty was amplified within both the agonist and antagonist condition, with the largest difference seen between the control and antagonist conditions. Thus, following an ‘Unknown’ cue, participants demonstrated reduced attentional flexibility during both the antagonist condition when compared to placebo (i.e. a reduced ability to perceive the strength of the subsequent stimulus following the ‘Unknown’ cue).

The activity of the midbrain dopaminergic neurons has previously been shown to be involved in the anticipation of uncertain cues of motivational-related outcomes (Bromberg-Martin, Matsumoto and Hikosaka, 2010). Thus one population of dopamine neurons has been demonstrated to code the uncertainty of a forthcoming reward whereas a different population codes the reward prediction errors (Fiorillo, Tobler and Schultz, 2003). By coding uncertainty as well as reward prediction errors, phasic and tonic increases in dopamine neurotransmission may encourage exploratory ‘risk-taking’ behaviour as well as attentional flexibility (Whitaker 2017). We propose that the cue of Unknown evoked a more uncertain state which requires a higher salience value (Seidel et al., 2015a). It could then be predicted that the coding of both uncertainty and reward prediction errors is impaired in the antagonist condition (due to blocking dopamine responses). This impairment in both the coding of uncertainty and of reward prediction might explain the reduced attentional flexibility of our participants in the antagonist condition (Whitaker 2017). Hence, the interaction of the dopamine manipulation with the effect of Certainty on pain rating provides further evidence for a role of dopamine in salience processing.

5.5.b. EEG results

5.5.b.i. Drug induced effect during anticipation

One of the present study’s main findings was a drug-induced reduction in neural activity during the mid-anticipation phase. The agonist and antagonist both induced a region-specific reduction in activity during the anticipation of pain when
5. An investigation of the role of the dopamine D2 receptor in pain processing

compared to the control condition. Although the result being in the same direction could be considered to be unexpected, this result could be explained by the fact that dopamine signalling is well-known to have an inverted-U type relationship (Cools and D’Esposito, 2011; Vijayraghavan et al., 2007; Levy, 2009; Floresco, 2013; Beggs and Plenz, 2003; Finke et al., 2010; Li and Backman, 2010; Goto, Otani and Grace, 2007) (See Figure 5.8). The peak of the inverted-U curve is indicative to be the optimum level of dopamine, with both low and high levels of dopamine being sub-optimal. This relationship has been demonstrated in cognitive and memory tasks (Cools and D’Esposito, 2011; Floresco, 2013), and understood further with help from research into Parkinson’s disease and Schizophrenia.

During mid-anticipation, both the agonist and antagonist conditions induced a reduced activity in the right mid-temporal lobe, a region involved in sensory integration and semantic processing (Herath, Kinomura and Roland, 2001; Ishai et al., 1999; Chao, Haxby and Martin, 1999; Tranel, Damasio and Damasio, 1997). D2Rs are located within the mid-temporal lobe (Goldsmith and Joyce, 1996) and the

Figure 5.8: A schematic diagram to signify the inverted-U relationship between Dopamine level and performance. The vertical dashed lines are representative of the ‘optimum’ range of dopamine for many cortical processes.
5. An investigation of the role of the dopamine D2 receptor in pain processing

binding potential of D2Rs within the right medial temporal cortex has been inversely correlated with cold pain tolerance (Hagelberg et al., 2002). In addition, a reduced abundance has been proposed to explain the aberrant information processing in cognitive tasks in dementia (Joyce, Myers and Gurevich, 1998). Therefore, the region has previously been demonstrated to have a role in the integration of sensory and cognitive processing, and be central to an individual’s pain tolerance. Therefore, this study’s finding of altered processing prior to pain perception following dopamine D2R manipulation may indicate a potential role of the mid-temporal lobe in top-down mechanisms associated with pain processing.

We have also highlighted differences within regions of the parietal lobe in both the agonist and the antagonist conditions. The agonist condition showed a reduced activity within the right angular gyrus, a region located within the parietal lobule and connected with the mid-temporal lobe. Whilst the left angular gyrus is responsible for language processing (Seghier, 2013; Binder et al., 1996; Pugh et al., 2000), the right angular gyrus is associated with body representation (Spitoni et al., 2013), attentional reorientation (Taylor et al., 2011), and the integration of vestibular and somatosensory input (Blanke et al., 2002). Hence, we propose that the dopamine manipulation may have altered these processes.

The differences seen in the parietal and temporal regions following dopamine modulation may be linked with the orienting attention which is associated with salience detection (Legrain et al., 2011). The network of regions associated with pain processing is often referred to a salience detection system, and includes the temporal and parietal regions (Legrain et al., 2011). The participants of this study were also assessed for their attentional reorientation in a separate assessment (Whitaker, 2017). The results of this assessment showed that both the agonist and antagonist reduced attentional flexibility, and this aligns with the reduced activation seen within the right parietal lobe in this study. The results also support the inverted-u relationship between dopamine and performance. Hence, our results indicate a potential disruption in the salience and orientating attention network which is activated during the anticipation of the noxious stimuli.
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The antagonist induced a lower activity within the right postcentral gyrus, located within the inferior parietal lobule and the location of the primary somatosensory cortex. This area is responsible for tactile and pain processing (Backonja, 1996; Bushnell et al., 1999; Corkin, Milner and Rasmussen, 1970) and has previously been seen to be involved in the anticipatory response to tactile and painful stimuli (Roland, 1981; Brown, El-Deredy and Jones, 2014). A reduced activation within the right somatosensory cortex has been shown in Fibromyalgia patients during the anticipation of pain (Brown, El-Deredy and Jones, 2014; Loggia et al., 2014). In addition, an fMRI study has previously shown a reduced BOLD response in the right somatosensory cortex within Fibromyalgia patients in comparison to controls during painful pressure (Gracely et al., 2002). Hence a reduced activity within the right somatosensory cortex has been associated with abnormal pain processing and we have shown that the D2Rs could be involved in this process.

5.5.b.ii. Drug induced effect during laser perception

During the post-stimulus time window, the antagonist induced a reduced degree of activity in the right insula in comparison to the control condition, and when compared to the agonist, also showed reduced activity in the right hippocampus, mid- and inferior-temporal lobe, and the Heschl. The change in activity during the perception of pain was not correlated to any change in pain rating of intensity or unpleasantness. Thus indicates that the processes affected were not involved in the coding of these aspects of pain, and instead could be related to the integration of sensory information.

The activation of the contralateral insula during pain perception has previously been shown to be increased in patient groups whom have a reduced level of baseline dopamine; Fibromyalgia (FM) patients (Gracely et al., 2002; Bradley et al., 2000), Parkinson’s disease (Brefel-Courbon et al., 2005) and people with cluster headaches (Hsieh et al., 1995). It would be predicted that the D2R antagonist, would induce a response in healthy controls that is similar to that seen in these patient groups. However, we have shown a contrasting result whereby the antagonism of the D2Rs reduced the activity within the ipsilateral (right) insula.
during nociception. The specific manipulation of D2Rs is thus affecting the insula’s processing during pain differently than the effect of global loss of dopamine. Our results may be explained by the differences between acute manipulation of dopamine receptors as seen in our study compared with the chronic reduction in dopamine levels seen in PD and FM. It is well recognised that chronic, but not acute, alteration in dopamine levels can lead to long term changes in synaptic plasticity and may explain this difference (Jay, 2003; Shen et al., 2008; Arbuthnott, Ingham and Wickens, 2000).

The insula has been considered to be involved in intensity coding (Derbyshire et al., 1997), however, there is more evidence to show that the insula may play a much more complex role in integrating both the sensory and affective aspects of pain (Mazzola et al., 2010; Lu et al., 2016; Singer et al., 2004a), and integrating cognitive and emotional processing. This parallels with the developing story for the role of dopamine and D2Rs in pain, such that dopamine is important for correct interpretation and integration of sensory stimuli. Hence, a persistent dysfunction of the D2R (Martikainen et al., 2015) could amplify the altered insula activity observed in this study and result in an impaired integration of sensory processing.

5.5.c. Future Considerations

An important consideration of the interpretation of this study is whether the changes in the activity during anticipation are directly linked with pain processing and whether long term dopamine dysfunction would impair the top-down modulation of pain. The reduction in the activity within the aforementioned regions could be independent of pain processing. Therefore, a future investigation which uses salient (i.e. noxious) and non-salient (i.e. visual) stimuli could further decipher D2Rs role in pain perception. In addition, the acute administration of D2R agonist and antagonist does not fully enable us to understand how long term dopaminergic transmission (with resultant changes in synaptic plasticity) can impact pain processing. The fact that we have shown significant differences following acute D2R manipulation within multiple aspects of pain processing, suggests that prolonged changes in dopamine might magnify these changes.
5.6. CONCLUSION

In summary, these data demonstrate that manipulating the activity of D2Rs alters aspects of pain processing. Firstly, we have shown for the first time, that D2R antagonism induced a greater effect of uncertainty on pain rating, and that neither agonism nor antagonism affected pain sensitivity, pain unpleasantness or pain rating. The EEG recording highlighted that sub-optimal dopamine activity following the agonist and antagonist evoked a reduction in activity within the right parietal and temporal lobes during the anticipation of pain. The antagonist also induced a reduction in the activity within the right insula during the perception of pain. Therefore, we propose that the dopaminergic activity via the D2Rs is likely to be involved in the top-down processes associated with pain perception, rather than directly coding the intensity of pain. The strong role of dopamine neurons in attention and salience, plus the participants’ showing impaired attentional flexibility (Whitaker, 2017), and the importance of these factors in top-modulation supports D2R neurotransmission as a strong candidate for involvement in the top-down modulation of noxious stimuli.
An EEG investigation of age-related differences in acute pain

Sarah Martin, Anthony Jones, Christopher Brown, Christopher Kobylecki, Monty Silverdale
6.1. ABSTRACT

The tolerance to experimentally induced pain has previously been shown to increase with age, however, chronic pain rates increase with age. Therefore, within this study we investigated whether age-related changes in central processes of pain can help to explain the increased prevalence of chronic pain in the older population. Previous research has shown that pain tolerance increases with age, however, there is limited information on changes within the central processing of pain. Participants were divided into a Young and Old group and underwent an EEG recording whilst acute heat stimuli were delivered to their forearm. Our results concluded that the Older cohort were significantly more tolerant of the pain stimuli, and showed a reduced degree of brain activity during the anticipation and perception of the pain stimuli. We have highlighted a possible age-related change in central processing of nociception which will help to further understand the natural changes in pain processing over time. In addition, the age-related difference in pain processing highlights the importance for chronic pain conditions to be researched within age-groups in which the disease is prevalent. Nevertheless, additional research is required to confirm our findings due to differing protocol efficacy between the two age groups.

6.2. INTRODUCTION

There is a substantial increase of chronic pain with age (Andersson et al., 1993; Blyth et al., 2001a; Rustøen et al., 2005). Whilst the increase in chronic pain in the aged population can be partly explained due to an accumulation of negative lifestyle choices, such as a sedentary lifestyle, and age-related diseases such as arthritis, there is emerging evidence of the deterioration of the pain system leading to a heightened susceptibility to chronic pain (see review: Yezierski, 2012).

Studies investigating the age-induced changes in pain perception have mainly focused on acute pain tolerance. In contrast to the increased rates of chronic pain, the sensitivity of acute pain has been shown to reduce with age in the majority of
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studies demonstrating that with age comes a higher pain tolerance (see review: Gibson and Farrell, 2004). The cause of altered pain tolerance could be explained by the age-related changes in the peripheral nervous system such as slower conductance of sensory nerves (Voitenkov et al., 2017; Palve and Palve, 2018), and changes in the Aδ- and C-fibres (Chakour et al., 1996). Therefore, the development of chronic pain within the older population is not merely due to increased sensitivity to noxious stimuli, and is likely to be linked with altered central top-down processing.

The processing of pain is multidimensional and depends on the integration of a number of cortical processes. Abnormalities in central processing have been associated with chronic pain conditions (Brown, El-Deredy and Jones, 2014; Dick, Eccleston and Crombez, 2002; Gracely et al., 2004; Baliki et al., 2006; Latremoliere and Woolf, 2009) which demonstrate the importance of cortical processing prior and during pain processing. There is evidence that maladaptive top-down modulation of pain, which takes into account the subjective aspect of pain processing, is central to the development of chronic pain conditions. Hence, the age-related changes seen in the central nervous system may negatively affect the top-down modulation of pain and help to explain the increased susceptibility of chronic pain.

Age-related changes have been demonstrated within regions associated with pain processing, including a reduced brain volume within the prefrontal cortex, hippocampus and brain stem structures, whilst the insula and putamen have shown to reduce in activity during pain perception (Farrell, 2012). In addition, there is evidence that there is an age-related deterioration in the cortical networks associated with sensory processing, cognition and salience (Onoda, Ishihara and Yamaguchi, 2012; La Corte et al., 2016; Eckert et al., 2012). Furthermore, the efficacy of the endogenous pain control systems such as the descending pain pathway and the diffuse noxious inhibitory controls (DNICs) reduce with age (Lariviere et al., 2007; Grashorn et al., 2013) and potentially contributes to the
development of chronic pain (Ossipov, Dussor and Porreca, 2010). These findings indicate an age-related alteration in central top-down modulation of pain.

The investigation of the top-down modulation can be investigated by studying the anticipation of noxious stimuli (Brown et al., 2008b; Brown, El-Deredy and Jones, 2014; Brown and Jones, 2008; Clark et al., 2008a; Jones, Brown and El-Deredy, 2013). The anticipation of pain has previously shown to be abnormal within chronic pain conditions (Brown, El-Deredy and Jones, 2014; Jones et al., 2012) and is indicative of a maladaptive pain network. In addition, the activity during the anticipation of a noxious stimulus is associated with the assignment of a salience value to the forthcoming stimulus (Seidel et al., 2015a; Wiech et al., 2010; Menon and Uddin, 2010) and has been shown to be correlated to the pain perceived. Therefore, for the first time, we have compared the anticipatory processing to a noxious stimulus in young and old cohorts via EEG recording and the delivery of noxious stimuli. Any differences observed aim to improve our understanding of how pain perception changes over time. Also, it will improve the translatability of pain studies using EEG conducted in young healthy volunteers to chronic pain conditions which are more prevalent in the older population.

Importantly, we will address methodological considerations when using laser induced pain stimuli and EEG recordings in the different age groups. We will also discuss the possible limitations of interpretation of EEG recordings between age groups due the changes seen in the aging nervous system.
6.3. METHODS

The study was approved by the local ethics committee and all participants gave informed consent according to the Declaration of Helsinki to participate in the study.

6.3.a. Participants

A total of fifty healthy participants took part in the study and were divided into two age groups. The young age group consisted of twenty-six participants aged between 18 and 32 years old (mean age 22.2 years, SD 3.7 years). The older age group included twenty-four participants aged between 45 and 82 years old (mean age 62.92 years old, SD 8.76 years).

The datasets included in this report were from two separate studies using identical experimental methods. The young group reported in this paper are the participants within Chapter 5 of this thesis during the control condition of the study\(^4\). The old group in this study are the healthy control participants reported in Chapter 3 of this thesis. The data used is from their first session completing the experimental protocol. A difference within the recruitment of the participants is that the Young cohort was selected for a middle score of the BIS-11 questionnaire with the aim of limiting baseline dopamine variability, whereas the Old cohort did not complete the questionnaire. Therefore, there is a potential impact of different variability within the baseline dopamine levels within the two groups.

6.3.b. Experimental design

6.3.b.i. Pain Stimuli

A CO\(_2\) laser was used to deliver acute pain to the dorsal forearm surface. The CO\(_2\) laser delivered a beam with a diameter of 15 mm and 150 ms duration. For each

\(^4\) Participants were part of randomised, repeated measure, double-blinded study design. The study involved the administration of a D2 dopamine receptor agonist (Cabergoline) or antagonist (Amisulpride), or an inactive control (Sodium Chloride) (20mg) on three separate visits. The data collected from the control condition was used in this analysis. The control visit was visit 1 for 9 participants, visit 2 for 11 participants and visit 3 for 8 participants.
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test, the stimuli were delivered in an area measuring 4 x 5cm and was delivered in a predetermined randomised path (Brown et al., 2008a). This was to avoid habituation, sensitization, or skin damage.

6.3.b.ii. Psychophysics

Before starting the experimental protocol, psychophysics was used to calibrate the laser to the individual’s pain sensitivity. An ascending method of limits procedure was used, starting from 0.6 V with 0.06 increments each time. The participant used an eleven point NRS (0 - 10) to rate the intensity of the pain perceived for each stimuli (0=no sensation, 4=pain threshold, 7=moderately painful, 10=unbearably painful). The rating scale was introduced to the participant via these standardised descriptives to ensure that no explanation altered their interpretation of the scale. The procedure was repeated three times to allow participants to get used to the laser and was used to calculate the voltage used for the Low and High laser intensities in the main experiment.

The High laser intensity was calibrated to be the voltage required to induce level 7 (moderate pain) and the Low laser intensity was calculated to be level 4 (just painful) in both groups.

6.3.b.iii. Main experiment

The participants received 120 laser stimuli at the two intensities (low and high) separated into four conditions; Low (level 4), High (level 7), Unknown Low (level 4) and Unknown High (level 7). To investigate the anticipation of a painful stimulus, a 3 second auditory countdown preceded the laser stimuli (see Figure 6.1). The first auditory cue was presented concurrently with an anticipatory cue to indicate the forthcoming laser stimuli. The participant was either shown ‘Low’, ‘High’ or ‘Unknown’. The presentation of the word ‘Unknown’ indicated that the laser stimulus has an equal chance of being low or high. This was to investigate the importance of certainty in the anticipation of the laser stimuli. The image was also used as a visual fixation cue to discourage eye movements. After the laser stimuli, the 0-10 numerical rating scale was shown on the screen and the participant rated
the intensity of the pain. The Young group used a handheld keypad to enter a pain rating, whilst the Old group verbally reported their pain rating. The order of the stimuli was randomised and separated into three blocks with short breaks in-between.

Figure 6.1: A schematic diagram of a single trial of the experimental paradigm. A computer monitor showed the participant a visual cue of; Low, High or Unknown from -3s to +2s. A three second countdown of beeps at -3, -2 and -1 allowed accurate anticipation of the laser stimuli at time 0s (red bar). The presentation of the visual cue at -3s was concurrent with the first auditory cue. The visual cue was consistent throughout the anticipation and laser stimulus. At +2s, an eleven-point number rating scale (NRS) was presented for the participant to rate the laser stimulus. For each condition (Low, High, Unknown Low and Unknown High) there were thirty trials. The trials were presented in a randomised order and divided into three blocks of forty trials.

6.3.b.iv. EEG recording

A BrainVision MR EEG cap was used to record from 63 scalp electrodes using a BrainVision-cap system [Standard BrainCap-MR with Multitrodes]. The arrangement of the electrodes was modelled on the extended 10-20 system. Recording parameters were set at: Filter (DC to 70 Hz), Sampling rate (1000 Hz), Gain (500). To reduce electrical interference, a 50Hz notch filter was applied. Prior to starting the laser protocol, resting states were recorded with eyes open and closed for two minutes in all participants. This ensured that the experience prior to the experiment was consistent for all participants.

The difference in pain rating methodology was due to the participants within the Old group were the control group for the investigation of pain processing in people with Parkinson’s disease. As the Parkinson’s patients had limited movement, the pain rating was completed verbally in both groups. The addition of the keypad for pain rating within the Young cohort was the return to standard practice of the experimental protocol.
was identical. The three experimental blocks were recorded separately to allow for better artefact rejection of the EEG data.

6.3.c. Analysis Methods

6.3.c.i. Statistical analysis of behavioural data

Statistical analyses of the behavioural measures were carried out using IBM SPSS Statistics 22 software. Prior to using statistical tests, the data was assessed for normality using a combination of Q-Q plots, histograms, and the values of skew and kurtosis. Normally distributed data was analysed using independent t-tests and ANOVA tests, whilst data reported to be not normally distributed, the appropriate non-parametric test was utilised. Specific statistical tests for each analysis step are reported in the results section.

6.3.c.ii. EEG analysis method

EEG pre-processing was carried out using EEGLAB toolbox (Delorme and Makeig, 2004) in MATLAB version R2015a (The Mathworks Inc) whilst statistical analysis was carried out using SPM12 toolbox (Wellcome Department of Imaging Neuroscience, Institute of Neurology, UCL, London, United Kingdom) running in MATLAB.

6.3.c.ii.a. EEGLAB Pre-processing

Pre-processing consisted of; removal and interpolation of bad channels, down-sample to 500, low-pass filter of 20Hz and re-reference to the common average. The four conditions were separated and -3500ms to 2000ms epochs extracted and Linear detrend applied. Independent Component Analysis (ICA) was carried out on all datasets using the SemiAutomatic Selection of Independent Components for Artifact correction (SASICA) toolbox to select components to remove via pre-determined thresholds. The thresholds were set to; Autocorrelation (threshold = 0.35 r, lag = 20 ms), Focal (threshold = 3.5 z), Focal trial (threshold 5.5 z), Signal to noise (period of interest (POI) = [0 Inf], baseline (BL) [-Inf 0], threshold ratio = 0.5), and Adjust Selection enabled. The thresholds were sufficient to remove artefacts
from the majority of the datasets; however, a number of datasets required further manual removal of eye-blink components where not picked up by SASICA.

6.3.c.ii.b. SPM EEG Analysis

The pre-processed datasets were converted to Statistical Parametric Mapping (SPM) compatible files. Statistical analysis was carried out to investigate the anticipation evoked potentials and the post-stimulus LEPs using scalp-level and source localisation analysis techniques available in the SPM toolbox.

SPM scripts for batch processing were used to analyse the EEG data at the scalp level and source localisation. Two baselining methods were applied to the data analysis for the anticipation phase and were applied for scalp-level and source localisation analysis (see 2.1.b.ii). Primary analysis applied distinct baselines (BLs) of 500 ms, occurring prior to each of the three auditory cues respectively, to analyse each of the three anticipation phases. The BLs and time window of interest (TWOI) were as follows; Early [BL: -3500 ms -3000 ms: TWOI: -2500 ms -2000 ms], Mid [BL: -2500 ms -2000 ms: TWOI: -1500 -1000 ms] and Late [BL: -1500 -1000 ms: TWOI: -500 0 ms] anticipation phases. The secondary analysis method applied a single baseline of 500 ms prior to the first auditory cue [BL: -3500 ms -3000 ms] that was common to every TWOI anticipation phase (early, mid and late). This second analysis was conducted to enable comparison to previous studies (Brown et al., 2008a; Brown, El-Deredy and Jones, 2014). All statistical analysis for the anticipation phase was adjusted for multiple comparisons.

All analysis for the post-stimulus phase TWOI [200 ms 600 ms], centred on the LEP, was baseline corrected to -500 ms prior to the laser stimulus [BL: -500 ms 0 ms].

6.3.c.ii.c. Source Localisation analysis parameters

SPM12 EEG and MATLAB scripts were used to estimate the sources of the anticipation- and laser-evoked potentials using Low Resolution Electromagnetic Tomography (LORETA). The forward model was created using an 8196 vertex template cortical mesh coregistered to the electrode positions of the standard 10-
A three-shell boundary element model (BEM) EEG head model available in SPM12 was used to compute the forward-model. The images were smoothed with a 12mm full-width-at-half-maximum (FWHM).

### 6.3.c.ii.d. EEG Analysis Statistical analysis

The EEG analysis was analysed via an analysis of covariance (ANCOVA) with covariates of laser energy and average pain rating. All data was assessed for outliers using SPM’s boxplot function and only outliers labelled as extreme outliers were removed. The linear relationship between the covariates and the EEG data was assessed via extraction of non-adjusted Eigenvariate data from the significant clusters reported in the SPM statistics output. The homogeneity of regression slopes was assessed between age and each covariate for each group. The data had no outliers and the assumptions required for an ANCOVA were met.

For the analysis of the anticipatory TWOIs, a three way repeated measures ANOVA was applied, with one between-subject factor of Group (Young vs Old) and two within-subject factors of Certainty [Known (Low/High) v Unknown] and Expectation [Low v High (Known)]. For the analysis of the post-stimuli TWOI, a three way repeated measures ANOVA was applied, with one between-subject factor of Group (Young vs Old) and two within-subject factors of Certainty [Known (Low/High) v Unknown] and Intensity [Low v High (Known and Unknown)].

Source localisation results were reported as follows. To control for multiple comparisons, a cluster-forming threshold of p<0.001 was used and resulting clusters were considered significant at FWE (p<0.05). Significant clusters were also restricted to >100 voxels in size and regions labelled using the Anatomical Automatic Labelling (AAL2) toolbox in SPM.
6.4. RESULTS

6.4.a. Laser behavioural results

The previous research showing an age-related reduction in sensitivity to experimentally induced pain was investigated via comparing the sensitivity to the laser in the Young and Old groups. An independent t-test reported a significantly higher tolerance in the older group (Mean ±SD: 2.20±0.25) in comparison to the young group (Mean ±SD: 1.93±0.43), F(1,48)=9.52, p=0.009.

To assess any differences in the rating of pain between the two groups, a mixed three way ANOVA was carried out on the pain rating with a between factor of Age (Young v Old), and within factors of Certainty (Known v Unknown) and Intensity (Low v High). A group difference was reported showing that the old group had a lower rating of the pain stimuli than the young group [F(1, 48)=34.90, p<0.000] (Figure 6.2). This indicates that although the laser was calibrated to induce a subjective moderate pain prior to the main experiment via the psychophysics procedure, the older group significantly reduced their rating for the same intensity when it was delivered in the main protocol.

The effect of certainty on the rating of the Low and High pain stimuli was consistent between the two groups [F(1,48) = 55.27, p<0.000] such that the certainty (Known (Low or High) v Unknown) of the visual cue affected the rating of Low and High stimulation with contrasting results. The known Low intensity was rated lower than the unknown Low condition, whilst known High intensity was rated higher than the unknown High condition (Figure 6.2).
6. An EEG investigation of age-related differences in acute pain

Figure 6.2: Pain rating of laser stimuli. A) The pain rating of the laser stimuli is shown for each laser condition. The Young (blue) group rated the pain significantly higher than the Old (red) group. B) The effect of certainty on the rating of Low and High was consistent in both groups, such that the Unknown Low was rated higher than known Low, whilst Unknown High was rated lower than known High. NRS: Number rating scale, Low: ‘Low’ visual cue and low laser intensity, High: ‘High’ visual cue and high laser intensity, UnLow: ‘Unknown’ visual cue and low laser intensity, UnHigh: ‘Unknown’ visual cue and high laser intensity. Data is plotted as mean ± SD.

To assess whether either group habituated to the laser stimuli within the main experiment, a linear regression was applied to establish whether the rating of the Known High stimuli could be predicted by trial number. The known high stimuli was selected for the habituation analysis due to it being calculated by identical protocols within the psychophysics in each group, and so that the effect of uncertainty did not need to be accounted for. We concluded that trial number did not predict pain rating for the Young [F(1, 28) = 1.042, p=0.316] or Old [F(1, 28) = 3.325, p=0.079] (see Figure 6.3).
Figure 6.3: The pain rating for *Known* High stimuli was averaged across participants within each group at each trial number. There was no association between the trial number and pain rating within either group which is indicative of no habituation to the laser stimuli during the main experiment. NRS: Number rating Scale.

6.4.b. EEG Results

6.4.b.i. Sensor-level

The waveforms at Cz are presented in Figure 6.4 and present the individual waveforms for participants in the Young and Old groups. The plots present the spread of the data and the average for each group (black line). The averaged waveform for each group is shown in Figure 6.5 and shows that the Old group had a distinctively lower degree of activity during the stimulus preceding negativity (SPN), a representation of anticipation, and a smaller LEP. This difference in the activity between the Young and Old groups can also be seen in the topography plots in Figure 6.5.
Figure 6.4: Waveforms at Cz electrode for all participants in the Young and Old groups. Both plots show the stimulus preceding negativity (SPN) during the anticipation of the laser stimuli (0 ms) and the subsequent laser evoked potential (LEP). Each waveform represents a participant’s waveform averaged across all trials and the solid black line shows the averaged response within each group. Data is baselined to -3500 -3000.
A) Grand average waveforms for each group

![Waveform Graph]

B) Grand average topographic plots for each group

![Topographic Plots]

Figure 6.5: ERP waveforms and topography plots. A) The average waveform at Cz electrode of the anticipatory and post-stimulus response. Red: Old, Blue: Young. The data is presented as an average of all laser-conditions and baselined to [-3500 – 3000 ms]. The waveforms of the young and old groups are distinctively different, with the young group showing a higher stimulus preceding negativity (SPN), and larger laser-evoked potential (LEP). B) The topoplots represent the average activity at each electrode for all participants in each group. The difference between Young and Old is also shown.
6. An EEG investigation of age-related differences in acute pain

6.4.b.i.a. SPM analysis

Both baselining methods reported the same sensor-level differences between the young and old groups. For clarity and to allow reference to results from Chapters 3, 4 and 5, here we will show statistical results using the primary baseline method using baselines prior to auditory cues.

The Young group showed a significantly higher degree of activity during anticipation. The characteristic topography of anticipatory processing is a negative response over dorsal regions and a positive response over posterior regions. Our results of comparing the Young and Old groups show two distinct locations of significant clusters which are located in the centre of the top of the head (dorsal) and the back of the head (posterior (Figure 6.6). Hence, the statistical results along with the topographic plots (Figure 6.5) show that the Young group have a significantly higher anticipatory response compared to the Old group. This interpretation is supported by the SPM T-contrasts which report which factor (i.e. Age group) had a more positive EEG amplitude in the significant clusters (Table 6.1). The Young group showed a more negative dorsal response (as the Old group showed a more positive response: Old>Young), and a more positive response over posterior regions (Young>Old).

<table>
<thead>
<tr>
<th>Anticipation</th>
<th>Early</th>
<th>Mid</th>
<th>Late</th>
<th>Post Stimulus</th>
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</thead>
</table>

Figure 6.6: Topographic plots of the SPM F-statistics for the main group effect during anticipation and post-stimulus time windows. The results are restricted to FWE correction and clusters of >100 voxels. The red arrow indicates the highest peak value.
Table 6.1: Scalp-level statistical results for whole-scalp analysis. Group differences were found within all time-windows. The significant clusters were reported at FWE correction with a threshold of p<0.025 to account for multiple comparison (due to two baseline methods). The results are divided into main F-contrast and post-hoc T-contrasts. The MNI coordinates are reported as the peak voxel response within the cluster and described with x and y coordinates and time (ms). The interpretation of the T-contrast comparisons are such that the > sign denotes which factor (i.e. Age group) had a more positive EEG amplitude within the cluster. Group = Young v Old, FWE = Family-wise error K = Number of voxels, F/T = F-contrast/T-contrast, Z = Z-score.

<table>
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<th>Time (ms)</th>
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6.4.b.ii. Source localisation

The source localisation analysis using the LORETA technique did not highlight any significant contrasts between the two groups.
6.5. DISCUSSION

Here we have indicated that there may be an age related difference in pain processing. We have shown that the younger cohort were more sensitive to the laser stimuli and rated the pain higher than the older group. We have also shown via scalp-level EEG analysis that the younger cohort showed a higher degree of anticipation related activity and larger response during perception of pain. Although caution must be taken when interpreting the results due to several limitations of the investigation (see section 6.4.c) the results are indicative that the central processing of pain may change with age.

6.5.a. Pain sensitivity

Firstly, the older group were less sensitive to the laser stimuli during the psychophysics procedure and required a higher voltage of the laser. The finding that the older cohort was less sensitive to the laser stimulation supports previous knowledge of increased tolerance of a range of experimentally induced stimuli. The experiments are reviewed in detail by Gibson and Farrell, 2004, and collates research to show either an age related increase, or no change, in pain threshold to heat, electrical and mechanical stimuli.

The psychophysics procedure allowed for the calibration of the laser voltage to the individuals’ subjective sensitivity. Although the psychophysics protocol was successful within both groups to induce a high (level 7) pain, the Old group reduced their rating of the pain when the main experiment was started using the previously calibrated voltage. Therefore, we have demonstrated that age can affect the efficacy of the psychophysics protocol used to calibrate to a subjective experience. This highlights the benefit of using a strict age range in pain research to limit the variability. The reduction in the rating of the laser stimuli must be considered when interpreting the results and are discussed in Section 6.4.c.
6.5.b. Interpretation of anticipation phase in EEG results

Here we have shown a reduced degree of activity during anticipation in the older group compared to the younger group. One potential explanation of this finding is to consider the age-related changes in an individual’s relationship with pain stimulation. The relationship with pain changes with the experiences that we have, and as such our response to pain will ultimately change as we grow older. The top-down processes such as context, prior experience and emotional state, are all able to modulate the perception of pain and are likely to change with age. There is a theory coined as the ‘positivity effect’ whereby older people are more likely to favour positive stimuli over negative stimuli within cognitive tasks (Reed and Carstensen, 2012). An fMRI study reported a reduced activation within the striatum and insula during the anticipation of monetary loss in the older cohort in comparison to the younger group (Samanez-Larkin et al., 2007). This age-related reduced activity was not observed during the anticipation of monetary gain. Therefore, the reduction in brain activity during anticipation of the laser stimuli that we have seen in this study may be analogous to the age-related difference seen associated with the ‘positivity effect’ (Reed and Carstensen, 2012; Samanez-Larkin et al., 2007), i.e. older subjects have a tendency to pay less attention to negative cognitive input. In addition, there is evidence to show that psychological resilience is increased within the older population (Cicchetti and Cohen, 2006; Ong, Bergeman and Boker, 2009). As such, within the older population, the noxious stimuli may have been assigned a lower salience value in comparison to the younger cohort, and resulted in a reduced level of activity during anticipation.

The development of chronic pain has been associated with dysfunctional brain activity during the anticipation of pain (Gracely et al., 2004; Brown, El-Deredy and Jones, 2014; Loggia et al., 2014). Therefore, in people with chronic pain, the normal age-related reduction in the anticipatory activity to pain stimuli could be different and represent abnormal top-down processing of pain. It would be interesting to establish whether older people with chronic pain show the same ‘positivity effect’ seen in the healthy population. It could be hypothesised that older people with
chronic pain would not show the typical disfavour for negative stimuli which is seen in the healthy population. As such, if a higher salience was assigned to the negative stimuli, and a higher degree of activity occurred, this may help to explain the persistence of chronic pain.

6.5.c. Limitation of interpretation

The interpretations of these results are constrained by several limitations associated with the comparison. Here we will outline the limitations and provide suggestions of interpretation.

6.5.c.i. Recruitment

One constraint of interpretation includes the recruitment of the participants being from two different studies. The participants within the Young age group were part of a repeated-measures, double-blind and placebo-controlled study which investigated the role of dopamine D2 receptors in pain perception. The data used for this comparison is taken from the control condition when they received the inactive compound Sodium Chloride (20 mg). Although this would have no direct physiological effect, there is potential that the knowledge that it could be a dopamine modulating drug could induce a difference in their response to the experiment. In contrast, the Old group were the control group in an investigation of pain perception in Parkinson’s disease, and no intervention was administered.

6.5.c.ii. Difference in Pain Rating

Although the psychophysics procedure successfully calibrated the laser voltage to induce a high pain in both age groups, the resulting pain rating during the main experiment was significantly lower within the older group and demonstrates that the older participants habituated to the laser stimuli between the psychophysics and main experiment. Importantly, the rating of the stimuli within the main experiment did not show any habituation within either group. The drop in pain rating was not shown in the Young group and the pain rating was hence used as a covariate within the EEG data analysis. Although the covariate was included, it is
difficult to conclude whether the decrease in activity during anticipation in the Old group was not merely due to the participants anticipating a lower intensity of pain.

Whilst the young group rated the pain via a numeric keypad, the older group reported their ratings verbally. The different techniques may have led to the difference in pain rating due to the impact of psychological and social factors when rating verbally in comparison to via a personal keypad. There is evidence that the environment and social context of pain experiments can influence pain rating. For instance, males are likely to reduce their pain rating when the researcher is female in comparison to a male researcher (Levine and De Simone, 1991). Furthermore, stoic behaviours of hiding pain severity have been shown to be a good predictor of reductions in self-reported pain intensity (Yong, 2006; Cantwell et al., 2013). Therefore, the verbal pain rating in the Old group may have been sensitive to the additional psychological factors such as resilience (Ong, Bergeman and Boker, 2009), stoicism and rapport with the researcher, in comparison to the Young groups use of the keypad.

6.5.c.iii. Age-related changes in EEG recording

Another consideration of comparing younger and older participants is the differences in the EEG recording. A study by Duffy et al., (1984) reported no age-related change in alpha frequency or amplitude, however, did report a dysfunction of EEG desynchronization. In addition, a study which investigated the visual evoked potential (VEP) in EEG recordings of young and middle aged adults concluded that the older cohort showed a lower VEP amplitude and delayed latency (Dustman et al., 1990). Although there is limited information regarding EEG age-related changes, there are widely discussed differences in fMRI recording. A comparison in the overall functional response to cerebral activity in fMRI highlighted that the older participants showed a lower amplitude in the BOLD response compared to the younger participants (Ross et al., 1997; Madden et al., 2004; Juckel et al., 2012). Therefore, an age-related decline in neuroimaging may have produced the reduced response seen in the older cohort. Further investigation is needed to establish
whether the reduced response is a consequence of reduced anticipation of pain, or a result of a decline in neuroimaging quality.

6.6. CONCLUSION

Our investigation further strengthens the knowledge that tolerance to experimentally induced pain is increased with age. We have also highlighted a potential age-related reduction in brain activity during the anticipation and perception of pain, and highlighted methodological considerations for experimental paradigms in different ages. Although there are limitations which impair a confident conclusion of the comparison, the reduced response may be highlighting the ‘positivity-effect’ within older cohorts whereby they show a reduced neural response to negative outcomes. We have also shown that the current psychophysics procedure is sensitive to age and is an important protocol choice for future studies. Further investigations are required to understand the differences in pain perception in young and older cohorts.
Chapter 7

General Discussion
7.1. MAIN AIM OF THESIS

The main aim of the research conducted in this thesis was to further understand pain processing in Parkinson’s disease and to establish whether abnormal top-down processing could help to explain the high prevalence of chronic pain seen in Parkinson’s disease. The main focus of the research was to investigate the brain activity during anticipation of pain as a technique to research top-down modulation during pain processing.

The first study investigated pain perception in people with Parkinson’s disease whilst off (Chapter 3) and on (Chapter 4) their medication. The second study explored the role of dopaminergic transmission in pain perception in young healthy volunteers (Chapter 5). And finally, age-related changes in pain perception were explored (Chapter 6).

7.2. SUMMARY OF EXPERIMENTAL WORK

Chapters 3 and 4 describe the Parkinson’s disease patient study which investigated the brain activity during anticipation and perception of pain in the patient group and healthy age-matched controls.

Firstly, Chapter 3 reviews the results from the study conducted whilst the patients were withdrawn from their medication. The behavioural results reported that the PD group, compared to the healthy age-matched controls, were more sensitive to the laser stimuli, showed higher scores in pain catastrophising, and yet showed no difference in their mood or cognitive states (assessed via HADS and MoCA respectively). Brain activity was assessed using LORETA source localisation and concluded that there was an increased activation in the midcingulate region within the PD group during mid and late anticipation in comparison to the control group. This amplified response was independent of the PD motor severity, PCS, HADS and chronic pain severity.

Chapter 4 (on) summarises the results from when the experimental protocol was repeated in participants from the study reported in Chapter 3 (off) whilst the PD
patients were on their Parkinson’s medication. The behavioural results showed a higher sensitivity to the laser in the PD group, which is consistent with conclusions drawn in Chapter 3 (off). The EEG data reported no differences between the PD group on their medication and the healthy controls. Caution must be taken in drawing firm conclusions due to the following limitations of the study: no randomisation of experiment order, repetition of the experiment on the same day, a potential time-of-day effect and a low n number. However, the study indicates that the higher pain sensitivity seen in the PD participants is not affected by increasing dopamine and suggests that dysfunction of other neurotransmitters such as Serotonin or Noradrenaline may be to blame. The lack of difference in the EEG results between the HC and PD on their medication could indicate that the increase in dopamine ‘normalised’ the anticipatory response in PwPD seen following withdrawal of their medication, but it did not ‘normalise’ their pain sensitivity.

Chapter 5 (D2R) reviews the pharmacological manipulation of dopamine D2 receptors to assess the role of dopamine in pain processing. The investigation was carried out in young healthy volunteers and conducted using a repeated-measures, randomised, double-blind, placebo-controlled protocol. The dopamine manipulation did not change sensitivity to the laser, but EEG source localisation analysis did show a significant effect on brain activity during the anticipation and the perception of pain. The agonist (Cabergoline) and antagonist (Amisulpride) induced similar reductions in the parietal and temporal lobes during anticipation in comparison to the control condition. In addition, the antagonist induced a reduced activation in the insula during the receipt of pain stimuli in comparison to the control and agonist. Interestingly, the dopamine manipulation evoked a larger effect of certainty on pain rating, with the antagonist inducing a significantly amplified effect of the Unknown cue on the rating of the pain.

Chapter 6 (Age) reviews the differences in the behavioural and neural responses to the experimental protocol. The aim of the analysis was to shed light on the age-related changes in pain processing due to the higher degree of chronic pain within the aging population. The comparison highlighted a higher pain sensitivity and
anticipatory response in the Young group compared to the Old group. The reduced pain rating in the Old group and age-related difference in EEG recording may have influenced the results, and caution is needed when interpreting the results. Nevertheless, the study indicated that there is an age-related reduction in anticipatory processing to noxious stimuli.

The conclusions drawn from the EEG analysis were mainly from the pre-auditory cue baselining method. The method was carried out in conjunction with a single baselining method which did not highlight significant differences within the studies (except for the Age comparison: Chapter 6). A potential reason for this is due to the timing of the single baseline period (-3500 -3000 ms) being when a higher degree of movement of the participants would be expected which increased variability in the baseline time window. The PD participants especially were likely to use the interval between the trials to relax their suppression of tremors or to move into more comfortable condition, prior to the visual/auditory cue. The pre-auditory cue baselining method, which highlighted significant differences, may have shown differences due to the mid- and late- baselines being during focused attention on the experiment (i.e. low variability). Due to two baselining methods being used, the statistical significance was adjusted accordingly, and ensured reliability of the results.

The conclusions drawn from each chapter and how they contribute to the understanding of pain processing, both in Parkinson’s disease and the general population, will be discussed in the following sections. Considerations for future research and details of potential further research will also be discussed.

7.3. AN INTERPRETATION OF THESIS STUDIES

We propose that the dysfunctional dopaminergic system within the Parkinson’s brain results in a disruption in the top-down modulation of pain, which predisposes PwPD to develop chronic pain.
The three main studies *(Chapters 3, 4 & 5)* which investigate pain in Parkinson’s patients off and on dopamine medication, and the effect of dopamine modulation on healthy participants, contribute to the understanding that dopamine plays a role in top-down modulation in pain processing, whilst demonstrating that it is unlikely to be central in coding the intensity of pain. This thesis can draw this conclusion because we have shown no change in pain sensitivity following dopamine manipulation, yet have shown changes in brain activity prior to pain stimulation, a representation of top-down processing, following dopamine modulation. Top-down modulation encompasses various neural processes associated with processing nociceptive information such as: cognition, attentional orientation and emotional aspects. Therefore, we conclude that changes in dopamine levels present in Parkinson’s disease is likely to cause disruption within these processes and may result in long-term changes in the brain’s ability to integrate nociceptive information accurately and increase susceptibility to the development of chronic pain.

**7.3.a. Behavioural response**

Firstly, the sensitivity to the laser stimuli was not shown to be modulated by dopamine levels in the PD study or D2R study. The PD participants had a higher sensitivity to the CO\textsubscript{2} laser whilst both off and on medication. This finding was also shown by Tinazzi et al., (2008) using CO\textsubscript{2} laser stimulation, and Djaldetti et al., (2004) using a heat thermode. In contrast, (Brefel-Courbon et al., 2005) showed a reduction in sensitivity to the cold pressor test (CPT) in PwPD following Levodopa medication. The difference in the effect of dopamine medication may be due to the differences in the method of inducing experimental pain. The CO\textsubscript{2} laser stimulation is comparable to an acute pain, whereas the CPT is regarded to be more analogous to chronic pain (Rainville et al., 1992). Therefore, due to dopamine’s potential role in modulating top-down processes during pain perception, it would be more likely that the PD dopaminergic medication would improve CPT tolerance in contrast to acute CO\textsubscript{2} laser stimulation.
In addition, the manipulation of D2Rs, via **Cabergoline** and **Amisulpride**, in healthy participants also did not modulate the individual’s sensitivity to the laser. The higher sensitivity to the laser in the PD participants (both off and on medication), and no differences seen in the D2R study participants, indicates that the sensitivity in PD patients is likely to be associated with other neurotransmitters such as serotonin, noradrenaline and/or opioids.

Furthermore, the D2R study concluded that the manipulation of dopamine changed the effect of certainty on pain rating of the two pain intensities. This shows that there is dopamine-dependent processing during the anticipation of pain which results in the alteration of the pain rating and strengthens our theory that dopamine is involved in the cognitive/top-down processes related to pain perception. However, it is important to state that the PD group did not show evidence of an altered effect of certainty on pain ratings which was present following the D2R manipulation. The translatability of results from a young healthy cohort to Parkinson’s disease is limited due to age-related differences in nociceptive processing, and the multitude of neurological changes present in the PD brain. Yet a better understanding of dopaminergic transmission in nociceptive processing will help to better understand, and treat, dysfunctions of pain processing.

**7.3.b. Neural response**

A difference in brain activity during the anticipation of the laser stimuli was present in the PD patients off their medication, and the D2R agonist and antagonist conditions, and thus indicates that there are dopamine dependent processes prior to the perception of pain. As previously discussed in Chapter 2, brain activity which occurs prior to the perception of pain can represent top-down modulation of pain perception, whereby factors such as cognition, attention and emotional state can manipulate the perception of the pain.
7.3.b.i. Neural response in PD

The increased anticipatory activity within the MCC/SMA of the PD patients whilst off their medication is hence highlighting an underlying dysfunction of pain-related processing. The amplified response was not observed following levodopa and suggests that dopaminergic hypofunction may be responsible for the amplified response. Therefore, the degeneration and long-term disruption in the dopaminergic pathways seen in the PD brain are likely to result in persistent disruption of top-down processes. In addition to the deterioration of the dopaminergic systems potentially impairing central processing of pain, a possible ‘side-effect’ of dopamine treatments for patients in the early stages of PD is dopamine hyper-function following L-DOPA administration (Poletti, 2018). Dopamine hyper-function has been linked with aberrant salience assignment whereby a neutral stimulus is attributed as a salient event (Poletti, 2018; Nagy et al., 2012; Poletti et al., 2014). Therefore, the fluctuations between hypo- and hyper-function of dopaminergic processes are likely to disrupt the interpretation of pain.

7.3.b.ii. Neural response following D2R manipulation

The manipulation of the dopamine D2R in young healthy participants also induced changes in central processing during the anticipation of the laser stimuli and highlights a potential role of dopamine activity in the central processes related to pain. A common compensatory response to the loss of dopamine in PD is a significant increase in the abundance of striatal D2 (and D1) receptors (Hassan and Thakar, 1988; Ryoo, Pierrotti and Joyce, 1998), and drug-naïve PD patients have shown supersensitivity in the D2R binding. A study by Christopher et al., (2014) have also shown a reduction in D2R in the striatum and insula, within PwPD who also presented with mild cognitive impairment (MCI). In theory, the change in the anticipatory activity within regions associated with sensory integration and pain perception in young healthy volunteers following D2R manipulation may highlight that the pathophysiological changes in the D2R in the PD brain could also disrupt pain processing within these regions. The potential reasons for why the neural
response during anticipation was different in the PD patients and young healthy volunteers following D2R manipulation are discussed in the forthcoming section.

The D2R study also showed a reduction in the Insula during the perception of the laser stimuli within the antagonist group. The Insula is a region sometimes considered to be involved in coding pain intensity, however, there is also evidence that it has a wider role in the integration of sensory and affective processing in pain (Derbyshire et al., 1997; Mazzola et al., 2010; Lu et al., 2016; Singer et al., 2004b). The fact that the altered activity within the Insula within the antagonist condition did not alter pain rating or pain sensitivity, supports the theory that the Insula is not solely intensity coding, and further supports our theory that dopamine is not central to intensity coding.

7.3.c. Parkinson’s Disease vs D2R manipulation in healthy participants

The D2R manipulation in the young healthy group induced a different alteration in neural activity that was shown in PD patients whilst off their medication (Chapter 3). Therefore, the amplified response in the MCC/SMA within PwPD is likely to not be solely due to altered D2R activity. This would be expected as PD is not a disorder of D2Rs, and in reality is the combination of impaired signalling of numerous neurotransmitters and structural changes across the brain. In addition, the acute manipulation of D2Rs in healthy volunteers cannot be directly compared to the chronic changes that have occurred in the PD brain. In addition, the action of taking long-term medication, and the action of medication withdrawal will have also produced unique differences in the PD group.

The PD participants off their medication have low striatal dopaminergic processing and Chapter 3 concluded that they have an increased anticipatory activity within the MCC/SMA prior to the pain stimuli. In contrast, the dopamine D2R antagonist in young healthy volunteers evoked a reduction in activation in the temporal/parietal regions during the anticipation of pain stimuli. The global loss of dopamine and the antagonism of the D2R hence resulted in opposing effects on the degree of activity during anticipation, and within different regions. There are numerous reasons for the differences observed. Firstly, the global loss of dopamine in PwPD is different to
the specific agonism/antagonism of the D2R, and therefore, cannot be directly compared. In addition, the neurodegeneration associated with the PD brain such as the deterioration of the dopaminergic, serotonergic and noradrenergic systems, along with any age-related differences, mean that a direct comparison cannot be carried out. Nevertheless, as stated previously, the D2R-related changes in the healthy brain during anticipation of pain, and the changes in the D2R within the PD brain, may further suggest an impaired central processing of pain in the PD brain.

7.3.d. Age, Pain and Dopamine

Chapter 6 discussed the finding of reduced brain activation in the older cohort compared to the young control participants. Due to the focus of this thesis being on the dopamine’s role in pain perception, an extra consideration to discuss is the age-related decline in dopamine (Volkow et al., 2000, 1996b; a; Suhara et al., 1991). There is evidence that dopaminergic activity changes with age. For instance, the D2 (Volkow et al., 1996b) and D1 (Suhara et al., 1991) receptors, and dopamine transporters (Volkow et al., 1996a) decline with age, and the positive correlation between midbrain dopamine level and frontal cortex activity changes to a negative correlation (Dreher et al., 2008). In addition, within healthy aging, there is a reduction in the D2R within the putamen and caudate, which correlates with a reduction in motor and cognitive tasks (Volkow et al., 1998). Thus demonstrates that the age-related reduction in the dopaminergic system can result in the changes in the performance of dopamine-dependent processes.

We can only speculate that the natural reduction in dopamine and the decline of dopaminergic processes may indicate that if the dopamine level in the healthy aged population becomes too low, and drops below the ‘optimal’ level for performance, there is an impairment in the top-down aspects of pain processing. Theoretically, this could explain why chronic pain is more prevalence in the older population however, dopamine is only one of many age-related changes in the brain and no evidence as yet has investigated this relationship.
To the best of my knowledge, there are no investigations which specifically investigate the relationship of age-related changes in dopamine and its effect on pain processing. Future investigations would be valuable to establish whether age-related decline in dopamine contributes to the higher susceptibility of chronic pain in the older population.

7.4. SUMMARY OF INTERPRETATION

In summary, acute modulation of dopamine transmission affects the processing prior to pain perception. And although no change was seen in the pain rating, long term changes in dopaminergic transmission and the top-down processes which are dependent on dopamine, is likely to result in an impaired pain system which creates persistent and augmented perception of pain. Previous research has shown an inverted-u relationship between dopamine and the performance of central processes. Therefore, Figure 7.1 summarises the potential effect that dopamine level has had on central processing.
Figure 7.1: A schematic diagram of the relationship between dopamine level and performance of dopaminergic processes. It is important to state that the ‘performance’ is not directly correlated to the brain activity during the anticipation of the laser stimuli; rather that ‘performance’ is the effectiveness/efficacy/accuracy of dopamine neurotransmission. We suggest that a dopamine level outside of the optimal may induce an impaired top-down processing of pain.

Firstly, the low dopamine level in the Parkinson’s patients reduces the optimal performance of the dopaminergic processes, such as the precise prediction of stimuli, which may have resulted in the augmentation of the anticipatory activity prior to pain stimulation (can’t be for sure until we rule out serotonin, noradrenaline etc). However, following the administration of the dopaminergic medication, the dopamine levels were increased and anticipatory processing did not differ to the HC group. The age-related decline in dopamine has been taken into account such that the older Healthy controls (Blue Square) within the Parkinson’s study (Chapters 3 & 4) are positioned on the lower side of the optimal dopamine level. We propose that a de novo PD patient group would show a similar response to our off-medication group; yet variability would be expected based on the severity of their diagnosis, and the unknown impact of medication withdrawal and compensation mechanisms has had on our results.

The modulation of dopamine level via the D2 receptor agonist and antagonist is likely to have resulted in an increase and decrease of dopamine respectively. Both low and high dopamine has been reported to reduce performance in cognition, memory, attention due to sub-optimal dopamine levels. Therefore, the sub-optimal dopamine level induce a change in the central top-down processing of the forthcoming stimuli and also alter the effect of certainty on pain ratings.
7.5. NEUROIMAGING RESEARCH OF PAIN IN PARKINSON’S

The motivation of the research within this thesis was to address the concerning high prevalence of chronic pain in people with Parkinson’s disease, and to establish whether dysfunctional central processing within PD contributes to the persistence of pain. To establish the potential significance of the research within this thesis in the clinical setting, we need to understand where it fits in with the wider research and how together the research could be clinically important. Therefore, here we will summarise the research studies which shaped our theory of altered central processing prior to the commencement of the PhD (pre-2015), and also discuss the research which has been published during the PhD (post-2015).

At the beginning of the PhD, there was limited neuroimaging research published on central pain processing in PD. An early publication by Schott, 1985 highlighted the potential role of the dysfunctional dopaminergic pathways in altered pain in Parkinson’s disease, however, focused research on central pain processing is limited until the last decade.

7.5.a. Pre-PhD

Prior to starting the research within this thesis (pre-2015), there were few neuroimaging studies which focused on the central processing of pain in PwPD. A PET imaging study by Brefel-Courbon et al., (2005) showed that PwPD had an increased activity within the right insula and PFC, and the left ACC, during the cold pressor test compared to the healthy age-matched group. The augmented activity was attenuated following Levodopa administration. The research conducted by Schestatsky et al., (2007) and Tinazzi et al., (2008) investigated the LEP response to CO₂ laser stimulation using single or pairs of electrodes on the scalp. Schestatsky et al., (2007) concluded that the LEP response at the electrode position of Cz was amplified in PD patients who suffered from chronic pain, but not in those who did not have pain and controls. The difference was attenuated or disappeared following L-Dopa medication. In contrast, Tinazzi et al., (2008), recorded LEPs from electrodes at Cz and Fz positions, from pain-free PD patients, and reported a reduction in LEP
amplitude which was not affected by Levodopa administration. Therefore, the study by Brefel-Courbon et al., (2005) highlighted a difference in central pain processing in PD patients during the perception of pain. The research by (Schestatsky et al., 2007a) and (Tinazzi et al., 2008) applied similar techniques to conclude differences in the LEP in PD, however, the LEP is unlikely to shed light on alterations in top-down processing.

7.5.b. Post-PhD start

Following the start of the PhD (2015) there have been several neuroimaging studies published which strengthen the theory that PD pathophysiology alters pain processing and increase the likelihood of developing chronic pain. fMRI has been the most widely used technique and has highlighted differences in regions associated with pain processing in PwPD. Tan et al., (2015a) showed a reduced functional connectivity in the putamen and midcingulate cortex, and between the basal ganglia network and the salience network, during heat pain stimuli in drug-naïve PwPD without pain. Also a reduced activation of the temporal gyrus and insula was shown in subsequent analysis (Tan et al., 2015b). An fMRI and DTI investigation of PwPD with chronic pain indicated a thinning in several regions which are associated with pain processing (i.e. PFC, cingulate cortex & parietal areas), and an amplified response within the cerebellum and right inferior temporal areas, plus a reduction in the activity within the left frontal inferior orbital cortex (Polli et al., 2016). There was one study which also investigated the anticipatory response to laser pain in PD, and concluded that PD patients showed a reduced activation in the ACC and dorsal lateral PFC during anticipation and an augmented response in the MCC during the receipt of the pain (Forkmann et al., 2017). And finally, a study by Tessitore et al., (2018) showed that drug-naïve, pain-free PD patients showed an increase in neural activity within the left somatosensory cortex, left cerebellum and right pons, during heat pain stimulation.

Therefore, the research within this thesis in combination with other research also concluding altered central processing in PwPD (with or without pain) strongly indicates a role of altered central processing in chronic pain in PwPD. There is
therefore, a scientific rationale to promote the use of treatments which aim to improve central processing (i.e. meditation, cognitive behavioural therapy etc) in the treatment of pain in PwPD. For example, mindfulness meditation has successfully been used to reduce pain and is considered to improve cognitive processing important in modulating pain (Zeidan et al., 2012). Furthermore, EEG imaging has shown that meditation can alter anticipatory neural responses to painful stimuli and reduce the resultant unpleasantness ratings of the pain (Brown and Jones, 2010). In addition, there is increasing evidence of Cognitive Behavioural Therapy (CBT) as a treatment option for chronic pain. Along with improvements in pain severity, fMRI has highlighted the beneficial changes in connectivity between regions associated with nociceptive processing (Shpaner et al., 2014). Hence there is converging evidence that non-pharmacological ‘alternative’ treatments may be a valid treatment option for chronic pain which is considered to be due to dysfunctional top-down processes such as cognition, expectations, attention and emotion.

7.6. FUTURE CONSIDERATIONS

The limitations within the studies have been discussed within the individual chapters, however, there are general considerations which should be taken into account for future research.

7.6.a. Selection of pain stimuli

The use of the CO2 laser for pain stimulation was chosen for its optimal delivery speed and activation of nociceptive fibres and previous use in pain research (Brown and Jones, 2008; Clark et al., 2008b; Brown, El-Deredy and Jones, 2014). However, the technique does not come without limitations. Although the laser selectively activates the Aδ- and C-fibres which process pain, the activation of the C-fibres can sometimes result in a dull burning sensation which reduces the ability to distinguish the laser stimuli resulting in habituation. In contrast, due to the laser heating up the skin over time, there is also the potential development of sensitisation to the laser. The movements within a grid aim to avoid the development of sensitisation,
however, due to the limited size of the grid, it is likely that the same area of skin will receive more than one laser stimulus.

A further limitation of the laser which needs to be considered is the potential to cause long-lasting pigment change or skin damage. All participants included in laser experiments are informed of the likelihood of temporary pigment changes which last up to 6 weeks. However, there is a small chance that the pigment changes can last longer than this in a very small number of cases. Although there is a maximum of energy that the laser can be set to, the individual differences in skin sensitivity results in different reactions to the laser. There were no long-term changes in pigment change or skin damage within the studies included in this thesis.

There are several other options to induce pain experimentally which could be considered for future research. Firstly, the use of an electric shock allows for event related potentials (ERPs) analogous to the laser evoked potentials (LEPs). However, the stimulation activates all nerves and muscles below the electrodes and is not specific to pain fibres. Also, the anticipatory response to electric pain is different to that evoked by laser stimuli (Hird et al., 2018), plus the habituation of electrical stimuli is common (Bingel et al., 2007).

Another consideration is whether the use of acute pain stimulation is optimal to investigate chronic pain. A benefit of using acute pain is that it allows for the investigation of anticipation prior to the stimuli, a technique to assess the top-down processes that occur prior to pain which have been shown to be abnormal in chronic pain conditions (Brown, El-Deredy and Jones, 2014). In addition to acute pain, tonic pain induced via the cold pressor test (CPT) is also beneficial to research chronic pain due to it inducing persistent pain which requires motivational, attentional and emotional control (Verhoeven et al., 2010; Ahles, Blanchard and Leventhal, 1983), and can investigate coping strategies of individuals (Berntzen, 1987; Efran et al., 1989; Geisser, Robinson and Pickren, 1992). The CPT involves the submersion of the hand into cold water and has been used for the investigation of affective and cognitive factors which influence pain perception (Birnie et al., 2014). However, there is a high variability in the protocol of CPT between researchers and
leads to a limited ability to compare results between studies. The CPT has previously shown that Parkinson’s patients have a difference cardiovascular response during the CPT, and the tolerance of the CPT has been shown to be reduced in PD patients. However, to the best of our knowledge there have been no investigations of neural activity during CPT in Parkinson’s disease. Therefore, it would be beneficial to include the CPT in further neuroimaging investigations into chronic pain in PD.

7.6.b. Protocol Design

The laser paradigm has been used in both experiments and no changes were made to allow direct comparison between studies. The laser paradigm is a replication of previous research into chronic pain conditions; however, improvements could be made to the paradigm.

The current laser paradigm would be improved by including both innocuous and noxious stimuli to allowing conclusions to be drawn that the neural response is unique to the anticipation of noxious stimuli. For instance, the innocuous stimuli could be a tactile stimulus and be cued by a visual cue of ‘No Pain’.

In addition, the reduction in the pain rating observed in the older participants highlighted that an improvement to the protocol is needed so that the perception of the pain is maintained throughout the experiment so that the anticipation is to a consistent perceived intensity. Firstly, the psychophysics procedure could include a more randomised up/down method to calibrate the laser to the participants’ subjective level 7. Also, the voltage of the laser could be updated throughout the main experiment. Experience with the laser has led to the development of a formula (see below) which allows for a standardised change in laser voltage that is related to the average pain rating within each block of 30 laser stimulations.

\[ w \times (x - y) = z \]

\[ w = \text{Two increments of laser voltage} \ (0.12 \ V) \]
\[ x = \text{Average pain rating of High} \]
\[ y = \text{Desired pain rating} \ (i.e. 7) \]
\[ z = \text{Voltage increase} \]
7.6.c. Study Recruitment

7.6.c.i. Parkinson’s patients

For PD versus HC study, a possible improvement of the study design would have been to use additional control groups which divided the PD group into people with and without chronic pain, and healthy control group with no pain. This would allow any differences observed in the PD group to be independent of the presence of chronic pain and any abnormal brain response would hence be unique to PD physiology. An additional comparison with a chronic pain group would also be interesting to establish whether any maladaptive central processing within PD is common to all chronic pain conditions (such as FM) or whether the abnormalities are unique to PD.

In addition, the PD group could be better controlled by solely recruiting drug-naïve participants, single medication usage, and/or similar disease progression or duration. However, to remove the variability within the PD group would remove the true representation of the variety seen in the PD population. The recruitment of a small-specific participant is timely and often very difficult at a single-site experiment. The recruitment criteria being restricted to people able to be withdrawn from their medication already created a limited recruitment pool, and hence a further restriction of recruitment would have resulted in delays and a false representation of the PD population.

7.6.c.ii. D2R study

Within the Dopamine D2R modulation study, the participants attended three visits and carried out the same laser protocol. The order of the drug condition (agonist, antagonist or control) was randomised; however, there is a potential effect on neural processing when a protocol is repeated. A possible effect is that the participants’ response to the laser stimulation would be different on each visit, especially due to the stimuli being novel to the participant on their first visit. This potential effect was controlled by the participants receiving the three drug conditions in a randomised order. However, for future research, we propose the
use of a pre-study assessment to introduce the pain stimuli to the participants. This may reduce potential chance of a higher sensitivity to the laser being seen on their first visit compared to subsequent visits.

The choice to use a within-subject repeated-measure study design was selected over a between-subject design as the positives outweighed the negatives. For instance, a significantly higher n number would have been required for a between-subject study design. Plus there is a high degree of variability within individuals in their baseline dopamine, D2R properties, and pain perception, which could not have been controlled for and could have led to high variability within and between drug condition groups.

7.7. FUTURE RESEARCH

7.7.a. The role of Dopamine, Serotonin and Noradrenaline in pain perception

The focus of the research within this thesis is on dopamine’s role in pain processing due to PD being characterised by low dopamine levels. However, PD is also a result of other neurotransmitter changes, such as serotonin, noradrenaline, choline, and glutamate transmission. Therefore, understanding of the role of these neurotransmitters in pain perception will increase our understanding of what causes the potential dysfunction of central processes in PD.

Future research to compliment the findings within this thesis includes the investigation of tryptophan (precursor of Serotonin) and tyrosine (precursor of Dopamine & Noradrenaline) depletion in healthy volunteers. The design will be a repeated-measure, triple-crossover, placebo-controlled, double-blind study. The aim is to use the same laser and EEG protocol to investigate the effect of low levels of Serotonin and Dopamine/Noradrenaline, with additional testing of the CPT response due to its potential translatability to central processing associated with chronic pain. Results of the study will hopefully help to explain how low levels of
serotonin and noradrenaline (in addition to low dopamine), could contribute to impaired central pain processing in PD.

Preliminary findings of the study indicates that the depletion of serotonin and dopamine via their precursors Tryptophan and Tyrosine respectively, does not change pain tolerance to the laser stimuli or the cold pressor test. The lack of difference induced in pain perception in dopamine depletion is in line with our results presented in the D2 study within this thesis. However, the serotonin depletion has previously been shown to result in a decreased pain tolerance to laser stimuli (Martin et al, 2017). Therefore, our results may indicate that there is a degree of variability in the role of serotonin pain tolerance. EEG imaging indicated that serotonin depletion evokes amplification in the anticipation-evoked neural response to pain during the late anticipation time-window, plus increased activity during the receipt of the laser. In addition, there was evidence that serotonin and dopamine depletion reduced expectation- and certainty-evoked neural responses compared to the control condition. Therefore, preliminary findings are in agreement with our conclusions drawn within this thesis that dopamine and serotonin are involved in the central processing associated with processing nociceptive information, rather than coding the intensity of acute pain.
7.8. FINAL CONCLUSION

In summary, the research conducted within this thesis supports the theory of altered central pain processing in PD; and is likely to contribute to the high prevalence of pain in PD. The role of dopamine in causing the abnormalities is not confirmed, however, a possible normalisation of anticipation following dopamine medication, and altered pain anticipation during D2R manipulation, may indicate that dopamine is involved in the disruption in top-down processing seen in PD. Our conclusions are strengthened by the recent neuroimaging studies investigating pain in PD which have also confirmed altered central processing. Further investigations of global loss of dopamine, noradrenaline and serotonin will help to further understand the cause of these abnormalities. A better understanding of pain in PD is essential to provide better treatment and improve the quality of life for people with PD.
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