

2015 Phi Beta Kappa Honors Society
2015 Graduate with honors, Magna Cum Laude, Carleton College

Teaching and Outreach

2017-2020 Anatomy and brain labs instructor, University of Maryland School of Dentistry
2017 Collaborative teaching fellowship, Goucher College

Publications

Peer-reviewed journal articles

1. Abrams, K., **Krimmel, S.**, Johnson, S., Cieslowski, K., Strnad, H., Melum, A., & Kryder, C. (2017). Nicotine deprivation attenuates panic reactivity in smokers: Findings from a placebo-controlled nicotine patch study. *Depression and Anxiety, 34*(11), 996–1005.
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Abstract

Dissertation Title: Finding Islands of Structure in a Sea of Variance: Dimensions of Covariance Between Migraine Symptoms and Brain Connectivity

Samuel Raymond Kimmel, Doctor of Philosophy 2021

Dissertation Directed by: David A. Seminowicz, PhD

Professor, Department of Neural and Pain Sciences

Migraine is a heterogeneous disorder with variable symptoms and responsiveness to therapy. Current attempts to capture migraine variability through migraine subtyping are not informed by biology, ignore many migraine symptoms, and are not predictive of treatment responses. Taking advantage of neural network organization captured with resting-state functional connectivity (RSFC) and advanced statistical analysis, sophisticated symptom-brain mapping can now be performed. In aim one, I use a multivariate approach to relate clinical variability in migraine to RSFC, and find three dimensions of covariance between symptoms and the brain. Additionally, I show that the current subtyping of migraine does not adequately capture clinical heterogeneity. Instead, using the three identified dimensions of covariance, biotyping of migraine can be performed that does a better job of capturing migraine variability than the current field norm. In aim two I examine how RSFC can help to predict variability in migraine patient response to the mind-body therapy Mindfulness-Based Stress Reduction (MBSR), in the hopes of developing precision medicine for migraine. Finally, in aim three, I examine the mechanisms of MBSR by analyzing how MBSR changes functional connectivity to reduce the frequency of headaches. These findings suggest that novel approaches can

better capture migraine variability, paving the way for the development of personalized treatment of migraine.

Finding Islands of Structure in a Sea of Variance: Dimensions of Covariance Between
Migraine Symptoms and Brain Connectivity

by
Samuel Raymond Krimmel

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Dedication

To Joel Greenspan, for his guidance and mentorship.

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List of Abbreviations

aCompCor	Anatomical CompCor
AROMA	Automatic Removal of Motion Artifacts
BOLD	Blood Oxygen Level Dependent
CGRP	Calcitonin Gene-Related Peptide
CCA	Canonical Correlation Analysis
CLR	Calcitonin-like Receptor
ComBat	Combining Batches of Microarray Data
CSD	Cortical Spreading Depression
CSF	Cerebrospinal Fluid
CV	Canonical Variate
DAN	Dorsal Attention Network
DMN	Default Mode Network
EPI	Echo Planar Imaging
fMRI	Functional Magnetic Resonance Imaging
FPN	Frontoparietal Network
GAD-7	Generalized Anxiety Disorder 7
GM	Gray Matter
GPCR	G Protein-coupled Receptors
GSR	Global Signal Regression
ICA	Independent Component Analysis
ICHD-3	International Classification of Headache Disorders 3 rd Edition

LASSO	Least Absolute Shrinkage and Selection Operator
LASSO-PCR	Least Absolute Shrinkage and Selection Operator Principal Component Regression
LOOCV	Leave-One-Out-Cross-Validation
MBSR	Mindfulness-Based Stress Reduction
MBSR+	Enhanced Mindfulness-Based Stress Reduction
MNI	Montreal Neurologic Institute
MRI	Magnetic Resonance Imaging
NHA	Number of Headaches
PCA	Principal Component Analysis
PCS	Pain Catastrophizing Scale
PSQI	Pittsburg Sleep Quality Index
PHQ-9	Patient Health Questionnaire 9
PHQ-15	Patient Health Questionnaire 15
RAMP1	Receptor Activity Modifying Protein 1
R_c	Canonical Correlation
RDoC	Research Domain Criteria
RSFC	Resting State Functional Connectivity
ROI	Region of Interest
SMH	Stress Management for Headache
SMN	Sensorimotor Network

SN	Saliience Network
TE	Echo Time
TR	Repetition Time
T1w	T1 Weighted
5-HT	Serotonin

Chapter 1: Introduction

Epidemiology of Migraine and Clinical Characteristics

A migraine is a headache typically with a unilateral frontotemporal localization, pulsating quality, and of moderate to severe pain. During a migraine headache, nausea/vomiting, and/or phonophobia/photophobia occur. Left untreated, these headache attacks last 4-72 hours. To meet the current diagnostic threshold for migraine disorder using the International Classification of Headache Disorders 3rd edition (ICHD-3), five such attacks must have occurred (International Headache Society, 2018). However, symptoms can occur outside of the migraine attack. Beginning 1-2 days before a migraine 12-16% of patients experience prodromal symptoms like fatigue, difficulty focusing, stiffness, photophobia and phonophobia, nausea, visual difficulties, and yawning (Rasmussen & Olesen, 1992). For 1-2 days following an attack, some patients may experience postdromal symptoms, which are similar to prodromal symptoms. In Addition to pro-/post-dromal symptoms, approximately a third of migraine patients experience aura (Rasmussen & Olesen, 1992), which is almost always visual in nature (Cutrer & Huerter, 2007). An aura typically involves the appearance of scotoma or odd visual patterns that progress across the visual field with time (Queiroz et al., 1997). For those with aura, these symptoms may occur before the onset of any pain and serve as a warning of an impending headache.

Migraine is diagnosed using a categorical-polythetic approach, a standard approach in much of disease diagnosis that is currently hotly debated in psychiatry (Hengartner & Lehmann, 2017; Krueger & Markon, 2006). In categorical-polythetic approaches, there is a binary diagnostic outcome (i.e. a patient can or cannot have the

illness) and a diagnosis is made based on fulfilling a series of criteria. ICHD-3 recognizes four broad categories of migraine: episodic migraine with aura, episodic migraine without aura, chronic migraine, and probable migraine. Probable migraine stands out from other migraine subtypes because it is not a categorical diagnosis. Migraine with aura is further divided into typical aura with headache, typical aura without headache, brainstem aura, familial hemiplegic migraine (of which there are 3 types and an additional other loci version), sporadic hemiplegic migraine, and retinal migraine. Therefore in total there are 12 different categories of migraine diagnosis. Chronic migraine is distinguished from episodic migraine based on having more than 14 headache days a month for more than 4 months, with at least 8 migraine attacks each month (International Headache Society, 2018).

These migraine subtypes have important implications for migraine treatment and research. Clinical trials may only recruit chronic or episodic migraine patients, leading The United States Food and Drug Administration to approve therapies for only specific migraine subtypes. For instance, botulinum toxin and Calcitonin Gene Related Peptide inhibitors are only approved for chronic migraine patients (Edvinsson et al., 2018; Escher et al., 2017). This requires episodic migraine patients to seek off label prescriptions for these medications. Migraine subtyping has also influenced research, where it is common to recruit only specific subtypes of migraine patients for studies, limiting clinical heterogeneity. For instance, a study only including episodic migraine patients has a fairly narrow band of migraine frequency data, limiting the between subjects variability, potentially making within-subjects analyses more challenging. Therefore, the current subtyping of migraine greatly influences migraine research and treatment.

Approximately 15% of the global population is diagnosed with migraine (Stovner et al., 2018), with prevalence reaching as high as 20% in some regions. There is a consistent sex difference in prevalence, with a 2-3:1 female to male ratio (Rasmussen, 1995). With 73% of migraine patients experiencing more than 1 severe monthly attack (Lipton & Bigal, 2005), the illness confers immense disability (Steiner et al., 2013). In the United States of America, 13 billion dollars in lost productivity from migraine occurs annually (Lipton, Stewart, & Scher, 2001). In fact, migraine disorder accounts for 3% of all global disability associated with a specific illness (Leonardi & Raggi, 2013). The cost of therapy is generally inexpensive, and the majority of the economic burden of migraine is associated with indirect costs (Lipton, Stewart, Diamond, et al., 2001).

Pathophysiology of Migraine

The cause of migraine is unknown, and with over several centuries of study, many theories have been proposed (Eadie, 2005). The most traditional model of migraine is the neurovascular hypothesis arguing that an initial vasoconstrictive event in meningeal vasculature gives rise to vasodilation, stimulating trigeminal sensory nerves producing painful sensations. Vasodilatory peptides released by the trigeminal ganglia are centered in this process and could create a feed forward loop that results in a migraine attack (Silberstein, 2004). In the neurovascular model, the brain responds to a peripheral event and does not drive the migraine headache itself. As has been noted, the vascular hypothesis is a “great story wrecked by the facts” (Goadsby, 2009). One intuitive argument came from the pulsatile nature of migraine pain, which appeared similar in rhythm to the heart beat. It was argued that arterial pulsation and the resultant increase in vascular volume would transiently increase pain during migraine attacks, suggesting that

dilated vessels were already mechanically stimulating ganglia during migraine attacks. However, the rate of migraine pain pulsation is not related to arterial pulse rate (Ahn, 2010). Additional arguments for the vascular hypothesis was that vasoconstrictors treated migraine, whereas vasodilators caused migraine. While some vasoconstrictors are indeed therapeutic (Humphrey et al., 1990), many effective compounds have no effect on vascular diameter (Goadsby, 2009). Also, vasodilation alone does not appear necessary to trigger a migraine, as equally vasodilatory compounds pituitary adenylate cyclase activating peptide and vasoactive intestinal polypeptide both do and do not trigger migraines respectively, indicating no effect of vasodilation (Goadsby, 2009; Schytz et al., 2009). Finally, magnetic resonance angiography of spontaneous migraine attacks indicates migraine attacks are not accompanied by vasodilation (Amin et al., 2013). Therefore the earliest and perhaps best tested model of migraine, the neurovascular hypothesis, has been falsified.

Most recent models of migraine emphasize the brain more so than the neurovascular hypothesis did. There exist two hypothalamic models of migraine. Hypothalamic neurons are widely believed to be important for homeostasis (Waterson & Horvath, 2015; Williams et al., 2000, 2001). Unlike the neurovascular model that was only explanatory for headache pain, hypothalamic models attempt to explain a wider range of migraine symptoms, including prodromal ones. Prodrome symptoms like fatigue, depression, yawning and food craving, are all functions linked to the hypothalamus (Burstein et al., 2015). In one hypothalamic migraine model, hypothalamic neurons activate meningeal nociceptors by changing the parasympathetic/sympathetic tone in the meninges to favor parasympathetic (Burstein et al., 2015). The mechanism for

this is hypothalamic excitation of the superior salivatory nucleus which then activates parasympathetic ganglia, which in turn may release vasodilators and also sensitize the trigeminal ganglia, leading to migraine. In a second hypothalamic model, hypothalamic neurons detect deviations from emotional and physiological homeostasis and lower the threshold for trigeminothalamic transmission to the cortex, facilitating pain (Burstein et al., 2015). As noted above, these models can factor in migraine symptoms beyond headache pain, like prodromal symptoms. However, there are several major criticisms of these models. First, the general process described should not be selective for migraine as hypothalamic neurons are well established to be involved in pain across the body (Razavi & Hosseinzadeh, 2017), and therefore there should exist other disorders with prodromal symptoms that instead involve a spontaneous painful attack to other parts of the body (in a sense a non-facial migraine); however, no such disorder exists. Also, the model is based on the most stereotypical migraine symptoms, but not all patients experience prodromal symptoms (Rasmussen & Olesen, 1992). Overall, the hypothalamic models have not been rigorously tested relative to the neurovascular hypothesis.

Most other theories of migraine center on aura. Approximately one third of migraine patients experience aura and relative to other disorders, the comorbidity of migraine and aura is striking. Many in the field have assumed a causal link between the mechanisms of aura and migraine. Most theories of migraine centering aura focus on the presumed cause of aura, cortical spreading depression (CSD). CSD is conceptualized as a wave of increasing voltage followed by a sustained reduction in electrocortical activity (Borgdorff, 2018). Accompanying CSD is vasodilation and then vasoconstriction. Importantly, this theory of migraine can only be assumed to be causal in the minority of

migraine patients with aura, though some do argue for a ‘silent’ aura that is unobserved (Pietrobon & Moskowitz, 2013), which would seem to be an unfalsifiable hypothesis since it cannot be measured. The original work on CSD and aura came from Lashley in 1941 who characterized his own aura travelling at approximately 3 mm/min across his visual field (Lauritzen, 1994; Tfelt-Hansen, 2009). Later work by Leao in rabbits observed the now well characterized CSD, where mechanical stimulation of cortex while measuring electroencephalography produced a wave of excitations followed by inhibition (Tfelt-Hansen, 2009).

The literature on CSD has naturally focused on animal research given the invasive nature of the work. However, major differences exist between humans and standard research animals. One major difference is that primates are gyrencephalic whereas mice, rats, rabbits and birds are lissencephalic. Levels of stimulation capable of evoking CSD in lissencephalic organisms have failed in primates (McLachlan & Girvin, 1994). And the folding of cortex in gyrencephalic organisms greatly limits the spread of CSD across sulci (Borgdorff, 2018). Consistent with these results in non-human primates, mechanical, electrical, and chemical stimulation of the human cortex does not evoke CSD reliably (Borgdorff, 2018). CSD can occur in humans (Drenckhahn et al., 2012; Hartings et al., 2014), however, when CSD has been observed it does not appear to co-occur with aura (Dreier et al., 2006). Perhaps the best argument against CSD causing aura comes from magnetoencephalography studies where two studies observed DC-shifts in migraine patients, but after aura, thereby challenging a causal argument (Bowyer et al., 1999, 2001). Even if CSD is not the cause of aura, it could still play a causal role in migraine attacks. However, established anti-migraine medication fails to diminish CSD in rats

(Read et al., 2001). Therefore, the link between aura and CSD is tenuous as is the causal role of CSD and migraine.

Even if CSD was not the cause of aura, it is still possible that aura, or an aura related process, causes migraine (in at least the minority of patients with aura). However, there is also strong evidence that the mechanisms of migraine and aura are largely dissociable. For instance, cilostazol, a selective inhibitor of cAMP-degrading phosphodiesterase 3, evokes headaches in healthy people (Birk et al., 2006) and migraine like attacks in migraine patients (Guo et al., 2014), but it does not evoke aura in patients with migraine with aura (Butt et al., 2018). Other compounds like nitroglycerin (Afridi et al., 2004; Bonuso et al., 1989; Christiansen et al., 1999) and calcitonin-gene related peptide (Hansen et al., 2010) also trigger migraine like attacks in migraine patients without causing aura. This dissociation between migraine and aura indicates that aura is not necessary to trigger migraine attacks even in migraine patients with aura. Additionally, aura can occur without migraine, and aura does not always precede migraine attacks, arguing against aura causing migraine (Goadsby, 2001). Together, these data suggest that a clear causal link between aura and migraine is lacking.

In conclusion, despite many theories of migraine (Eadie, 2005; Goadsby, 2001; Waterson & Horvath, 2015), the causes of migraine are unknown. The most likely account for this major gap in knowledge is that migraine is an extremely heterogeneous and complex disorder, and a singular mechanism is unlikely to explain migraine attacks. Moreover, many theories have assumed that any symptom correlated with migraine (like aura) must be causal. Research into the causes of migraine would benefit from being more aware and skeptical of epiphenomenon. It appears clear that there is no single

mechanism of migraine attacks in the tens of millions of people with migraine. What is unclear is if current theories of migraine can explain migraine attacks in even a single person. It is possible that there are many different causal subtypes of migraine, and therefore every theory of migraine attacks will be insufficient when applied to a large and variable sample. Were this to be the case, superior subtyping of migraine may facilitate research into the underlying causes of the disorder.

Migraine as More than a Headache Disorder

Despite being characterized as a headache disorder, migraine patients often display other pathologies beside headaches, aura, and premonitory symptoms. For instance, migraine has a well-established link to depression with migraineurs being 3 times more likely to be diagnosed with depression than the general healthy population (Breslau et al., 1994, 2000). Also, anxiety disorder are the most common psychiatric comorbidity among migraineurs with prevalence estimated to be between 25-50% of patients (Fuller-Thomson et al., 2017; Oh et al., 2014). Sleep disturbances are also common, with 67% of migraine patients having sleep abnormalities relative to less than 10% in healthy controls (Karthik et al., 2012). Together, these comorbidities indicate that a more holistic view of migraine symptoms is warranted.

Aside from comorbidity studies, few researchers have examined the inter-relatedness of chronic pain symptoms in migraine. Sleep, affect, headache, disability, and pain related cognitions are clearly related to one another in migraine (Breslau et al., 2000; Lebedeva et al., 2017; Lin et al., 2016; Minen et al., 2016; Oh et al., 2014; Seng et al., 2017) and in other chronic pain disorders (Altindag et al., 2008; Arnstein et al., 1999; Finan et al., 2013; Kelman & Tanis, 2006; Lillis et al., 2017; Magni et al., 1994).

Unfortunately, many studies only examine relationships between pairs of symptoms (e.g. headache frequency and anxiety). One study has examined the relationship between 12 clinical features in migraine, including sleep, affect, pain severity, disability, general health, allodynia, and pain related cognition, and found widespread relationships between symptoms, with disability, pain severity, and depression being the most likely to associate with other symptoms (Krimmel et al., 2021). Therefore, migraine is more complex than simply a ‘headache disorder’ and often displays a wide range of associated clinical features from sleep disturbances to affective well-being that relate to one another.

Attempts to Capture Variability in Migraine

Despite the strong evidence that migraine consists of a complex range of symptoms, most migraine research has focused on only the most stereotypical symptoms, headache and aura, while relatively little effort has been made to characterize symptoms holistically. This can be seen by the current ICHD-3 subtyping of migraine, which is based almost exclusively on aura and headache frequency, and does not consider additional psychosocial variables. There are also many examples illustrating whether or not patients have aura is unrelated to treatment response: there were no differences in treatment response between patients with or without aura for topiramate (Silberstein et al., 2006a), zolmitriptan (Rapoport et al., 1997), or sumatriptan (Ashford et al., 1998a). Aura status also provides little information about the severity of other migraine symptoms. For instance, grouping patients into subtypes through latent class analysis of migraine symptoms did not support migraine with and without aura being distinct categories (Nyholt et al., 2004). Instead, different classes had roughly equal proportions of migraine with aura patients in them. An additional study did not report differences in

migraine attack characteristics like severity and location between migraineurs with and without aura (Rasmussen & Olesen, 1992). Therefore, the current stratification does not help to explain variability in other migraine symptoms, nor does it help to explain variability in clinical responsiveness. This leads us to conclude that migraine variability is not adequately characterized using ICHD-3 subtypes alone.

Treatment for Migraine

Triptans are serotonin (5-HT) agonists with a high affinity with two serotonin receptors, 5-HT_{1B} and 5-HT_{1D} (Loder, 2010), both of which are G protein-coupled receptors (GPCR) (Nichols & Nichols, 2008). Triptans constitute the major first-line therapy for migraine patients and can be successfully abort a migraine attack taken early abortive when taken early in the attack (Cady et al., 2009). This short window of opportunity for successful treatment limits the overall impact of triptans for migraine (Burstein et al., 2004). Even with this limitation, sumatriptan (a commonly prescribed triptan), is still reasonably effective. In a meta-analysis of 53 randomized controlled trials, 59% of patients receiving sumatriptan did not choose to take an additional abortive medication 2-24 hours post-dose compared to 30% with placebo (M. D. Ferrari et al., 2002). However, it is worth highlighting that patients choosing not to take an additional dose of medication does not mean that they are entirely pain free. Notable limitations of triptans include the small therapeutic window, abortive (not preventative nature of medication), presence of triptan non-responders, and headache from overuse (Limmroth et al., 2002).

One recent development in migraine therapy comes from work on Calcitonin Gene-Related Peptide (CGRP). CGRP is a 37 amino-acid neuropeptide coded for by the

CALCA gene and is expressed in the brain (Hökfelt et al., 1992) and in trigeminal ganglia that innervate cerebral blood vessels (Lassen et al., 2002). While there is debate as to the exact number of receptors for CGRP (Ho et al., 2010), it is generally accepted that the seven-transmembrane GPCR calcitonin-like receptor (CLR) and receptor-activity-modifying protein 1 (RAMP1) form a major CGRP receptor in both central and peripheral nervous systems (Lassen et al., 2002). RAMP1 is involved in the trafficking of CLR, is necessary for CGRP receptor functionality, and appears to be a rate limiting step in CGRP receptor availability (Ho et al., 2010).

Interest in CGRP and migraine was piqued by data showing that CGRP levels in the blood increase during trigeminal nerve stimulation, (Goadsby et al., 1988), increase during migraine attacks (Goadsby et al., 1990), and reduce in concentration from migraine abortive medication (Durham, 2004). Additionally, CGRP infusion into humans with migraine causes migraines, suggesting that an increase in CGRP release may actually generate migraines, contributing to migraine frequency (Lassen et al., 2002). Importantly, multiple clinical trials have shown CGRP antagonism mitigates the amount of migraine pain at two hours (Diener, 2014; Ho et al., 2008) and reduces frequency of headache attacks (Dodick et al., 2014; Ho et al., 2014; Sun et al., 2016). Recently the Food and Drug Administration has approved multiple CGRP monoclonal antibodies (Tepper, 2018). With several drugs that have now completed phase 3 clinical trials, it can now be confidently stated that CGRP antagonists can be used as a preventative migraine therapy. However, the magnitude of difference in reduction in headache frequency (the standard migraine clinical trial endpoint) is minimally different when compared to placebo, and the approved drugs typically reduce headache frequency around 2.5 days

relative to placebo (Spindler & Ryan, 2020). While progress has undeniably been made, a great deal of work is unfortunately still necessary.

Not all migraine therapy is pharmacologic. One non-pharmacologic therapy is mindfulness-based stress reduction (MBSR), a treatment developed in the later 1970s for stress management (Niazi & Niazi, 2011). Since its initial limited scope, its applications have greatly expanded to be used to treat more than a dozen disorders (McCubbin et al., 2014). Standard MBSR therapy takes place for 2.5 hours a week over 8 weeks with a 1 day retreat and patients are encouraged to practice mindfulness outside of treatment sessions (Niazi & Niazi, 2011). During treatment sessions patients are taught to attend to sensations with a nonjudgmental attitude, to recognize un/pleasant emotions and sensations, to be mindful, to modulate reaction to stressors, and to understand attitudes and unskillful thought patterns (McCubbin et al., 2014). Due to being non-pharmacologic, MBSR does not suffer from potential dangerous combination effects as can occur when taking an additional pharmacotherapy, meaning that MBSR can potentially complement existing therapies.

Given MBSR's focus on reappraising unpleasant sensations, it would seem to apply quite naturally to chronic pain therapy. MBSR trials for fibromyalgia (Kaplan et al., 1993), chronic lower back pain (Cramer et al., 2012), and migraine (Bakhshani et al., 2016) indicated feasibility and efficacy. However, many of these studies suffer from serious methodological shortcomings like the absence of control groups and small sample sizes. Fortunately, well-powered and appropriately designed clinical trials have now been conducted that clearly show that MBSR is effective in treating chronic pain disorders. A trial comparing MBSR, cognitive behavioral therapy and standard care with more than

300 patients with chronic lower back pain found that MBSR patients were more likely to show meaningful improvement relative to standard care receiving control subjects (Cherkin et al., 2016). Unfortunately, there was no control group in the study to account for placebo effects from non-standard care, tempering optimism. Recently, a well powered and designed randomized clinical trial assessing enhanced MBSR (MBSR+) (Seminowicz et al., 2020). In addition to standard MBSR, MBSR+ randomized patients received an additional four biweekly session. Importantly, this study compared MBSR+ to stress management for headache (SMH) condition. SMH subjects received migraine education and also received equal clinical interactions over the same time course. This study found that MBSR+ patients had a larger reduction in headache frequency than did SMH patients after therapy, indicating utility of MBSR+ for migraine.

Resting State Functional Connectivity Studies and Migraine

Functional Magnetic Resonance Imaging (fMRI) measures the blood oxygen level dependent (BOLD) signal. BOLD signal is used as a non-invasive proxy measure for neural activity, given the well-established relationship between directly recorded neural activity and BOLD (Logothetis et al., 2001; Schulz et al., 2012; Shmuel et al., 2006). Much of fMRI research has been task based, where an explicit task is performed by a participant and BOLD signal is modelled based on a theoretical model the task. It was originally thought that only task-based fMRI was useful and that non-task related (i.e. spontaneous) BOLD signals were driven by noise. However, a landmark study found that spontaneous, low frequency, BOLD signals were correlated across hemispheres and corresponded with motor networks (Biswal et al., 1995). This finding was termed functional connectivity. Since then, resting state functional connectivity (RSFC) has

exploded in popularity. Similar to task based fMRI studies, spontaneous BOLD signal has also been shown to be of neural origin (Mitra et al., 2018; Schölvinck et al., 2010; Turchi et al., 2018). While there is still debate about the exact biological meaning of RSFC, it is generally regarded as an index of information transfer (McIntosh, 2000), with recent work showing that patterns of activation mirror patterns of functional connectivity (Cole et al., 2016). Moreover, RSFC is mainly dominated by group and subject specific factors and therefore can be thought of as a trait measure stable enough for prediction (Gratton et al., 2018).

RSFC has been instrumental in shifting the view of the brain from a series of independent and highly specialized nodes, into a conceptualization as being organized into a series of networks with component nodes. While the number of networks identified varies, the most common large-scale networks include frontoparietal network (FPN), dorsal attention network (DAN), sensorimotor network (SMN), salience network (SN), and default mode network (DMN). The FPN is a major executive network and contains posterior parietal cortex and dorsal lateral prefrontal cortex. FPN is well established to be involved in cognitive operations and is widely implicated in chronic pain. Multiple studies have found reduced FPN connectivity in migraine (Coppola, Di Renzo, Tinelli, Lepre, et al., 2016; Russo et al., 2012; Tessitore et al., 2015). The dorsal attention network is another executive network primarily associated with attention orienting and contains the intraparietal sulcus and frontal eye fields. Similar to FPN, DAN also appears to display reduced connectivity in migraine (Coppola, Di Renzo, Tinelli, Di Lorenzo, et al., 2016). The sensorimotor network (SMN) contains pre/post central gyri and has a well-established role in processing noxious stimuli and is also a locus of pathology for

chronic pain. One study reported that SMN has increased connectivity in migraine (Ke et al., 2020). SN is generally implicated in executive processes and likely constitutes a core for cognitive function (Dosenbach et al., 2006; Menon & Uddin, 2010). It contains the dorsal anterior cingulate cortex and anterior insula. Multiple studies have also shown SN abnormalities in chronic pain. Some studies have shown increased SN connectivity in migraine (Hubbard et al., 2014; Tso et al., 2015), whereas another study had reduced SN connectivity to FPN (Russo et al., 2012). DMN is a task negative network first characterized due to its stereotypical deactivation from tasks (Raichle, 2015). Since then, the DMN has been shown to activate from internally guided thought (Smallwood et al., 2013). The DMN contains the rostral anterior cingulate cortex/medial prefrontal cortex, posterior cingulate cortex/precuneus, and angular gyri. Many studies also show a role for DMN and chronic pain. Differences in DMN connectivity are found in migraine (Xue et al., 2012) with reduced DMN connectivity (Coppola, Di Renzo, Tinelli, Lepre, et al., 2016; Hubbard et al., 2014; Ke et al., 2020), whereas others have found increased DMN connectivity (Buono et al., 2017; Coppola et al., 2018).

Other studies have used brain wide association in an attempt to relate migraine symptom variability to neural variability. The most common clinical parameters used are pain intensity, headache frequency, and disability related measures. Pain intensity has been shown to have a negative relationship to DMN-SN connectivity (Coppola et al., 2018), but a positive relationship to DMN-DAN/SMN connectivity (Hubbard et al., 2014). Pain intensity also negatively correlates with FPN connectivity (Russo et al., 2012). Headache frequency anti-correlates with DMN-FPN connectivity (Hubbard et al., 2014), and SN and SMN connectivity correlates with frequency (Mainero et al., 2011).

Disability related metrics relate to SN-FPN connectivity (Jin et al., 2013) and DMN-SN (Hubbard et al., 2014).

Given the extensive variability across studies, it is very challenging to synthesize key takeaways from the RSFC migraine literature (Skorobogatykh et al., 2019). The small sample sizes that dominates the field (often around 15 subjects per group) has diminished the reproducibility of between-subjects studies. Furthermore, the use of very different RSFC approaches, especially from the volumes used (i.e. parcellations or not) limit the ability to perform meta-analysis. It is also worth pointing out that between-subjects designs may be of limited utility to understand clinical variation within an illness. In the between-subjects RSFC study, healthy controls and migraine patients are contrasted in an attempt to identify differences in RSFC between the two groups. Large effects from a two-sample t-test will depend on the average difference between groups, the within group variance, and sample size (Hogg et al., 2005). Therefore, between-subjects studies select, in part, for functional connections with smaller group variance, since this will yield a larger test statistic. Any feature with limited between subject variability is a poor candidate to be associated with other variables with large between subject variability (Enkavi et al., 2019), such as clinical measures. As a result, standard approaches for migraine neurobiology research select against neural features with the greatest potential to explain clinical variability. To understand the biology of clinical variation in migraine, researchers must instead use within-subjects designs.

Unfortunately, studies performing within-subjects brain wide association in migraine also suffer from important limitations. First, these studies are often extremely too small in sample size to yield reliable measures of clinical/neural association (Marek

et al., 2020). Additionally, these studies typically use a mass-univariate approach, that treats all functional connections as independent of one another, which is not the case (Yeo et al., 2011). Moreover, at times in mass-univariate studies only a single clinical variable is associated with functional connectivity, ignoring the many symptoms that migraine patients experience. When the brain wide association for multiple symptoms is examined, univariate analyses do not factor in the complex and shared structure between clinical symptoms by instead examining only one variable of interest. Moreover, these studies almost never examine how estimates of motion relate to the clinical variable of interest, which if true, would confound results, given the impact of motion on RSFC (Power et al., 2012). Even though within-subjects designs are an essential tool to understand how clinical variability in migraine relates to neural processes, analytic shortcomings have limited such research.

A better approach would be to examine how many symptoms relate to many functional connections, which can be achieved with Canonical Correlation Analysis (CCA). In CCA, two domains of variables measures in a single set of individuals are associated with one another in one multivariate step (Hotelling, 1935). Linear combinations of input variables for each domain are made (canonical variates) with the aim of maximizing the correlation between the canonical variates. CCA allows for structure to exist within each domain of data (clinical and RSFC), giving it a major advantage over mass univariate brain wide association studies. In the case of migraine, CCA can be used to examine how a diverse range of symptoms associates with a diverse range of neural networks (see chapters 2 and 3).

Specific Aims

The overall goal of this project is to understand various domains of migraine variability. The first aim examines how holistic migraine symptoms are related to RSFC. The second aim examines how RSFC variability before treatment predicts response to treatment. The final aim seeks to determine mechanisms of mindfulness therapy by examining how variability in RSFC changes from therapy mediates treatment outcomes. While each of these aims use, at least in part, exploratory techniques, there is room for synthesis across them. For instance, RSFC associated with headache frequency (identified in aim 1 potentially), might be used to predict treatment outcomes (aim 2). Just as RSFC mediators of therapy (aim 3) may overlap with pre-treatment predictors of responsiveness (aim 2). Overall, this project works to adopt a new research framework for migraine that instead of relying on the flawed ICHD-3 subtypes, embraces and takes advantage of migraine symptom and neural variability.

Aim 1: Identify multivariate association between RSFC and diverse migraine symptoms

- a) Use canonical correlation analysis (CCA) in a multi-site dataset to examine how seven migraine symptoms relate to RSFC across six networks
- b) Examine the empirical support for clustering patients into episodic and chronic migraine based on seven clinical symptoms
- c) Determine if clustering based on RSFC dimensions associated with migraine symptoms would identify biotypes

Hypothesis: Dimensions of association between RSFC between specific ensembles of networks and symptoms will exist and can be used to biotype patients

Aim 2: Determine baseline (pre-treatment) RSFC predictors of MBSR+ responsiveness

- a) Use machine learning on pre-treatment RSFC to predict the percentage reduction in headache frequency for MBSR+ randomized patients
- b) Examine how RSFC dimensions associated with symptoms (from aim 1) predict the percentage reduction in headache frequency for MBSR+ randomized patients

Hypothesis: MBSR+ outcomes can be predicted using pre-treatment RSFC and models will weight RSFC most associated with headache frequency at baseline

Aim 3: Determine how RSFC changes mediate clinical improvement from MBSR+

- a) Use mass univariate simple mediation to determine how changes in RSFC from pre- to post-treatment mediate percentage reduction in headache frequency for MBSR+ and SMH randomized subjects
- b) Use multivariate mediation to determine how changes in RSFC from pre- to post-treatment mediate percentage reduction in headache frequency for MBSR+ and SMH randomized subjects
- c) Use simple mediation to examine how changes in RSFC dimensions associated with symptoms (from aim 1) mediate percentage reduction in headache frequency for MBSR+ and SMH randomized subjects

Hypothesis: reduction in headache frequency will be mediated by RSFC changes primarily by changes in connectivity related to headache frequency at baseline

Chapter 2: General Methods

Participants and Data

Two datasets were acquired as part of this thesis, referred to here as the *chronic-Stanford* and *episodic-UMB* datasets. For the *chronic-Stanford* dataset, participants were recruited to participate in research studying biomarkers of chronic daily headache using clinical, behavioral, and magnetic resonance imaging (MRI) data at Stanford University. Subjects were over 18 years of age and met the International Classification of Headache Disorders ICHD-3 diagnostic criteria for chronic migraine as determined by a physician for a minimum of three months (International Headache Society, 2018). Subjects were excluded if they had a history of severe neurologic/psychiatric disorders or any MRI contraindications. In accordance with Stanford University guidelines subjects provided written informed consent. Prior to quality control, data from 49 participants were available (average age = 39.7 s.d. = 13.1, 40 female). For the *episodic-UMB* dataset, participants were recruited to participate in a clinical trial assessing MBSR+ treatment of migraine (Seminowicz et al., 2020). Subjects were between the ages of 18-65 years of age and met the ICHD-3 diagnosis for episodic migraine with or without aura for at least one year (International Headache Society, 2018). Subjects were excluded if they had a history of mindfulness meditation, severe psychiatric symptoms, and/or opioid use. In accordance with University of Maryland Baltimore guidelines, subjects provided written informed consent. Before any quality control, data for 124 subjects was available for *episodic-UMB* before clinical trial treatment arm randomization (average age = 37.4 s.d. = 12.1, 110 female). Of this original sample 98 were randomized to receive one of two

treatment conditions. Randomized subjects received additional scans at 10 weeks and 20 weeks.

Data Acquisition: Magnetic Resonance Imaging (MRI)

Chronic-Stanford MRI scans were a structural T1-weighted (T1w) scan (repetition time [TR] 5900 ms, echo time [TE] 28 ms, flip angle 15°, voxel size 0.9 x 0.9 x 1 mm) and also an eyes closed resting state function MRI (fMRI) scan (8 minutes, gradient echo spiral-pulse, TR 2000, TE 30 ms, slice thickness 4 mm, FOV 220 mm, flip angle 80°, voxel size 3.4 x 3.4 x 4.5 mm). *Episodic-UMB* scans used in this analysis were a structural T1w scan (TR 2300ms, TE 2.98 ms, slice thickness 1 mm, FOV 256 mm, flip angle 9°, and voxel size 1 × 1 × 1 mm) and also an eyes open resting state fMRI scan (10 minutes, echo planar imaging, TR 2000, TE 28 ms, slice thickness 4 mm, FOV 220 mm, flip angle 77°, voxel size 3.4 x 3.4 x 4 mm).

Preprocessing

MRI data underwent preprocessing using FMRIPREP. FMRIPREP uses a standardized and reproducible image processing pipeline. In the interest of reproducibility, FMRIPREP automatically generates a methods section based on the pipeline. Consistent with field norms, we paste this section below.

Preprocessing was performed using FMRIPREP version stable (Esteban et al., 2020), a Nipype (Gorgolewski et al., 2017) based tool. Each T1-weighted (T1w) volume was corrected for INU (intensity non-uniformity) using N4BiasFieldCorrection v2.1.0 (Tustison et al., 2010) and skull-stripped using antsBrainExtraction.sh v2.1.0 (using the OASIS template). Spatial normalization to the ICBM 152 Nonlinear Asymmetrical template version 2009c (Fonov et al., 2009) was performed through nonlinear registration

with the `antsRegistration` tool of ANTs v2.1.0 (Avants et al., 2008), using brain-extracted versions of both T1w volume and template. Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was performed on the brain-extracted T1w using `fast` (FSL v5.0.9 (Zhang et al., 2001)).

Functional data was slice time corrected using `3dTshift` from AFNI v16.2.07 (Cox, 1996) and motion corrected using `mcflirt` (FSL v5.0.9 (Jenkinson et al., 2002)). This was followed by co-registration to the corresponding T1w using boundary-based registration (Greve & Fischl, 2009) with six degrees of freedom, using `flirt` (FSL). Motion correcting transformations, BOLD-to-T1w transformation and T1w-to-template (MNI) warp were concatenated and applied in a single step using `antsApplyTransforms` (ANTs v2.1.0) using Lanczos interpolation. Many internal operations of FMRIprep use Nilearn (Abraham et al., 2014), principally within the BOLD-processing workflow. For more details of the pipeline see <https://fmriprep.readthedocs.io/en/stable/workflows.html>.

Quality Control of MRI

Two selection criteria were used to perform quality control of MRI data. The first was to visually examine the preprocessed data using FMRIprep automatic visual reports output of preprocessed data. We examined the performance of brain extraction by looking at the brain mask overlaid on the T1w anatomical image. We then examined the T1w registration to Montreal Neurologic Institute (MNI) space. Then we examined the brain mask applied to functional data, which created a skull stripped echo planar image (EPI). Following this we examined the EPI registration to T1 space. Any subjects with abnormalities in the visual inspection (e.g. brain mask removing cortex) were removed from analysis. A total of 3 subjects for baseline *episodic-UMB*, 2 for 10 weeks, and 2 for

20 weeks failed visual quality control inspection. These subjects had severe abnormalities following registration likely caused by brain abnormalities (e.g. lesions) and scans that excluded much of the cortex. Preprocessing errors were unfortunately more common in the *chronic-Stanford* dataset, and 6 subjects failed visual quality control inspection. These subjects had scans that removed large sections of the brain, or image intensity abnormalities brought on through preprocessing (slices with much larger intensity than other slices).

The second layer of quality control concerned motion. Motion severely impacts estimates of RSFC, which greatly complicated the interpretation of RSFC (Power et al., 2012b). While many methods have been created for the correction of motion effects on RSFC (Ciric et al., 2017; Satterthwaite et al., 2019), it is likely that some subjects have moved too much to be included in analysis. To examine each subject's motion characteristics, we took the average of the frame-wise displacement vector for each subject to get an estimate of subject motion during the resting state scan (Power et al., 2014). One challenge for removing subjects due to motion is the decision of a motion cutoff as the exact choice of a cutoff can be arbitrary. The decision for how conservative to be is ultimately related to the experimental question. For studies performing network analyses where functional connectivity is estimated and is not necessarily related to another variable of interest, then it is more important to have data free of motion artifact. However, in cases where RSFC is going to be related to variables unrelated to motion, there is a built in protection against positive effects being created by motion artifact. In

such a scenario, motion still unfortunately reduces sensitivity to true relationships, but the relative risk is attenuated. Given that this thesis work was relating RSFC to various clinical measures, we elected to use a liberal threshold for subject motion. To determine the exact cutoff, we examined violin plots of baseline *episodic-UMB* data for baseline and selected an average framewise displacement value that removed high motion outliers from the rest of the data (Fig. 1). We chose this dataset as it was

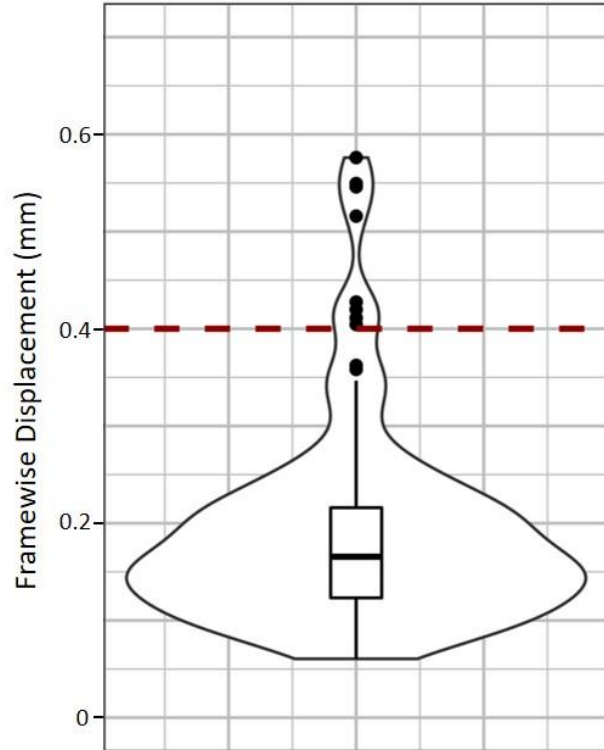


Figure 1. Distribution of average framewise displacement for *episodic-UMB* dataset. Average framewise displacement above 0.4 mm (shown in red) was used as a cutoff to remove subjects. mm=millimeter.

the larger of the two. This cutoff chosen was 0.4mm average framewise displacement and this cutoff was applied to all data. This resulted in one *chronic-Stanford* subject being removed. For *episodic-UMB* data, 8 subjects were removed at baseline, five at 10 weeks, and eight at 20 weeks.

Motion Correction

Relatively small amounts of motion during MRI produces spatially structured patterns of functional connectivity (Power et al., 2012a; Satterthwaite et al., 2012). Motion most impacts nearby voxelwise timeseries' increasing local correlations (Power et al., 2012a). In fact, differences in motion between groups of subjects has created the

illusion of ‘true’ neuronal differences when in fact structured noise was different (Power et al., 2012a). Many methods have now been proposed to correct for motion effects ranging from censoring high motion volumes (Power et al., 2014), tissue related signal regression (Behzadi et al., 2007), matrix decomposition approaches like independent component analysis (Pruim et al., 2015), and fairly standard regression of realignment parameters. However, even the most aggressive motion removal strategies still leave noise, like global signal (Power et al., 2017a). Global signal is a shared signal across all voxels in the brain and is a consistent source of controversy in RSFC denoising (Murphy & Fox, 2017). While global signal is influenced by motion and breathing it also represents a separate source of both noise and signal (Glasser et al., 2018; Schölvinck et al., 2010; Turchi et al., 2018).

Previous studies have attempted to benchmark various denoising approaches (Ciric et al., 2017). The major takeaway from this most thorough analysis was that no single denoising pipeline dominates all others, but there are some that are consistently worse than others and thus should be avoided. Importantly, the effect of global signal regression (GSR), a common approach to remove global signal, was found to both mitigate and exacerbate some quality control indicators in a pipeline specific manner. For instance, the pipeline Automatic Removal of Motion Artifacts (AROMA) had larger distance dependent motion effects with GSR. It is also possible that different datasets with different amounts of motion and respiratory artifact may perform best with unique denoising pipelines. Therefore, we compared 3 pipelines: a standard approach with 12 motion regressors (6 realignment parameters + temporal derivatives), AROMA (non-aggressive (Pruim et al., 2015)), and spike regression for any frame with a framewise

displacement greater than 0.5mm. We also performed GSR for each pipeline, yielding 6 total pipelines. Global signal was calculated based on the average timeseries of all gray matter voxels. All of these pipelines also used aCompCor with the first 5 principal components for a twice eroded CSF mask and a 4th eroded WM mask, used 0.008-0.15 Hz bandpass filter applied simultaneously to all regressors and voxelwise timeseries. These two levels of erosion have previously been shown to no longer contain global signal (Power et al., 2017b). All resting-state denoising was conducted using the CONN functional connectivity toolbox version 19f (Whitfield-Gabrieli & Nieto-Castanon, 2012: <http://www.nitrc.org/projects/conn>)

The primary metrics for quality were the correlation with RSFC, where we anticipated the magnitude of correlation with average framewise displacement to decrease with better denoising pipelines. An additional concern is that a pipeline could be too aggressive and remove both signal as well as noise. Therefore we additionally tested the average framewise displacement with 7 clinical parameters, anticipating that a good denoising pipeline would increase sensitivity. Consistent with previous benchmarking, no single pipeline dominated all others, however, just using 12 motion parameters performed the worst and had the highest average RSFC correlation with motion and the most number of edges significantly related to motion (Fig. 2A, B). AROMA without GSR had the lowest correlation with framewise displacement (average correlation 0.11). Each pipeline was fairly similar with correlation to the 7 clinical parameters, and all had an average absolute correlation between 0.08 and 0.09. Therefore the final denoising pipeline we used for all data was non-aggressive AROMA followed by aCompCor with simultaneously applied 0.008-0.15 Hz bandpass filter and linear detrending.

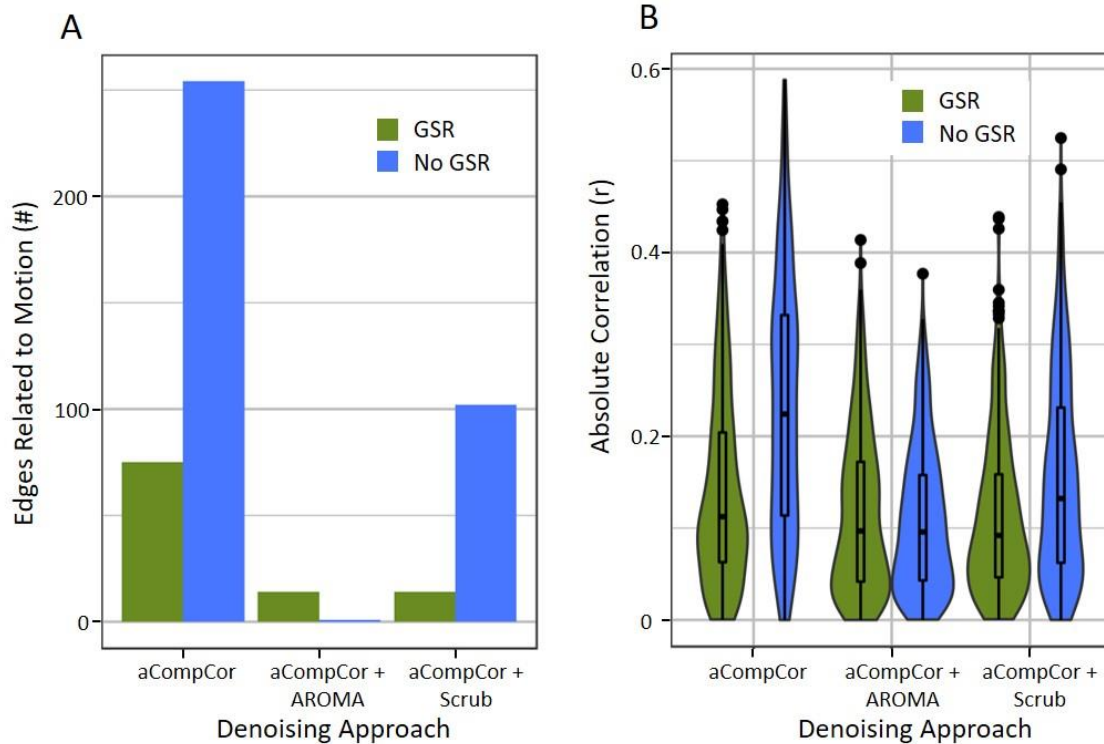


Figure 2. Relationship between edges and motion across denoising pipelines. Functional connectivity (edges) were correlated with average framewise displacement for the *episodic-UMB* dataset comparing three approaches: 1) only aCompCor and realignment parameters; 2) aCompCor and AROMA; or 3) aCompCor and realignment parameters, and motion scrubbing with spike regression. All approaches also underwent global signal regression. A) Edges with a statistically significant relationship to motion. B) Absolute value of correlation between average motion and edges. aCompCor with AROMA and without global signal regression had the weakest relationship with subject motion. aCompCor=anatomical CompCor; AROMA=Automatic Removal of Motion Artifacts, Scrub=regression of motion spikes; GSR=Global signal regression.

Parcellation and Feature Selection

Parcellations are a common tool used to average related voxels. This process improves sensitivity to signal and also aids interpretation of results. Parcellations are also an excellent dimensionality reduction tool, which is an especially serious problem given that correlations for RSFC increase the dimensionality of the data, massively increasing the dimensionality relative to the starting voxel space, which is already high in

dimensionality. After denoising, we averaged voxels within a 32 region of interest (ROI) parcellation based on an ICA of the Human Connectome Project. This parcellation, based on hundreds of subjects, is available via the CONN toolbox. These 32 ROIs were organized into standard networks: default mode, sensorimotor, visual, salience/cingulo-opercular, dorsal attention, frontoparietal, language and cerebellar networks. The timeseries from each ROI was correlated with all other ROI

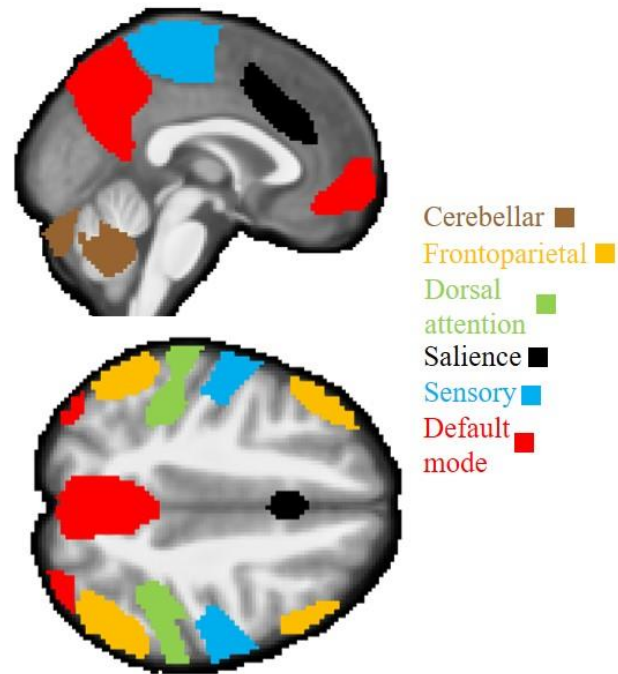


Figure 3. Parcellation based on Human Connectome Project. Resting State Functional Connectivity (RSFC) was estimated from 24 regions (shown above) spanning six networks.

timeseries creating Pearson correlation coefficients, which were subsequently converted to z scores.

We performed feature selection on the functional connections by excluding nodes that were part of the language and visual networks, because we did not believe these networks would be associated with the assayed migraine symptoms, nor to MBSR+ therapy. Nodes in the language network included the posterior superior temporal and inferior frontal gyri, consistent with Wernicke’s and Broca’s area. The visual network ROIs were located within the occipital cortex. The occipital cortex has been implicated in aura (Hadjikhani et al., 2001), which was not measured in any dataset. Additionally, as noted in Chapter 1, the relationship between aura and other migraine symptoms appears

to be small to non-existent. Following feature selection, there were 24 ROIs with 276 unique functional connections organized into six networks (Fig. 3).

Chapter 3: Three Dimensions of Association Link Migraine Symptoms and Functional Connectivity

Introduction

Migraine is a complex disorder displaying many interrelated clinical features ranging from sleep disturbances to affective pathology. Importantly, the intensity of symptoms is heterogeneous across migraine patients. By using mass-univariate approaches, much of the literature unfortunately ignores the complex and interrelated nature of clinical and neural data. In the current aim we used a multivariate statistical approach, canonical correlation analysis (CCA), to relate RSFC with clinical symptoms in a single multivariate step. CCA allows for dimensions of association to be found between clinical and RSFC, where these associations are formed through unique combinations of clinical and RSFC variables.

CCA has become increasingly popular in neuroimaging research (Wang et al., 2020; Winkler et al., 2020) and has been used to link RSFC with demographic characteristics (Smith et al., 2015), mind wandering (Wang et al., 2018), and pathology (Mihalik et al., 2019). One very promising application of CCA came in depression (Drysdale et al., 2017). This study attempted to use CCA to relate RSFC with specific item scores on a depression questionnaire, and ultimately argued for two dimensions of covariance between functional connectivity and depressive symptoms, one dimension relating to anhedonia, and a second to sleep disturbances. These two dimensions were then used to cluster patients into subtypes derived from biological data, called biotypes. While this was an exciting application of CCA that garnered an impressive citation count,

the approach has been extensively criticized due to concerns about generalizability (Dinga et al., 2019). Therefore, while CCA studies have yielded exciting results, some may have serious, though not insurmountable flaws, that future work can address.

In the current aim we sought to identify association between RSFC and seven clinical features of migraine using CCA. We worked to minimize dimensionality of the data through an unbiased feature selection process and principal components analysis. We show that with these steps that model performance in held out data was similar to in training data. We were additionally interested in testing if the categorization of chronic migraine chronic and episodic migraine subtypes is supported using the seven clinical features. If the current subtyping of migraine is not empirically supported, then major changes in migraine research and treatment are warranted. Finally, we attempt to establish a biotyping of migraine based on RSFC dimensions associated with clinical symptoms (for an overview of the study, see figure 4).

Aim 1: Identify multivariate association between RSFC and diverse migraine symptoms

- a) Use canonical correlation analysis (CCA) in a multi-site dataset to examine how seven migraine symptoms relate to RSFC across six networks
- b) Examine the empirical support for clustering patients into episodic and chronic migraine based on seven clinical symptoms
- c) Determine if clustering based on RSFC dimensions associated with migraine symptoms would identify biotypes

Hypothesis: Dimensions of association between RSFC between specific ensembles of networks and symptoms will exist and can be used to biotype patients

Methods

Participants and Data

Participants were from the previously described *episodic-UMB* and *chronic-Stanford* datasets (see chapter 2). This aim was interested in how clinical features related to RSFC. The analyses were based on initial (baseline) data, and thus some subjects from the *episodic-UMB* dataset were not subsequently randomized into one of the two treatment arms. For analysis on current subtyping of migraine, subjects only required complete clinical data. For this analysis 46 subjects were available from *chronic-Stanford* (average age of 39, s.d. = 13, 38 female)

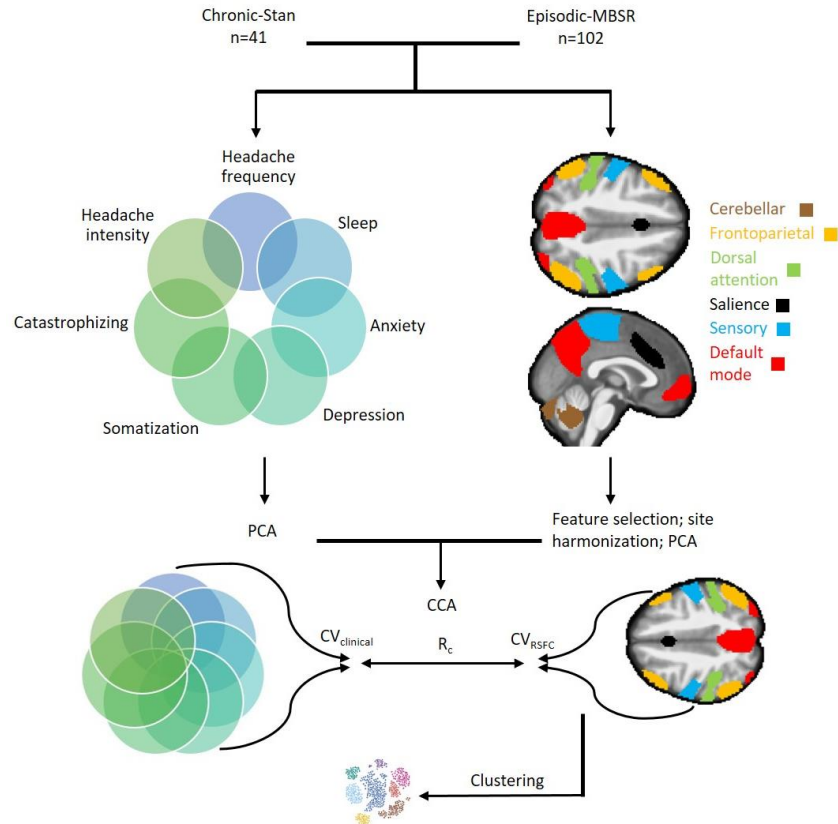


Figure 4. Study overview. For Canonical Correlation Analysis (CCA), 143 migraine subjects were collected from two sites. Seven clinical measures were acquired along with resting-state functional connectivity (RSFC) estimates from 24 regions spanning six functional networks. After site harmonization of RSFC and principal components analysis (PCA), CCA was used to associate clinical and biological data. CCA is used to study the relationship between variables from two domains of interest measured in one sample. In CCA, linear combinations of the input variables from both domains are created (called canonical variates) so that the correlation between the combined variables (canonical correlation, R_c) is maximized. RSFC canonical variates underwent clustering analysis to reveal biotypes of migraine. PCA=principal components analysis; R_c =canonical correlation; CV_{RSFC} =canonical variates for RSFC; $CV_{clinical}$ =canonical variates for clinical data.

and 120 subjects from *episodic-UMB* (average age of 38, s.d. = 12, 106 female). For analysis related RSFC to clinical features, subjects had to have complete clinical and RSFC data. From the *chronic-Stanford* dataset 41 subjects were available (average age of 38, s.d. = 13, 34 women) and from the *episodic-UMB* dataset a total of 102 subjects were used (average age of 38, s.d. = 12, 91 women).

Clinical Features

Subjects completed many self-report questionnaires to examine clinical features. The frequency of headaches were assessed using headache diaries completed by participants to calculate the number of headache (NHA; Seminowicz et al., 2020). The severity of headache attacks was the average pain measured in a 0-10 scale from participants (Mean Pain). We used the Pain Catastrophizing Scale (PCS; Sullivan et al., 1995) to assess maladaptive pain coping. Sleep quality and disturbances were measured using the Pittsburg Sleep Quality Index (PSQI; Buysse et al., 1989). Somatic symptom severity as well as somatization was measured with the Patient Health Questionnaire 15 (PHQ-15; Kroenke et al., 2002). Anxiety was captured using the GAD-7 questionnaire (Spitzer et al., 2006) and depression was indexed using the Patient Health Questionnaire 9 (PHQ-9; Kroenke et al., 2001). Where applicable, the total score for each questionnaire was used for all analyses. In total, seven clinical features were available for every participant.

Multi-site Harmonization

Scanner and site effects have been shown to greatly influence fMRI data and should be adjusted for when possible (Marek et al., 2019; Noble et al., 2017; Van Horn & Toga, 2009; Yu et al., 2018). A challenge when correcting for site effects is that unique

study locations typically have samples with unique clinical and demographic characteristics, meaning that site harmonization should be carefully employed so as to be specific for site effects. To harmonize for site across the *episodic-UMB* and *chronic-Stanford* datasets while controlling for age, sex, and clinical variables, we used Combining Batches of microarray data (ComBat; Johnson et al., 2007). ComBat uses an empirical Bayes method to remove effects of site in RSFC data while controlling for specified variables (in this case age, sex, and all clinical features). To determine that ComBat had not removed the association between RSFC and the seven clinical features we examined the absolute value of Pearson correlation coefficient between RSFC and the clinical features. ComBat consistently improved the magnitude of mass univariate association between clinical

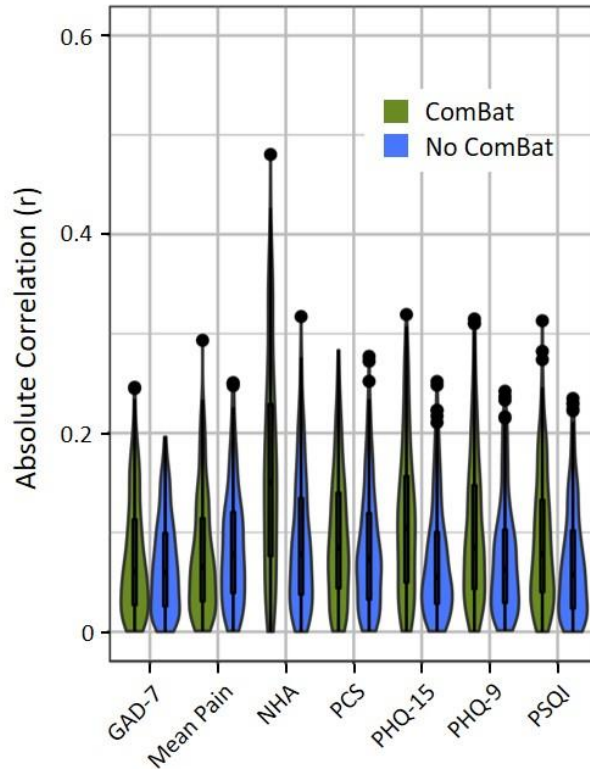


Figure 5. Effect of site harmonization of RSFC on clinical variables' association with RSFC. Edges were correlated with each clinical variable before and after ComBat site correction was applied. The absolute value of correlation between edges and clinical variables was consistently larger following ComBat (green) than before ComBat (blue). These results indicate that site harmonization did not damage the relationship between RSFC and clinical variables. Mean Pain=average headache pain; NHA=headache frequency; PHQ-15=patient healthy questionnaire 15 item; PSQI=Pittsburgh sleep quality index; PCS=pain catastrophizing scale; GAD-7=generalized anxiety disorder; PHQ-9=patient health questionnaire 9 item; r=Pearson correlation coefficient; ComBat=Combining Batches of microarray data.

features and RSFC, indicating that it is very unlikely that our multi-site harmonization approach removed clinical variance from RSFC (Fig. 5).

Principal Component Analysis

To reduce dimensionality while addresses multicollinearity, we separately performed Principal Component Analysis (PCA) on clinical and RSFC data. PCA is used to create orthogonal components that can be linearly combined to explain the complete variance in the original dataset. We used a singular value decomposition for PCA and selected the first four clinical components (Fig. 6A) and the first 35 RSFC components (Fig. 6B) that explained more than 80 percent of the clinical and RSFC variance respectively. Only these components were used for canonical correlation.

Regularized Canonical Correlation Analysis

Canonical Correlation Analysis (CCA) is a multivariate statistical approach to identify relationships between two sets of variables (Hotelling, 1935). With CCA, a pair of canonical variates is created (one per data domain) through a linear combination of input variables in order to maximize the correlation between canonical variates (canonical correlation, R_c). As is the case with PCA, when performing canonical correlation it is possible to seek additional pairs of canonical variates that are orthogonal to all preceding canonical variate(s). However, for each additional pair of canonical variates that canonical correlation between them decreases. The total number of pairs of canonical variates is determined by the smaller of the number of variables in each domain, which in this analysis was four. It is generally recommended when performing CCA that a very large subject to feature ratio exists (over 10:1 for the first canonical correlation (Pituch & Stevens, 2015; Tabachnick et al., 2007). Following PCA, the

subject to variable ratio was 3.4, which while improved over the pre- PCA ratio of 0.5, was still fairly small. When the subject to variable ratio is small, the likelihood of overfitting increases. It is very likely that in previous attempts to associate clinical features with RSFC using CCA that overfitting was so large that the results were completely unlikely to generalize given new data (Dinga et al., 2019). To further address overfitting, we used an L2 norm regularized CCA (Gonzalez et al., 2008) that shrinks canonical weights for each domain of variables based on user specified lambda values. To determine optimal regularization parameters we calculated used 10-fold cross-validation stratified for study site repeated 10 times where the CCA model was iteratively built using nine folds and the resultant canonical weights were used to extract canonical variates in the held out testing fold. In total, 100 training sets with unique canonical weights and 100 corresponding testing sets were created. In each training set we used the canonical variates to calculate R_c and selecting regularization parameters that yielded the best average R_c in held out data for the first canonical correlation.

Determining Support for Episodic vs Chronic Migraine Clustering on Migraine

Symptoms

Migraine patients are currently clustered into episodic and chronic subtypes based on a 15 headache day per month cutoff. The current subtyping does not examine migraine symptoms holistically. Using our combined datasets and clinical data only (n=166), we were interested in determining if there is empirical support for the field norm subtyping of migraine. Using Euclidean distance between the seven clinical measures, we calculated silhouette index, a statistic examining cohesion and separation between clusters that has been shown to accurately identify clusters in data (Petrovic, 2006).

Should the episodic/chronic clustering have a silhouette index no better than chance (i.e., likely coming from a multivariate normal distribution), then it would argue against the current field norm of episodic and chronic migraine subtypes (see below section: *Statistics*).

Data Driven Clustering of RSFC Canonical Variates

Using RSFC canonical variates from CCA we sought to identify biotypes of migraine using k -means clustering. We used Euclidean distance as the distance metric. To determine the number of clusters, k , we compared 26 clustering metrics using the NBclust package (Charrad et al., 2014) with k ranging from 2 to 10. With every value of k , a score for all 26 clustering metrics was computed, and cluster performance was ranked for each metric (e.g., the value of k with the largest silhouette index received a rank of one). For each metric, a value of k ‘won’ when it received the first rank and therefore displayed the best performance for that specific metric of clustering. We used a majority rule, where the cluster number that had the most ‘victories’ was deemed the best overall.

Statistics

To calculate statistical significance of CCA we performed a permutation test by creating 5000 bootstraps with replacement (Efron, 2000) using the final model lambda parameters. For every bootstrap, we calculated R_c values for the four canonical correlations and compared them against the null distribution using a one-sided t-test. To facilitate interpretation of the CCA model, we calculated canonical loadings through Pearson correlation between canonical variates and their respective model inputs (e.g. RSFC values). Calculating statistical significance of clustering can be very challenging as there is no perfect null hypothesis to test against (Dinga et al., 2019; Liu et al., 2008). We

treated a multivariate Gaussian distribution as the null hypothesis, given that such normal distributions should not be clustered. To generate null models we created 5000 multivariate Gaussian distributions based on the mean and covariance of the input data and used k -means clustering to cluster each distribution ($k=2$ for clinical clustering, $k=4$ for biotyping). We then computed silhouette index for the multivariate normal distribution clusters and compared the resulting null distribution of silhouette index against the true clustering silhouette index using a one sample t-test, in a process similar to ones previously described (Dinga et al., 2019; Liu et al., 2008). We used a Spearman rank correlation to correlate average framewise displacement with canonical variate scores. For all analyses, significance was determined using a 0.05 FDR-corrected threshold.

Results

Three Modes of Association Were Found Between RSFC and Migraine Symptoms

We used regularized CCA to identify dimensions of covariance between a diverse array of clinical symptoms and RSFC. We optimized lambda values for regularization using performance in held out testing data to maximize canonical correlation (R_c) for the first pair of canonical variates. The best performance in testing data for the first canonical correlation was an average $R_c = 0.6$ with lambda values of 0.3 for clinical data and 0.9 for RSFC. These regularization parameters were used for our final model. In the final model, the first canonical correlation was $R_c = 0.71$, $N = 143$, $p = 0.0002$ (Fig. 6C, D), the second was $R_c = 0.49$, $N = 143$, $p = 0.0023$ (Fig 6. E, F), the third was $R_c = 0.43$, $N = 143$, $p = 0.006$ (Fig. 6G, H), and the fourth was $R_c = 0.35$, $N = 143$, $p = 0.098$. The average R_c values for held-out testing data is as follows: first canonical correlation $R_c = 0.6$, s.d. =

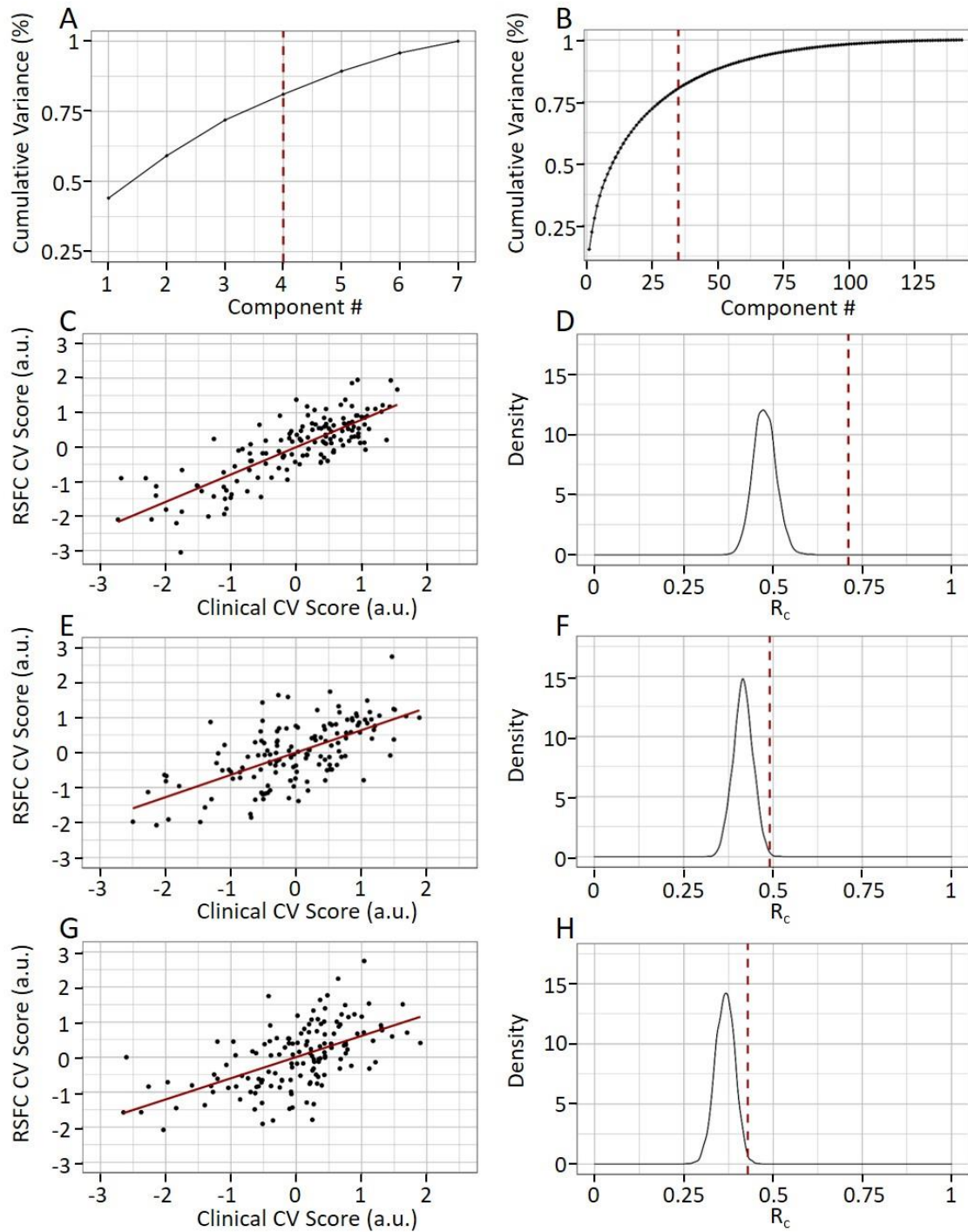


Figure 6. Using CCA to reveal three dimensions of association between clinical features and RSFC. Cumulative variance explained from clinical (A) and RSFC (B) principal components. Red lines indicate 80% variance explained. Canonical correlation (R_c) between RSFC and clinical canonical variates for first (C), second (E), and third (G) canonical correlations. Permutation test for significance of R_c value (shown in red) relative to 5000 permutations for first (D), second (F), and third (H) canonical correlations. R_c =canonical correlation; CV=canonical variate.

0.19, second $R_c = 0.14$, s.d. = 0.25, third $R_c = 0.19$, s.d. = 0.23, and fourth $R_c = 0.03$, s.d. = 0.23. We limited interpretation and further analysis to the first three significant canonical correlations only. Note that each canonical correlation is between a pair of normally distributed clinical and RSFC canonical variates. Therefore, from the three significant canonical correlations, each subject has 3 RSFC and 3 clinical canonical variates.

Association of RSFC Canonical Variates and Scanner Motion

Subject motion has profound effects on estimates of RSFC (Power et al., 2012b). We therefore ensured that the canonical variates for RSFC were unrelated to average framewise displacement. We did not find a significant correlation between average framewise displacement during the resting state scan with the first $r_s = 0.12$, $N = 143$, $p = 0.14$ (Fig. 7A), second $r_s = 0.08$, $N = 143$, $p = 0.3$ (Fig. 7B), nor third $r_s = -$

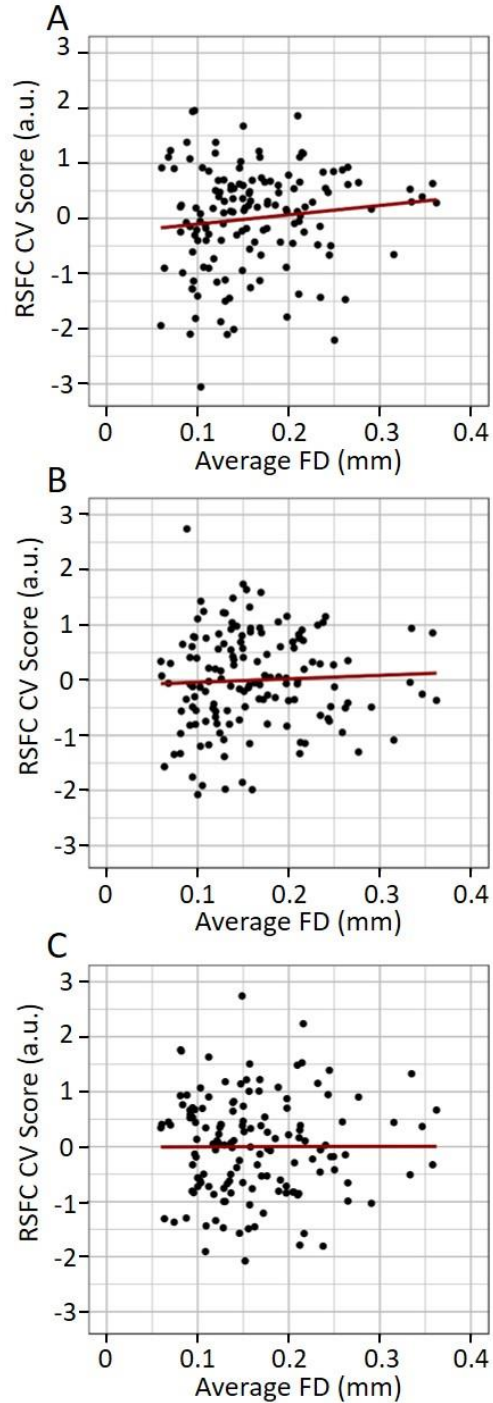


Figure 7. No association between canonical variates and subject motion. Scatter plot for first (A), second (B), and third (C) RSFC canonical variate relationship with average framewise displacement. CV=canonical variate, FD=framewise displacement.

0.04, $N = 143$, $p = 0.6$ (Fig. 7C) RSFC canonical variates. As a result, there is no evidence for a relationship between RSFC canonical variates and subject movement.

Interpretation of CCA Model

We created canonical loadings by correlating canonical variates with their respective RSFC/clinical symptoms to interpret the CCA model. While each of the three canonical correlations consisted of RSFC and clinical canonical variates, we chose to name each dimension with a clinically derived name for ease of discussion. The first canonical variate for symptoms correlated in the same direction with every clinical feature (Fig. 8A). We interpret the first clinical canonical correlation as the ‘global symptom dimension’. Patients with positive canonical variate values for the first canonical correlation had globally better symptoms (less severe symptoms overall). The associated first RSFC canonical variate related to frontoparietal network and dorsal attention network connectivity (Fig. 8B). The second clinical canonical variate reflected an inverse relationship between average headache pain and anxiety primarily, referred to here as the ‘inverse pain/anxiety dimension’ (Fig. 8A). Patients with positive values had above average headache pain and below average anxiety. The pattern of functional connectivity was sparse, but primarily related to anti-correlation between the DMN and other networks (Fig 8C). The third symptom canonical variate primarily related to pain catastrophizing (Fig. 8A) and related to salience, sensorimotor and default mode connectivity with one another (Fig. 8D). Patients with positive values had below average pain catastrophizing. We refer to this here as a ‘pain catastrophizing dimension’.

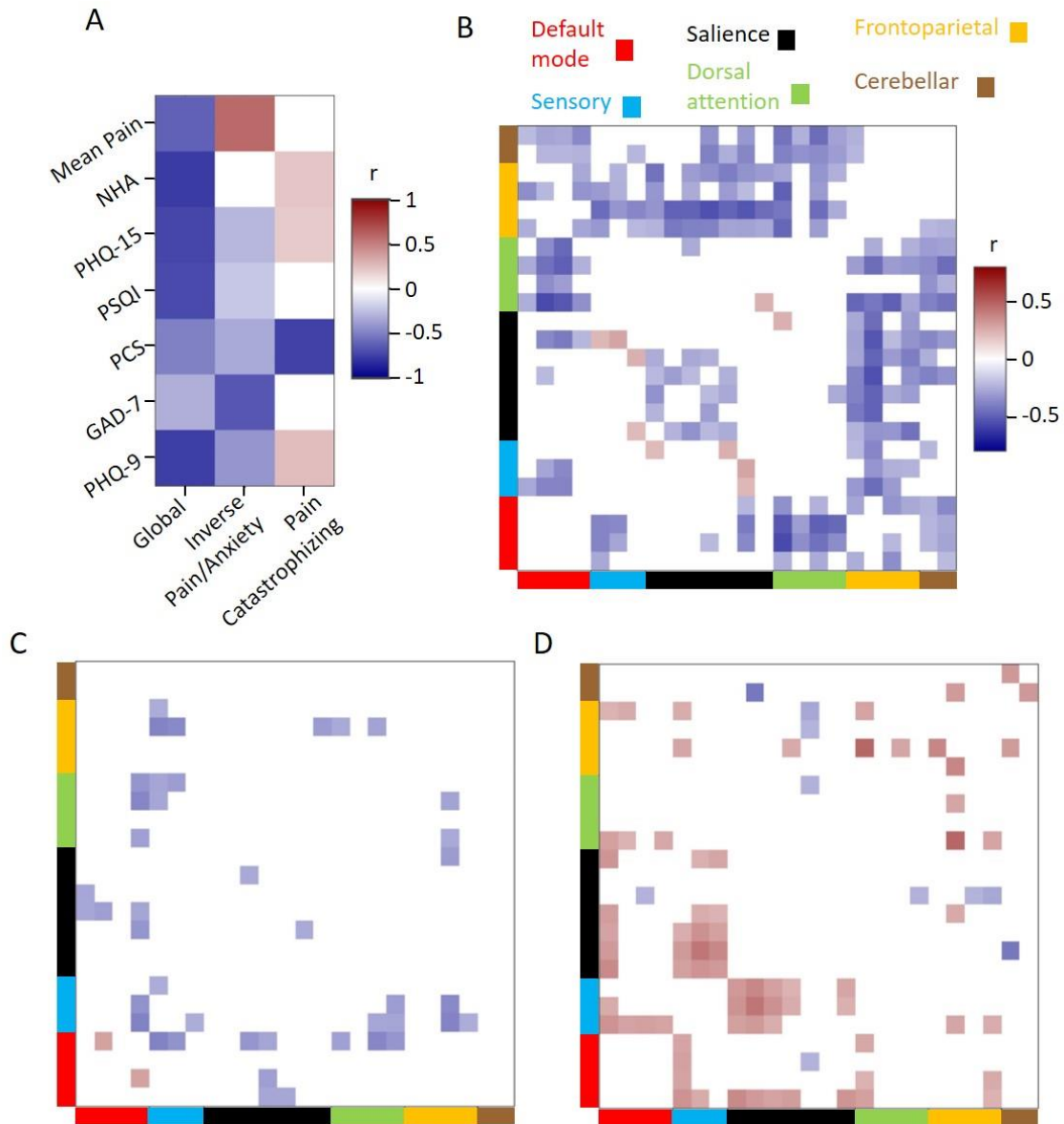


Figure 8. Interpretation of canonical variates. Canonical variates were correlated with input data to facilitate interpretation. A) For clinical data, correlations shown for the three identified dimensions. Correlation between RSFC and global dimension (B), inverse pain/anxiety dimension(C), and pain catastrophizing dimension (D) scores organized over 24 regions of interest in 6 networks. The global dimension was most associated with frontoparietal network and dorsal attention network connectivity with other networks. The inverse pain/anxiety dimension was most related to default mode network and sensorimotor connectivity. The pain catastrophizing dimension was most related to salience, sensorimotor and default mode network connectivity. The same color coding for networks and correlation color range is used in panels B, C, and D. All off-white correlation values are significant for all four panels. r =Pearson correlation coefficient. See Methods: *Clinical Features* for clinical abbreviations.

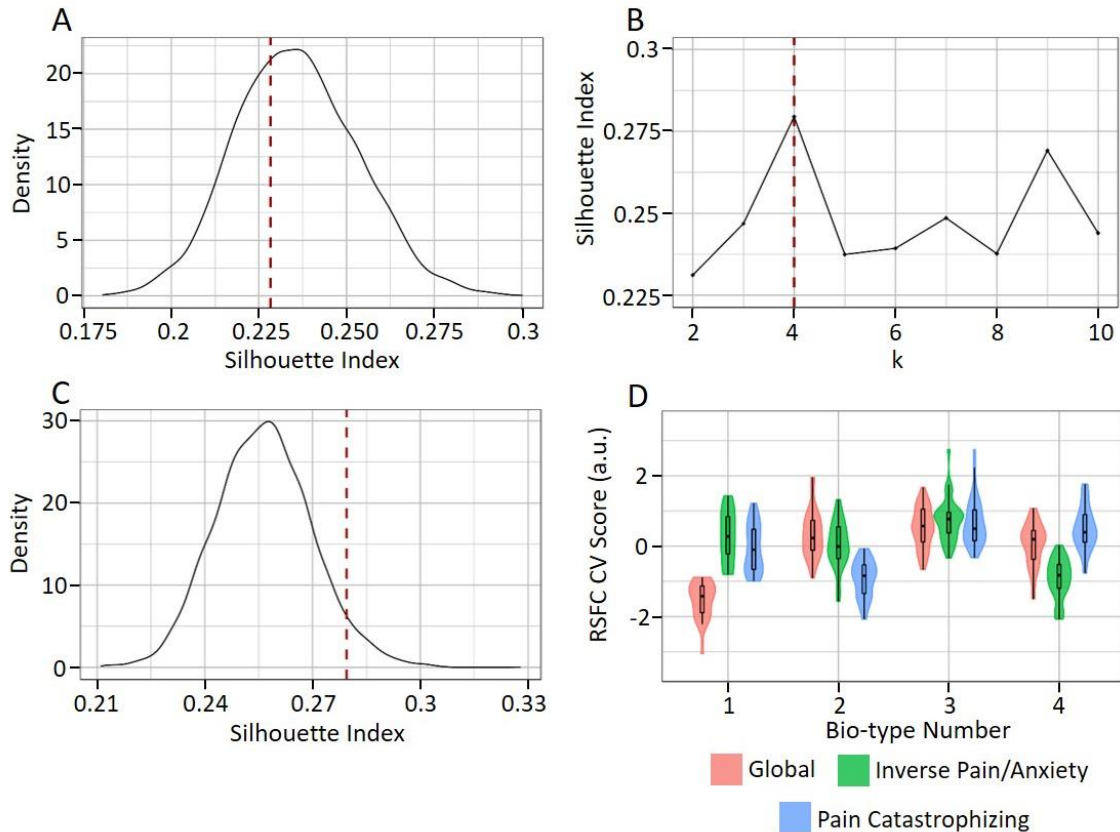


Figure 9. Lack of support for episodic/chronic migraine clustering and identification of a 4 biotype clustering of migraine. A) Lack of significance for clustering migraine into chronic and episodic subtypes based on silhouette index over 5000 multivariate Gaussian distributions of clinical data. B) Silhouette index for various cluster numbers (k) from three RSFC canonical variates indicating that $k=4$ had the best performance. C) Significance of clustering into four biotypes based on silhouette index over 5000 multivariate Gaussian distributions of RSFC canonical variate scores. D) Patterns of RSFC canonical variates for three dimensions over the four identified biotypes. Color coding indicates the dimension. Canonical variates are distributed with a mean of zero, therefore negative values indicate below average scores and positive values indicate above average scores. RSFC=resting-state functional connectivity; CV=canonical variate; k =number of clusters.

Testing the Support for Current Migraine Clustering

We tested if the current clustering of migraine patients into episodic (<15 headaches/month) and chronic (≥ 15 headaches/month) subtypes differs from a multivariate Gaussian distribution. Chronic and episodic migraine patients were collected

at different sites, creating a potential confound (see discussion). Using the seven clinical features used for CCA, we determined the silhouette index in 166 patients using the chronic/episodic distinction of 0.23. We then again compared this to 5000 multivariate Gaussian distributions based on the mean and covariance of the 7 clinical measures clustered with k value of two. This analysis indicated that the chronic versus episodic clustering was not significantly different from a multivariate Gaussian distribution, $N = 166$, $p = 0.66$ (Fig. 9A). Therefore, there is insufficient evidence to reject the null hypothesis that the episodic versus chronic clustering solution comes from a multivariate Gaussian distribution. This means that using a holistic catalogue of symptoms, we were unable to find support for subtyping migraine patients into episodic and chronic migraine.

Identifying Biotypes in Migraine

In order to identify subtypes of migraine based on biological data, called biotypes, we performed k -means clustering based on the canonical variates for RSFC for the three dimensions. The best performing solution was $k=4$, which had the best performance in 27% of metrics, though there were other solutions with similar performance ($k=2$ was best in 23%, no other solutions were consistently as strong; see Fig. 9B for silhouette index scores over various values of k). To determine significance of a four cluster solution, we estimated 5000 multivariate Gaussian distributions based on the mean and covariance of the three RSFC canonical variates. Each of these distributions then underwent k -means clustering into four clusters. We calculated the silhouette index, a measure of clustering quality, in each of these 5000 distributions and compared the true four cluster silhouette index of 0.28 against them (Fig. 9C). The true silhouette index of 0.28 was significant relative to the multivariate Gaussian distributions $N=143$, $p=0.049$

(Fig. 9C). These clusters had unique patterns of RSFC canonical variate scores (Fig. 9D). Cluster one had negative and therefore below average RSFC canonical variate values for the global symptom dimension, indicating patients in this group had worse than average symptoms overall. Cluster two had below average RSFC canonical variate scores for the pain catastrophizing dimension, meaning patients in this group had above average catastrophizing. Cluster four had below average pain/anxiety inverse dimension scores, indicating patients in this group had below average mean pain and above average anxiety. Cluster three had above average scores in all three dimensions, indicating patients in this group had the opposite pattern of symptoms as all other biotypes.

Discussion

Migraine is a variable disorder with heterogeneous response to therapy and expression of symptoms. This heterogeneity has been ignored in many studies that treat migraineurs as a single group in between-subjects analyses contrasting patients and healthy controls. When variability has been factored in, subjects are often subtyped into categories not derived from biological data that have also not been rigorously tested. When studies do perform within-subjects analyses to associate symptoms with biological data, they often examine only one variable of interest, ignoring the complex relationship between migraine symptoms. In the current study we addressed these existing limitations by using a large sample size, including a wealth of clinical data from well phenotyped subjects, and by relating these clinical profiles to RSFC in a single multivariate statistical step, as opposed to a standard mass univariate analysis. This approach identified three dimensions of covariation between clinical and RSFC data. The first was a global-symptom dimension that was associated with all symptoms and was primarily related to

frontoparietal network (FPN) and dorsal attention network (DAN) functional connectivity with other networks. Our findings suggest that cognitive networks relate to symptoms generally, and that they may function as targets to simultaneously treat all symptoms at once. Consistent with this, the normalization of FPN functional connectivity has been associated with clinical improvement in migraine (K. Li et al., 2015). The second dimension reflected inverse pain and anxiety scores and was related to default mode (DMN) and sensorimotor network connectivity. Finally, the third dimension was a pain catastrophizing dimension and was primarily associated with functional connectivity of salience network (anterior insula and midcingulate cortex) to DMN and sensorimotor networks, and to DMN connectivity with other networks.

The migraine RSFC literature consists mostly of studies with small sample sizes and inconsistent analyses, making it difficult to synthesize (Skorobogatykh et al., 2019). However, there do appear to be important points of convergence between our findings and previous work, which further supports the validity of our findings. Abnormal RSFC in migraine is often observed in the FPN, salience, sensorimotor, DMN, and DAN (Hubbard et al., 2014; Jin et al., 2013; Ke et al., 2020; Russo et al., 2012; Xue et al., 2012). We therefore anticipated that these networks would be associated with illness characteristics. Two clinical variables included in our analysis that have been the subject of multiple studies in migraine are headache frequency and pain catastrophizing. Frequency was most related to our first identified global dimension, and associations between headache frequency and functional connectivity of salience and FPN nodes (Hubbard et al., 2014; Mainero et al., 2011; Maleki et al., 2012). Pain catastrophizing is often linked to attentional processes (Quartana et al., 2009) and PCS scores are related to

anterior insula (key node of the salience network) and sensorimotor connectivity in migraine patients (Hubbard et al., 2014).

Our study has major implications to migraine research and clinical trial design. Migraine is currently split into episodic and chronic classifications based on a 15 headache a month cutoff (International Headache Society, 2018). There have been criticisms of the concept of chronic migraine in the literature (Medrea & Christi, 2018), and in particular over the exact cutoff point between episodic and chronic patients (Torres-Ferrús et al., 2017). We tested the quality of the current two cluster solution for migraine patients in our data based on seven clinical features, finding no evidence against the null hypothesis of a multivariate normal distribution (which does not have clusters). This is especially interesting because the episodic and chronic migraine patient cohorts in our study were collected at two separate sites, which if anything should bias the results toward a chronic versus episodic clustering solution. Additionally, we identified four biotypes of migraine that could be used for predicting therapeutic outcomes in future studies. Patients falling into a given cluster had unique brain-symptom relationships arguing for targeted therapy (Fig. 9D). For instance, patients in cluster one had the most extreme global symptom dimension score, which was associated with FPN and DAN connectivity, and perhaps these patients would benefit the most from interventions targeting these networks. On the other hand, cluster two patients who had more extreme pain catastrophizing might benefit the most from interventions that reduce catastrophizing and also target sensorimotor and salience networks. Together, these clustering results argue against the current episodic/chronic clustering that dominates

migraine research and clinical trial design, and suggest an alternative based on clinical and biological association.

This study is not without limitations. CCA is particularly susceptible to overfitting, and previously exciting results in depression (Drysdale et al., 2017) may have fallen victim to this issue (Dinga et al., 2019). In the current study we took several steps to mitigate overfitting. First, we reduced the number of features in the model through parcellations (instead of voxels) for RSFC, feature selection, and PCA. Altogether, this allowed us to greatly increase the ratio of subjects to model features, attenuating overfitting. We took the additional step of performing regularized CCA and tuned lambda parameters based on the held out strength of the first canonical correlation. This resulted in very minimal overfitting for the first canonical correlation. However, overfitting was larger for the second and third canonical correlations, indicating that some caution is warranted when interpreting and using them. Future work could expand on our results by testing this model in new data and benefit from data sharing to create larger sample sizes that are less prone to overfitting. Additionally, statistical inference for clustering is challenging (Dinga et al., 2019; Liu et al., 2008). In the current study we tested against a null distribution of a multivariate Gaussian distribution, which when true, would not merit clustering. However, even with rejection of this null hypothesis, it is possible that clustering is still unwarranted because not all multivariate non-Gaussian data are necessarily organized into clusters. Future work could examine the utility of this clustering solution by using the four biotypes to predict therapeutic outcomes from migraine treatment, and by examining if these biotypes exist in additional chronic pain disorders. Finally, our study is limited by using a parcellation without subcortical regions.

While the reduced number of nodes greatly attenuated dimensionality, ideally a larger sample size would have been available that allowed for a larger number of regions to be assessed.

In conclusion, we identify an association between migraine symptoms – including headache severity, quality of life, affective measures, and coping – and whole-brain resting state functional connectivity, yielding three dimensions of association between these two domains. These results may facilitate the development of personalized medicine, which is limited by treating illnesses as homogeneous groupings. Moreover, the biological association with our clinical data identifies potential targets for therapy and research. Finally, our data argue against the current clustering of migraine patients into chronic and episodic classifications and instead offer an alternative that is grounded in clinical presentation and biology simultaneously.

Chapter 4: Predicting MBSR+ outcomes with pre-treatment RSFC

Introduction

Clinicians and researchers alike view precision medicine as a critical long term goal for medicine (Ashley, 2016; Collins & Varmus, 2015; Mirnezami et al., 2012). In precision medicine, specific treatments are chosen for a specific patient based on individual characteristics (Ashley, 2016; Collins & Varmus, 2015; Council, 2011; Mirnezami et al., 2012). One gap that must be addressed for this long term objective is a better understanding of variability in treatment responses. In migraine, as is unfortunately the case across most illnesses, patients with identical diagnosis and treatment differ greatly in response. This is true for pharmacologic therapies like triptans (M. D. Ferrari et al., 2002), and for MBSR+ (Seminowicz et al., 2020). While we showed in the previous chapter that certain migraine subtypes are not evidence based, these subtypes suffer additionally from having limited predictive ability for therapeutic outcomes (Ashford et al., 1998a; Rapoport et al., 1997; Silberstein et al., 2006a). Altogether, the current state of treatment decision puts migraine clinicians in an unfortunate position where they are forced to make educated guesses for their patients.

Some progress has been made to better understand why migraine patients have heterogeneous responses to treatment. Demographic factors like sex and migraine pain severity are associated with acute migraine treatment (Lipton et al., 2016). Unsurprisingly, successful previous treatment is also predictive of triptan response (Rothrock et al., 2005). Brain structure across 10 regions was somewhat predictive of responder rates from acupuncture therapy (Yang et al., 2020). In a functional connectivity

study, anterior cingulate cortex functional connectivity was predictive of migraine patient response to cognitive behavioral therapy (Nahman-Averbuch et al., 2021). In general, these studies often fail to provide appropriate information to determine how predictive the models actually are, especially for performance in held out testing data. As a result, it is difficult to estimate how useful each of the studies on predicting migraine treatment responses would be in a new sample. No study has attempted to predict MBSR+ outcomes based on RSFC before treatment.

In the current aim, we sought to predict the percentage reduction in headache frequency using pre-treatment (baseline) RSFC. Two different sets of functional connections were used following two levels of feature selection. The first level of feature selection (described in chapter 2 and used in aim 1 [chapter 3]) was from removing language and visual regions of interest. The second level of feature selection was based on mass-univariate correlation between first level feature selection functional connections and headache frequency in the combined *episodic-UMB* and *chronic-Stanford* datasets. We used this second level of feature selection to further reduce the dimensionality of the data and because we believed that connectivity related to baseline headache frequency would be most likely to predict reductions in headache frequency from MBSR+ (see below section: *Feature Selection from Baseline Headache Frequency Association*). In addition to these features, we also used the first canonical variate score identified in aim 1, which was most related to headache frequency. Therefore, three predictions of percentage reduction in headache frequency were performed.

Aim 2: Determine baseline (pre-treatment) RSFC predictors of MBSR+ responsiveness

- a) Use machine learning on pre-treatment RSFC to predict the percentage reduction in headache frequency for MBSR+ randomized patients
- b) Examine how RSFC dimensions associated with symptoms (from aim 1) predict the percentage reduction in headache frequency for MBSR+ randomized patients

Hypothesis: MBSR+ outcomes can be predicted using pre-treatment RSFC and models will weight RSFC most associated with headache frequency at baseline

Methods

Participants and Data

Participants were from the previously described *episodic-UMB* dataset (see chapter 2). This aim was interested in how pre-treatment RSFC predicted clinical improvement, therefore, data were used for baseline scans (pre-therapy, 0 weeks). Only subjects who were randomized to a receive MBSR+ were included in this aim.

Additionally, subjects had to have available headache frequency data at baseline and 20 weeks, along with a resting state scan at baseline that passed quality control standards (see chapter 2). 42 subjects met these criteria.

Feature Selection from Baseline Headache Frequency Association

The RSFC underwent feature selection prior to all aims that removed visual and language nodes (see chapter 2). For aims two and three, there was an additional level of feature selection based on baseline univariate association with headache frequency. We performed spearman correlation between all subjects in aim 1 (n=143) between headache frequency and RSFC. We based this extended feature selection on baseline data from *episodic-UMB* and *chronic-Stanford* because we reasoned connections related to

headache frequency before treatment in a larger sample would be most likely to relate to headache improvements.

Machine Learning Prediction

Using pretreatment RSFC, we sought to predict the percentage reduction in headache frequency in MBSR+ randomized subjects. Given the fairly small sample size, we were concerned about over-fitting, where performance in training data would be far superior to model performance in testing data. Therefore, we used least absolute shrinkage and selection operator (LASSO) regression to predict reduction in headache frequency, given that LASSO performs well against overfitting (Friedman et al., 2001). Unlike other regularized regression approaches like ridge regression, LASSO regression typically selects a subset of predictors by shrinking regression slopes to zero for some predictors, yielding sparse models that are more interpretable (Franklin, 2005). The severity of shrinkage is controlled by a user specified parameter, lambda, which is the L1 norm of the regression coefficients. Lambda values of zero will yield a least squares fit and as lambda values approach 1, the model is increasingly regularized. LASSO regression has been used many times to predict behavior using neuroimaging data (Krishnan et al., 2016; Wager et al., 2011, 2013).

It is well established that functional connectivity can be organized into a series of networks (Yeo et al., 2011). Therefore, multicollinearity exists in any predictive model using functional connectivity. When multicollinearity of predictors exists, regression slopes become uninterpretable. To address this, we first performed Principal Components Analysis (PCA) on RSFC and used the resulting components, which are orthogonal to one another, eliminating concerns over multicollinearity. We elected to use components

explaining 80% cumulative variance. For prediction without feature selection (276 functional connections) 19 components were included and for the model with feature selection for baseline association with headache frequency (90 functional connections) 15 components were included.

To select optimal regularization parameters (λ) we used leave-one-out-cross-validation (LOOCV), where the LASSO-PCR model was built using all but one subjects, and the model coefficients were used to predict headache frequency reduction in the held out subject. This was repeated until every subject served as the testing subject, for all values of λ . λ values included 0 (least squares model) and moved sequentially from 0.01 to .3 (maximally regularized model) by increments of 0.005. To determine the optimal λ model, we examined the mean squared error for held out predictions for every value of λ .

RSFC 'Global' Canonical Variate Prediction

The global canonical correlation (1st canonical correlation) was related to headache frequency (see chapter 3). We therefore sought to determine if RSFC global canonical variate scores could be used to predict percentage reduction in headache frequency. Using the reconstructed weights we calculated canonical variates for every subject at baseline. We used a linear model to predict headache reduction in MBSR+ condition and evaluated over-fitting using LOOCV.

Statistics

To determine statistical significance of LASSO-PCR we performed a permutation test by constructing 5000 bootstraps with replacement (Efron, 2000) using the final model lambda parameters. For every bootstrap, we calculated mean squared error and then compared this null distribution against the true mean squared error using a

one-sided t-test. In the case of prediction with canonical variate values, the same process was used as for statistical testing of machine learning models, but without regularized regression. For feature selection from baseline headache association, significance was determined using a 0.05 FDR-corrected threshold (Benjamini et al., 2001).

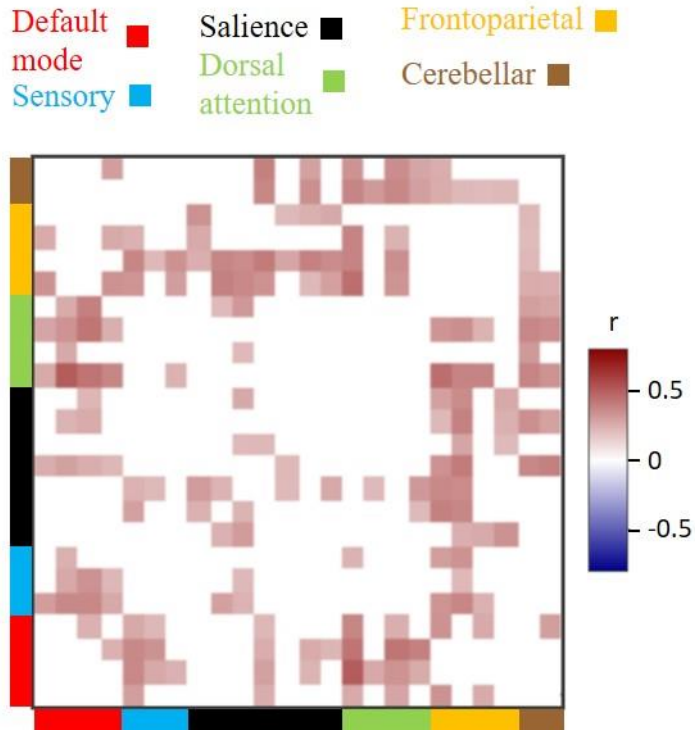


Figure 10. Significant mass-univariate association between headache frequency and RSFC in combined *episodic-UMB* and *chronic-Stanford* datasets. Results show that headache frequency was related to frontoparietal network connectivity with other networks.

Results

Feature Selection from Baseline Headache Frequency Association

We used a univariate filter to select edges related to baseline headache frequency. We found 90 functional connections that were significantly associated with baseline headache frequency and used this as an additional level of feature selection. These significant connections were primarily between the frontoparietal network and sensory, salience, and dorsal attention networks (Fig. 10). Therefore, models could use either 276 or 90 functional connections prior to dimensionality reduction from PCA.

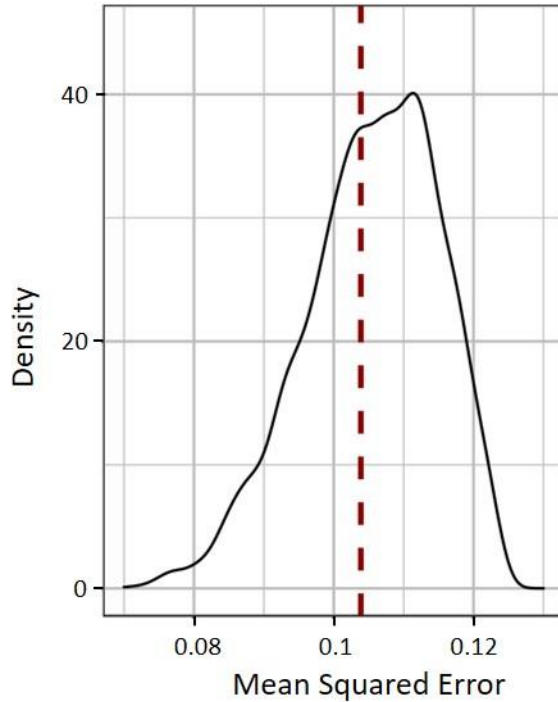


Figure 11. Permutation test for LASSO-PCR prediction of headache frequency reduction based on baseline RSFC. Final model performance mean squared error (shown in red) relative to 5000 permutations. This model only used RSFC that correlated to headache frequency at baseline.

Machine Learning Does not Predict Treatment Outcomes

For the model using only one level of feature selection (same as in aim 1) we determined that a lambda value of 0.3 for all functional connections and a lambda value of 0.07 for a second level feature selection based on pre-treatment headache frequency association was optimal. This level of regularization for the first level of feature selection (RSFC=276, 19 principal components) created a model where all coefficients were zero. This performance shows that a heavily regularized model that included no predictors (aside from the intercept) was the best, indicating poor model performance. Therefore, we made no attempt to estimate statistical significance, or to interpret this model. For

data with two levels of feature selection feature selection (RSFC=90, 15 principal components), a lambda value of 0.07 resulted in 2 RSFC components being included. The mean squared error for the testing data was .13 and for the full model was 0.1. Compared to this null distribution, the final model mean squared error was not significantly different, $p=0.41$ (Fig. 11).

RSFC ‘Global’ Canonical Variate Does not Predict Treatment Outcomes

We used a linear model to predict

headache reduction in MBSR+ condition subjects and evaluated over-fitting using testing data from LOOCV. Test data MSE was 0.12 and train data was 0.11. MSE was not significantly different from a null distribution $p=0.07$ (Fig. 12).

Discussion

Migraine is a disabling and poorly managed global health crisis (Lipton et al., 2007). A major gap in current migraine treatment is that patients are treated using a one-size-fits-all approach that ignores variability in responsiveness to therapy (A. Ferrari et al., 2008). Clinically, it is well appreciated that heterogeneity is the norm of migraine disorder and seemingly similar migraineurs display highly distinct responses from identical medications (Cutrer, 2003). This treatment heterogeneity is observed in

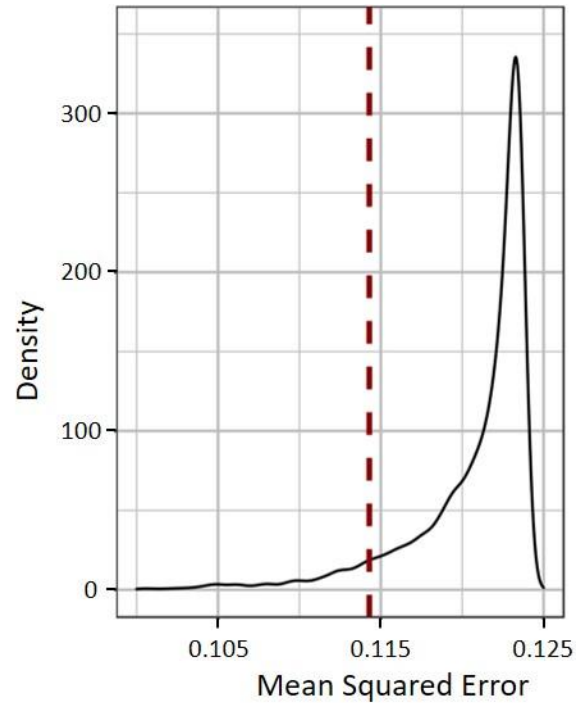


Figure 12. Permutation test for linear model prediction of headache frequency reduction based on baseline RSFC global canonical variate scores. Final model performance mean squared error (shown in red) relative to 5000 permutations.

pharmacotherapies (Ashford et al., 1998b; Silberstein et al., 2006) and from MBSR+ (Seminowicz et al., 2020). Ideally, patients would be treated using a precision medicine approach, where therapy is tailored at the individual level (Collins & Varmus, 2015). A potential strategy for precision medicine would be to input RSFC into a previously trained model, and to then use model output to either recommend or dissuade from pursuing MBSR+ therapy based on the likelihood of that individual's response. In the current aim, we attempted to build towards a precision medicine treatment of migraine by analyzing how RSFC before treatment is predictive of MBSR+ response. However, the promise of a predictor for MBSR+ proved elusive in this analysis as we were unable to predict MBSR+ response from functional connectivity.

We used a machine learning model (LASSO-PCR) with a variable number of functional connections based on two levels of feature selection for RSFC to predict reduction in headache frequency. One level of feature selection excluded language and visual regions (see chapter 2) and the second level of feature selection only included connections based on significant univariate association with headache frequency prior to treatment in the combined *episodic-UMB/chronic-Stanford* datasets. We reasoned that functional connections related to baseline (pre-treatment) headache frequency would be related to improvements in headache frequency from MBSR+. However, neither of these models were able to successfully predict treatment response. These null results suggest that functional connections unrelated to headache frequency may be predictive of responsiveness from mind-body therapy. As a result, feature selection using a pre-treatment clinical trial outcome measure only may be unlikely to yield successful models. Additionally, we used an ordinary least squares model to predict headache frequency

reductions using the RSFC global dimension canonical variate scores identified in aim 1 (see chapter 3). This global dimension was used as it was associated with headache frequency at baseline. Similar to the machine learning models, this model again was not able to predict headache frequency reductions from therapy.

There are several limitations and corresponding areas of improvement for this analysis. The sample size was small relative to recommendations for this type of statistical learning (Poldrack et al., 2020). Reducing the dimensionality through PCA and using a single predictor from aim 1 was not enough to ameliorate poor model performance. As a result of this limitation, it is difficult to convincingly conclude that functional connections associated with headache frequency prior to treatment are *unrelated* to functional connections predictive of headache frequency reduction. To address this limitation, improved data sharing in migraine research is necessary. An issue with the feasibility of increasing sample sizes is that clinical trials for MBSR+ treatment of migraine with RSFC are uncommon, therefore additional data collection is required. A separate and potentially more feasible question would be to examine if functional connectivity is predictive of treatment outcomes regardless of the therapy. That is, is there a common mode of variation across migraine therapies that predicts successful outcomes? This question allows for immediate consolidation of existing datasets. However, it does not exactly address the need for precision medicine, where a patient receives advice on a specific treatment to receive. Therefore, it seems that only additional data collection can likely determine if/what RSFC is related to MBSR+ outcomes.

Along with increasing the quantity of data (from larger sample sizes) an additional way to improve the current analysis is by strengthening the quality of the data.

It has recently been shown that individually stable RSFC estimates can be best achieved using longer duration resting state scans than the current analysis (Gordon et al., 2017). As a result, RSFC at baseline may be missing crucial elements of individual variability related to MBSR+ response prediction. To address this, studies can increase the duration of resting state scans, which would require novel data collection. A possible additional approach would be to leverage functional task scans. In the *episodic-UMB* dataset, subjects also performed pain and cognitive conflict tasks, neither of which were included for analysis in this thesis. Recent work indicates that it is also possible to achieve high quality functional connectivity estimates using task data (McCormick et al., 2021). It might therefore be possible to use task data derived functional connectivity combined with resting state derived functional connectivity to better capture individual variability.

In conclusion, we were unable to use pre-treatment RSFC to predict MBSR+ outcomes. This was true even when only using RSFC related to pre-treatment headache frequency. Therefore, it may be the case that functional connectivity related to headache frequency is not necessarily predictive of treatment that reduces headache frequency. To have more confidence in this claim, it is essential that larger sample sizes with higher quality RSFC estimates are made available. Previous studies that have predicted migraine treatment outcomes (Nahman-Averbuch et al., 2021) may be unreliable given certain inappropriate statistical practices like double-dipping (Vul et al., 2009). To increase sample sizes, improved collaborative projects featuring many study sites will be necessary. With such collaboration, hopefully it will be possible to provide precision medicine for migraine patients instead of recommending therapy that treats migraine patients as a homogeneous group.

Chapter 5: RSFC Mediation of Clinical Improvement from MBSR+ Therapy

Introduction

A common question in science is not simply if an independent variable exerts an effect on a dependent variable, but rather how the independent variable exerts such an effect. This is a question of central importance in clinical research. From our clinical trial on MBSR+, it is clear that MBSR+ exerts a clinically meaningful effect on headache frequency (Seminowicz et al., 2020), but the mechanism of this effect is unknown. To address this question of how effects are exerted, some researchers examine differences between experimental conditions following therapy. Using *episodic-UMB* data as an example, this would entail a contrast between stress management for headache (SMH) and MBSR+ randomized patients post-treatment. However, showing a significant difference in RSFC following therapy between MBSR+ and SMH patients does not show how MBSR+ reduced headache frequency, as it is unknown whether the identified functional connections are related to clinical improvement (e.g., the difference was from a non-specific effect of MBSR+ unrelated to headache frequency). For the same reason, a comparison within a clinical condition (e.g. MBSR+) pre/post-treatment also will not tell how MBSR+ exerted an effect on headache frequency. In the current aim, we examined how MBSR+ influences headache frequency reductions by testing a series of mediation models where changes in functional connectivity mediate MBSR+ effects.

Mediation analysis is interested in calculating the indirect effect. In a simple mediation model, the indirect effect is the product of two terms, a and b (Hayes, 2017). A is the regression coefficient for the independent variable (X) when predicting the

mediator using the independent variable. B is the regression coefficient for the mediator (M) when predicting the dependent variable using both the independent and mediator variable. Non-zero indirect effects are taken as evidence of mediation. Therefore, the indirect effect is the product of the relationship between the mediator and independent variable, and the relationship between the mediator and the outcome variable adjusting for the independent variable. In the context of mediation for clinical trial research, the independent variable (X) functions as the treatment, the dependent variable (Y) is the trial outcome, and the mediator (M) could be a change in the brain. Therefore, a significant mediator would indicate that a change in the brain was potentially responsible for how the independent variable exerts an effect on the dependent variable. Such a mediator is related not just to the treatment condition, but also to the outcome.

To my knowledge, no mediation analysis examining how changes in brain connectivity mediate clinical trial improvements in migraine has been performed. As a result, there is very little information about how treatment of migraine influences RSFC to improve symptoms. There have however been some studies that examine if treatment influences RSFC. An analysis of the *episodic-UMB* found some impact of treatment on anterior insula functional connectivity with posterior cingulate cortex and cuneus (Seminowicz et al., 2020). A separate clinical trial of migraine examined periaqueductal gray connectivity with a node of the DMN following acupuncture and found that compared to baseline all treatment conditions (including sham) had an increase in connectivity (Z. Li et al., 2016). Another clinical trial examining vagus nerve stimulation found larger DMN connectivity to thalamus relative to a sham condition, but this connectivity was unrelated to clinical improvement (Zhang et al., 2021). Therefore, very

little information exists as to how changes in functional networks might mediate clinical improvement in migraine.

The current aim examines three different mediation models. In all models, the independent variable (X) was the treatment condition, the dependent variable (Y) was the percent reduction in headache frequency. Previous analysis of this dataset indicates that MBSR+ has a significant effect on headache frequency (Seminowicz et al., 2020). We used percentage reduction in headache frequency as the dependent variable in mediation models given it was a pre-declared primary outcome for the clinical trial, and is typically treated as the most important indicator of migraine therapy (Tfelt-Hansen et al., 2012). For two of these mediation analyses (multivariate and mass-univariate) we used change scores in RSFC from baseline to post-treatment (20 weeks). Based on mass-univariate correlation between RSFC in the combined *episodic-UMB* and *chronic-Stanford* datasets we identified 90 RSFCs significantly correlated to headache frequency at baseline (see chapter 4). We used these 90 functional connections as an additional layer of feature selection. Therefore, in addition to the 276 RSFCs used for mass-univariate and multivariate mediations, we also performed these analyses using 90 functional connections. Finally, we tested a simple mediation model where changes in RSFC global dimension canonical variate scores (see chapter 3) mediated clinical improvements.

Aim 3: Determine how RSFC changes mediate clinical improvement from MBSR+

- a) Use mass univariate simple mediation to determine how changes in RSFC from pre- to post-treatment mediate percentage reduction in headache frequency for MBSR+ and SMH randomized subjects
- b) Use multivariate mediation to determine how changes in RSFC from pre- to post-treatment mediate percentage reduction in headache frequency for MBSR+ and SMH randomized subjects

- c) Use simple mediation to examine how changes in RSFC dimensions associated with symptoms (from aim 1) mediate percentage reduction in headache frequency for MBSR+ and SMH randomized subjects

Hypothesis: reduction in headache frequency will be mediated by RSFC changes primarily by changes in connectivity related to headache frequency at baseline

Methods

Participants and Data

Participants were from the previously described *episodic-UMB* dataset (see chapter 2). This aim was interested in how changes in RSFC mediated clinical improvement, therefore, data were used for baseline scans (pre-therapy, 0 weeks) and post-therapy scans (20 weeks). Only subjects who were randomized to a treatment group of MBSR+ or SMH were included in this aim. Additionally, subjects had to have available headache frequency data at baseline and 20 weeks, along with a resting state scan at both sessions that passed quality control standards (see chapter 2). 73 subjects met these criteria (35 SMH, 38 MBSR+).

Simple Mediation with RSFC Canonical Variates

Aim 1 identified three dimensions of covariance between RSFC and clinical symptoms. The first dimension was a global dimension that associated with headache frequency. Therefore, we sought to determine if changes in the global RSFC canonical variate values over therapy mediated clinical improvement. Using the reconstructed weights we calculated canonical variates for every subject at baseline and following therapy. We performed a simple mediation using a regression framework (Hayes, 2017), using treatment (MBSR+/SMH) as the independent variable (X), percentage headache

frequency reduction as the dependent variable (Y), and change in global RSFC canonical variate as the mediator (M). Path a was estimated using a linear model predicting canonical variate change (M) using treatment condition. Path b was estimated by predicting percentage headache frequency reduction (Y) using treatment condition (X) and canonical variate change (M) where b corresponded to the regression slope for M.

Mass-Univariate Mediation

We performed a simple mediation again using a regression framework (Hayes, 2017), using treatment (MBSR+/SMH) as the independent variable (X), percentage headache frequency reduction as the dependent variable (Y), and change in RSFC value as the mediator (M). The change in RSFC was calculated using simple subtraction of the post-treatment (20 weeks) RSFC values from the pre-treatment RSFC values. This simple mediation was performed on every RSFC value. We performed this process using 276 edges and 90 edges that were related to baseline headache frequency (see chapter 4).

Multivariate Mediation

The previous univariate mediation treats every functional connection as independent of one another. However, information is represented in the brain across diverse systems (Haxby et al., 2014), necessitating a multivariate analysis of data (Kriegeskorte, 2011). In multivariate mediation, a linear combination of many mediating variables (in this case RSFC changes) are created in order to maximize the size of the mediated effect (path $a * b$) (Chén et al., 2018; Geuter et al., 2020). This linear combination of mediating variables is a principal direction of mediation (PDM). Similar to PCA, multiple PDMs can be found, with each subsequent PDM having a smaller

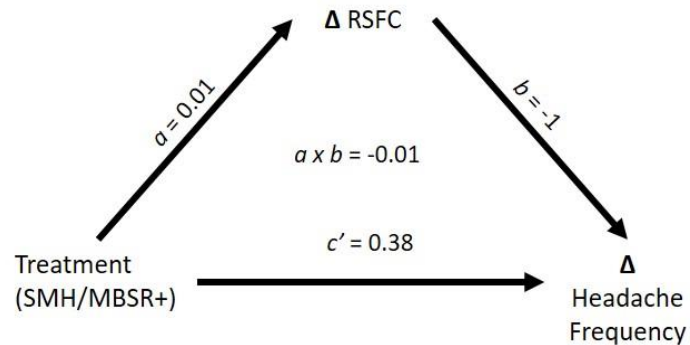
mediated effect, and also being orthogonal to any preceding PDMs. In the current context, any PDM would represent a whole RSFC mediator for treatment effects.

Multivariate mediation involves an initial dimensionality reduction step. We used singular value decomposition for dimensionality reduction. The difference values for RSFC (baseline RSFC values minus post-therapy RSFC values) were used (276 functional connections or 90 when additional feature selection was performed). We sought to determine the number of components that should be included. To determine the appropriate number, we determined the magnitude of the mediated effect ($a * b$) for the first PDM with varying levels of dimensionality reduction, moving from 5 to 64 components estimated. We used 10 fold cross validation stratified for treatment condition across folds and built multivariate mediation models with a single PDM estimated in 9/10 folds and tested the performance of this mediator in the training data and also the held out fold (testing data). This created 10 models tested in 10 folds of data. To determine mediation in held out data, we used the model weights from the training model to calculate the PDM value in testing data. We then calculated paths a and b using the same simple mediation regression based approach as described previously.

Statistics

Permutation tests were used to assess statistical significance of indirect effects calculated as the product of path a and b . To perform the permutation test, 5000 bootstraps with replacement were constructed for the mediator variable (Efron, 2000). For each bootstrap, we calculated the indirect effect ($a * b$) and compared this null distribution of indirect effects against the true indirect effect using a two-sided t-test. For

mass-univariate mediation, we corrected for multiple comparisons using a false discovery rate approach (Benjamini et al., 2001).



Results

Simple Mediation with RSFC

Canonical Variates

We performed a simple mediation using a regression framework (Hayes, 2017). We did not find evidence for changes in the global RSFC canonical variate scores mediating improvements from MBSR+ therapy ($ab=0.1$, $p=.3$, Fig. 13).

Figure 13. Changes in global RSFC canonical variate do not mediate clinical improvements from MBSR+. The indirect effect (product of $a*b$) was not significantly different from a null distribution composed of 5000 bootstraps made by shuffling canonical variate values. Δ = change.

Mass-Univariate Mediation

We examined how changes following treatment for 276 or 90 (with additional feature selection) functional connections mediated headache frequency reduction. After multiple comparisons correction, we were unable to find any functional connection that mediated treatment improvements.

Multivariate Mediation

We examined the held out performance in all 276 functional connections and the 90 baseline headache frequency associated functional connections. Unfortunately, we found that the median testing performance with both levels of feature selection was distributed around 0 and was always quite different from training data (Fig. 14A, B).

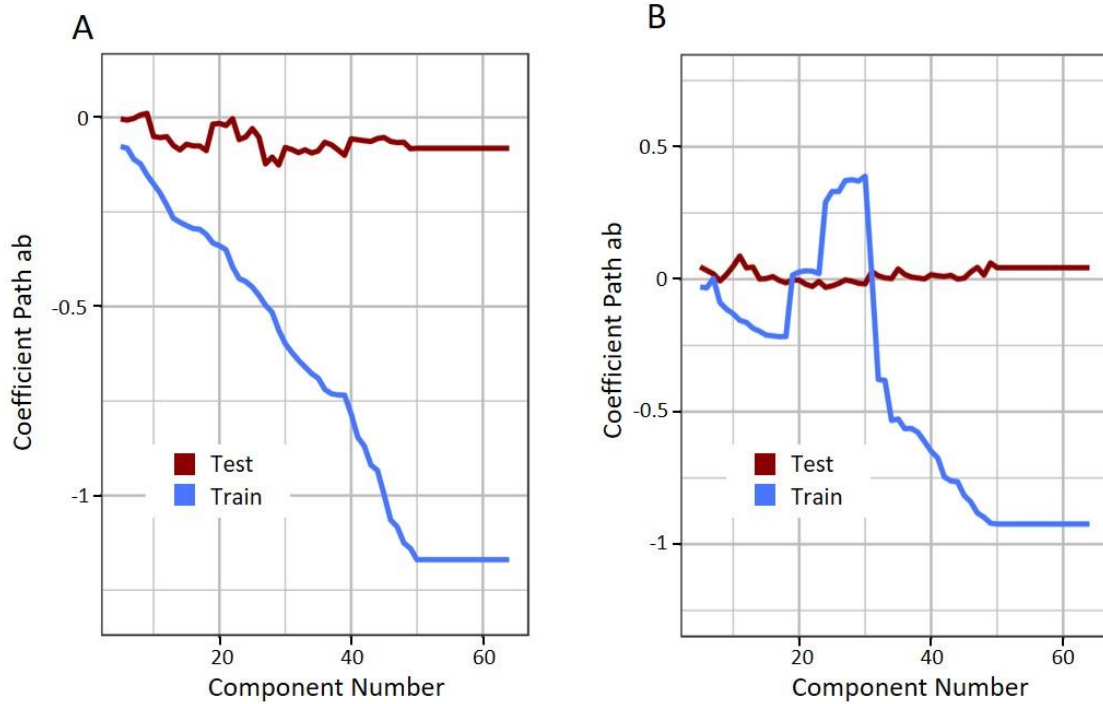


Figure 14. Overfitting for first principal direction of mediation (PDM) for multivariate RSFC changes mediating clinical improvements from MBSR+. A) First PDM performance in testing and training data for only one level of feature selection removing visual and language nodes. B) First PDM performance in testing and training data for an additional level of feature selection based on baseline association with headache frequency. Regardless of the dimensionality of the data and feature selection, the first PDM was always consistently different in training and testing data. This indicates that the first PDM would be unlikely to generalize to new datasets.

Therefore, there is no level of dimensionality reduction that would yield a model likely to generalize in a new dataset. As a result, we concluded that we were unable to find a multivariate mediator of clinical improvement.

Discussion

Previous analysis of the *episodic-UMB* dataset indicates that MBSR+ is more effective at reducing headache frequency than control condition treatment (Seminowicz et al., 2020). However, it is not entirely clear how MBSR+ achieved this success.

Determining how MBSR+ reduces headache frequency is of vital importance because we

will not be able to build better/supporting therapies for MBSR+ without first understanding how MBSR+ works. For instance, if we determined that MBSR+ reduces headache frequency through increasing DMN connectivity with FPN, then directly targeting DMN connectivity might serve as an aid for MBSR+ treatment of migraine, potentially making the therapy even more effective. We previously compared RSFC in patients randomized to MBSR+ or SMH (active control condition) following treatment and found some differences in connectivity between these two groups of patients (Seminowicz et al., 2020). However, this result alone does not indicate how MBSR+ reduced headache frequency, as it is unclear whether RSFC differences between MBSR+ and SMH after therapy are related to headache frequency reductions. Mediation analysis can help to identify how MBSR+ may reduce headache frequency from changing RSFC. Mediation analysis is an approach to understand how an independent variable (e.g. treatment) exerts an effect on a dependent variable (e.g. headache frequency reduction) through a mediating variable (e.g. changes in RSFC over treatment). In the current aim we sought to determine how MBSR+ reduced headache frequency by using a mediation analysis to determine if RSFC changes mediate MBSR+ effects. Unfortunately, we were unable to identify any RSFC changes that mediated MBSR+ effects on headache frequency.

Specifically, we used three categories of mediation analysis: mass-univariate, multivariate, and simple mediation. In the mass-univariate simple mediation, we separately compared how changes in each functional connection might mediate headache frequency reduction and did not find any RSFC change that mediated improvement. This was also true when using a second level of feature selection that selected functional

connections based on baseline association with headache frequency in the combined *episodic-UMB/chronic-Stanford* datasets. It is recognized that such mass-univariate approaches are limited, because they cannot treat the brain as a multivariate, network organized system (Kriegeskorte, 2011). To address this limitation, we also used a multivariate mediation approach (Chén et al., 2018; Geuter et al., 2020) with RSFC from two levels of feature selection. Using this approach, we were unable to identify a multivariate mediator for headache frequency reduction. Finally, we used the RSFC global dimension canonical variate change values in a simple mediation and were also unable to find any evidence for mediation. These null findings suggest that RSFC related to pre-treatment headache frequency may not be targeted by therapies that reduce headache frequency. Instead, such therapies may in fact target unique ensembles of functional connectivity orthogonal to pre-treatment headache frequency.

There are several limitations with the current analysis that future work should improve upon. In previous successful applications of multivariate mediation there have been many more subjects with single trial data, massively increasing the amount of data without an increase in the feature space (Geuter et al., 2020). Additionally, MBSR+ appears to exert fairly small effects on RSFC relative to control condition subjects in this dataset (Seminowicz et al., 2020). However, it is important to note that the previous analysis of the effect of treatment on RSFC in the *episodic-UMB* dataset was quite different than the current analysis. Therefore, our analysis suffered from both small effect sizes and small sample sizes, increasing the likelihood of null findings. Similar to what we noted in chapter four, there are many improvements that can be made to the RSFC data quality in this dataset, namely better individual functional connectivity estimates.

Such improvements would likely also improve the quality of the current mediation analyses by potentially increasing effect sizes for mediation.

In conclusion, we were unable to show that RSFC changes mediate MBSR+ reduction of headache frequency. These null findings were true even when only using RSFC related to pre-treatment headache frequency. It may be the case that functional connectivity related to headache frequency is not necessarily targeted by MBSR+. Larger sample sizes and improved data quality will be helpful to address this. Hopefully, larger datasets are created to test the effects of MBSR+ on RSFC in migraine patients. Such studies, when combined with the current dataset, may together determine the neural mechanisms of MBSR+ reduction in headache frequency. In the future, such work would provide candidate functional connections that could possibly be manipulated with brain stimulation to boost the performance of MBSR+.

Chapter 6: Discussion

Study Overview and Overall Project

Migraine is a major source of global disability and human suffering (Lipton et al., 2007). While new treatments are emerging (Seminowicz et al., 2020; Spindler & Ryan, 2020), comparatively little progress has been made towards precision medicine for migraine. A hurdle that exists for precision medicine is a poor understanding of migraine disorder itself, both in terms of why patients display variable symptoms and why seemingly similar patients have heterogeneous responses to therapy. As noted in the introduction, the pathophysiology of migraine is poorly understood and widely debated. Despite debate, most modern theories embrace the importance of central mechanisms for the disorder, necessitating neuroscience research of migraine (Akerman et al., 2011; Borsook et al., 2012; Goadsby, 2001). A major difficulty when studying migraine is that the illness is very challenging to preclinically model (Burma et al., 2017; Erdener & Dalkara, 2014; Mogil, 2009). Some aspects, like peripheral sensitization (Bergerot et al., 2006) and cortical spreading depression (Erdener & Dalkara, 2014) can be recapitulated; however, we are aware of no animal model that successfully captures a spontaneous and repeating migraine attack with variable psychosocial symptoms. As a result of this, and the widely accepted central mechanisms of migraine, it is essential to study migraine disorder in the human brain.

When studying the brain, many researchers currently adopt a network perspective where nodes of the brain are conceptualized as existing as part of larger coordinated networks (Bassett & Sporns, 2017). This viewpoint comes in contrast with highly modular and specialized conceptualizations of brain function emphasizing local

processing in specialized regions. These two perspectives are not always mutually exclusive, as coordinated activity across networks can contribute to highly specialized function in a region. For instance, visual data processed over a network ultimately gives rise to highly specialized processing of faces in the fusiform face area (Kanwisher & Yovel, 2006). To identify networks, researchers typically rely on correlated timeseries across nodes to estimate functional connectivity. This functional connectivity is believed to promote/inhibit information transfer (McIntosh, 2000). In fact, individual specific task activation can be modelled using the same individual's functional connectivity, indicating that functional connectivity establishes a scaffolding for task performance (Cole et al., 2016). Importantly, the network structure examined with functional connectivity is largely stable over periods of task performance and without tasks being performed (Gratton et al., 2018), making RSFC a trait measure with potential for probing other trait measures like clinical features in migraine patients.

Network analysis in migraine most typically compares migraine patients to healthy controls using a between-subjects design. Using this approach, many differences in functional connectivity have been observed. Several studies have found migraine abnormalities in task-positive networks like the frontoparietal (Coppola, Di Renzo, Tinelli, Lepre, et al., 2016; Russo et al., 2012; Tessitore et al., 2015), dorsal attention (Coppola, Di Renzo, Tinelli, Di Lorenzo, et al., 2016), and salience networks (Russo et al., 2012). The task-negative default mode network also displays differences between migraine patients and healthy controls (Xue et al., 2012). However, differences across studies exist, with some showing reduced default mode connectivity (Coppola, Di Renzo, Tinelli, Lepre, et al., 2016; Hubbard et al., 2014; Ke et al., 2020) and others showing

increased connectivity in migraine (Buono et al., 2017; Coppola et al., 2018).

Inconsistencies in approach from different research groups potentially accounts for these differences (Skorobogatykh et al., 2019). Unfortunately, most papers only report significant findings, which makes it difficult to consolidate findings across the field via meta-analysis. As a result, it is difficult to point to a consistent finding from between-subjects studies of migraine, beyond a general takeaway that distributed abnormalities in functional connectivity occur with migraine. However, because these are not within-subjects studies, it is unclear whether these identified abnormalities associate with migraine symptoms. Importantly, the between-subjects design selects for functional connections with both larger average differences between patients and controls and also small within group variance, making it a suboptimal approach to identify and to understand migraine variability.

Within-subjects approaches are better suited to understand the relationship between migraine symptom variability and networks. Most of these studies use mass-univariate approaches relating a single clinical variable to functions connectivity across the brain in a brain-wide association study. Such associative studies indicate a negative relationship between pain intensity and default mode/saliency network connectivity (Coppola et al., 2018), but a positive relationship with default mode-dorsal attention network connectivity (Hubbard et al., 2014). Headache frequency also has a relationship with frontoparietal (Hubbard et al., 2014) and saliency network connectivity (Mainero et al., 2011). Unfortunately, these within-subjects studies typically suffer from very small sample sizes, which increases the likelihood of findings that do not generalize (Marek et al., 2020). Additionally, the mass-univariate approach ignores the shared structure within

RSFC by treating each functional connection as independent of all others. Ultimately, due to analysis design decisions and poor sample sizes, the migraine-RSFC literature has not advanced our understanding of migraine variability as well as one would have hoped for. Additionally, there has been very little work done on understanding why patients have such variable responses to therapy, with nearly no studies working to do predictive studies of migraine response to treatment.

To use network analysis to better understand migraine variability, a change in research approach is needed. First off, it is important to critically evaluate the current clustering of migraine (International Headache Society, 2018) based on aura and headache frequency. Instead, migraine research would benefit from taking a dimensional perspective similar to Research Domain Criteria (RDoC, Cuthbert & Insel, 2013). RDoC has challenged the American Psychiatric Association view of mental illness by instead urging researchers to examine mental illness based on symptom dimensions that are related to biology (Cuthbert & Insel, 2013). By relating symptoms to biology, it will hopefully be possible to understand the neurophysiologic mechanisms of symptom dimensions and to potentially directly target the brain to modulate symptoms in the future. Additionally, such approaches may prove useful for explaining variability in treatment response. For instance, an RDoC consistent dimensional analysis of RSFC and depression symptoms proved useful in predicting patient response to brain stimulation (Drysdale et al., 2017). This approach applied to migraine could potentially prove useful in also understanding if clinical therapies, like MBSR+, achieve success through modification to functional connectivity that is related to migraine symptoms.

To address current limitations in understanding migraine variability, it is necessary to understand how migraine symptom variability and treatment variability relates to the brain. Greater understanding of this relationship will allow for new ways to group patients and to predict therapy. Unfortunately, previous within-subjects studies have serious methodological shortcomings, and typically examine a very limited band of migraine symptoms. Instead, a more holistic range of migraine symptoms must be related to neural variation. We address this limitation using multivariate analysis that relates symptom and neural variation with one another in a single statistical step. To better understand the variability in migraine treatment outcomes, we used data from a clinical trial on MBSR+. We used pre-treatment RSFC to predict MBSR+ outcomes and then examined how changes in RSFC might mediate clinical improvements from MBSR+. This work together holds the potential to fundamentally alter how we think about and research migraine and to move the field from one that eschews heterogeneity to one that embraces and capitalizes on it.

Summary of Main Findings

Migraine patients are currently researched and treated based on specific subtypes they belong to. One major diagnostic boundary in migraine is based on headache frequency of 15 headaches a month, which serves to create episodic migraine patients below the boundary and chronic migraine patients above. Research into migraine chronification often relies on this boundary (Bigal & Lipton, 2011) as do clinical trials and subsequent drug approval (Escher et al., 2017). Our research shows that the current clustering of migraine into episodic and chronic subtypes is not supported by our data. As a result, clinical trials specific to chronic or episodic migraine may be inadvisable.

Loosening the restriction on only having migraine patients of one subtype would make recruitment easier and allows for greater variability to be examined.

An additional finding from our work is that related to brain function. Using a dimensional approach consistent with RDoC philosophy, we identified three dimensions of association between seven clinical variables and RSFC distributed across six networks. The first dimension (global), related to all symptoms and to frontoparietal network connectivity with other networks. This suggests that frontoparietal targets could be used to both understand and to potentially modulate migraine symptoms globally. The second dimension (inverse pain/anxiety) related to default mode network and to sensorimotor network connectivity. The final dimension (pain catastrophizing) was related to pain catastrophizing and salience network connectivity. Using this dimensional approach allows for orthogonal elements with unique symptom/brain mapping to be identified, revealing potentially multiple targets for therapy. Using these three dimensions, we argue for a four biotype model of migraine that relates to neural and clinical variability simultaneously.

We were unable to produce predictors of migraine responsiveness to MBSR+ using exploratory approaches nor with our identified dimensions. Additionally, we were unable to find that RSFC changes mediated clinical improvement. This does not mean that RSFC is uninteresting from a treatment standpoint, and instead likely suggests that much larger sample sizes are needed. There are two fundamental challenges with increasing sample sizes. First, MBSR+ is a fairly recently accepted treatment for migraine, and therefore there are not additional studies that can be easily incorporated into an analytic pipeline. Additionally, there is a seeming unwillingness to share data in

migraine research. Despite more than a dozen requests to other labs to combine data in a mutually beneficial manner (reciprocal data sharing and co-authorship) only a single other lab at Stanford University was willing to share data. Some labs that refused to share data had even moved on from migraine research and had no plans to continue working on the data. To address this problem, funding agencies and journals should better enforce data sharing requirements, and a refusal to share data when contractually obligated should be reflected in investigator scores for future grant proposals.

Limitations and Future Directions

While we included more clinical features in our analyses than almost any other study, there are still clinical factors that were unassessed or were not included because of cross site differences. For instance, we were unable to include measures of allodynia in our analysis, nor were we able to include disability scores, because these constructs were not identically measured across sites. Additionally, aura was not evaluated in either dataset and therefore had to be excluded from all analyses. As noted in the introduction, aura has a limited relationship with other symptoms, therefore it is unlikely that the inclusion of aura would have changed our results.

One general issue with pain research is the challenge with determining what clinical features need to be used in analysis. Obviously, seven clinical measures are unlikely to capture the entirety of the migraine experience. However, it is unclear what additional features must be included. A major issue is that the shared structure across chronic pain symptoms is largely uncharacterized outside of general correlation patterns across symptoms. Future work would benefit from characterizing the structure of chronic pain symptoms. A possible starting approach would be factor analysis of clinical data.

This would require some ‘translation’ across disorders, so instead of factoring in the frequency of migraine attacks, one would have a frequency measure of primary pain symptoms. While some progress towards this objective could be made with existing datasets, it would likely be necessary to sample nationally representative chronic pain patients using a large battery of clinical measures. The results from factor analysis of such studies might allow for identification of latent variables that could be used in canonical correlation and related to biological data.

Conclusion

Our findings have major implications for drug approval, treatment, and research into migraine. By finding no evidence for the episodic/chronic migraine divide, clinical trials should no longer limit inclusion to either chronic or episodic migraine patients. This would allow the Food and Drug Administration to approve novel therapies for all migraine patients instead of just a subtype of patients. Our research also suggests that frontoparietal network connectivity with other networks could serve as a valuable target for modulating all migraine symptoms simultaneously. Additional networks, like the saliency network, appear important for understanding pain catastrophizing. These results will hopefully facilitate the development of personalized medicine for migraine.

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