Using Movies to Probe the Neurobiology of Anxiety

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Declaration

I, Peter Kirk confirm that the work presented in my thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Over the past century, research has helped us build a fundamental understanding of the neurobiological underpinnings of anxiety. Specifically, anxiety engages a broad range of cortico-subcortical neural circuitry. Core to this is a 'defensive response network' which includes an amygdala-prefrontal circuit hypothesized to drive attentional amplification of threat-relevant stimuli in the environment. In order to help prepare the body for defensive behaviors to threat, anxiety also engages peripheral physiological systems. However, our theoretical frameworks of the neurobiology of anxiety are built mostly on the foundations of tightly-controlled experiments, such as task-based fMRI. Whether these findings generalize to more naturalistic settings is unknown. To address this shortcoming, movie-watching paradigms offer an effective tool at the intersection of tightly controlled and entirely naturalistic experiments. Particularly, using suspenseful movies presents a novel and effective means to induce and study anxiety. In this thesis, I demonstrate the potential of movie-watching paradigms in the study of how trait and state anxiety impact the 'defensive response network' in the brain, as well as peripheral physiology. The key findings reveal that trait anxiety is associated with differing amygdala-prefrontal responses to suspenseful movies; specific trait anxiety symptoms are linked to altered states of anxiety during suspenseful movies; and states of anxiety during movies impact brain-body communication. Notably, my results frequently diverged from those of conventional task-based experiments. Taken together, the insights gathered from this thesis underscore the utility of movie-watching paradigms for a more nuanced understanding of how anxiety impacts the brain and peripheral physiology. These outcomes provide compelling evidence that further integration of naturalistic methods will be beneficial in the study of the neurobiology of anxiety.

Impact Statement

Impact within academia

Traditional task-based paradigms have revealed a myriad of neurobiological mechanisms underlying anxiety-relevant processes. Yet, these have been established within a narrow range of study-specific parameters (for instance, only a select number of ways in which anxiety is induced in experiments). Movie-watching as a more dynamic and ecologically-rich paradigm, can help determine the robustness of effects across a variety of contexts that may be more representative of daily life.

From a theoretical perspective, I demonstrate that results do show inconsistencies with previous task-based studies. Specifically, my results call into question exactly how anxiety impacts the perception of faces, how trait and state anxiety interact in more naturalistic contexts, as well as notions of 'intrinsic connectivity' in anxiety.

Irrespective of theoretical interpretation, the present thesis demonstrates the utility of movie paradigms as a novel methodological tool for naturalistically inducing and investigating anxiety. However, given the scarcity of research on this topic, much of the work throughout the thesis should be seen as a proof of concept. By demonstrating that individual differences in anxiety do appear to be associated with neurobiological responses to movies, this opens up avenues for further lines of inquiry within anxiety research. Moreover, by using a range of analytical tools I hope others can gain a better understanding of what techniques may (and may not) be sensitive to the detection of anxiety-relevant idiosyncrasies. This holds potential to advance both theoretically-driven studies of anxiety, as well as those interested in phenotypic prediction (biomarker-based research).

Impact outside academia

Anxiety bares a significant impact on public ill health and is prevalent globally. Yet, treatments are lacking. In the UK, less than half of those seeking help will respond to the first treatment they receive. As such, there is a strong patient-centric motivation for attaining a better understanding of the neurobiological and psychological underpinnings of anxiety. By developing a better understanding, we can help aid the development of research into pharmacological agents and psychological interventions

to treat anxiety. Moreover, there is little guidance for how current therapeutic interventions should be applied based on an individuals need. Consequently, there is a push for precision psychiatry, wherein the idiosyncratic responses of an individual can be used to tailor interventions to an individual. Movie-watching is already being applied in clinical contexts (e.g., pre-surgical mapping). Movie paradigms may eventually hold potential for aiding clinical evaluations of psychiatric disorders, including anxiety.

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Contents

1. Introduction

There is no terror in the bang, only in the anticipation of it.

– Alfred Hitchcock

1.1 Conceptualizing Anxiety

Adaptive State Anxiety

Anxiety is commonly a ubiquitous, evolutionarily adaptive emotional response to potential threats. A key function of anxiety is to promote the detection, identification, and avoidance of harm (Mobbs et al., 2015). Akin to states of fear, anxiety can help individuals mobilize defensive responses. While fear is typically characterized as being elicited by certain threats ('the bang'), anxiety is thought to arise in response spatially and/or temporally uncertain threats ('anticipation of the bang'; Mobbs et al., 2020); albeit, there is not necessarily a clear distinction between fear and anxiety, as both occur on a continuum and are supported by highly similar neural architecture as a way to engage defensive responding (Hur et al., 2020; Shackman & Fox, 2016).

When in potentially dangerous environments, anxiety can confer several advantages, including: dilation of the pupils (Leuchs et al., 2019), heightening sensitivity to faint visual input (Mathot, 2018) which may help detect potential threats; increased respiration and blood flow, providing oxygen to skeletal musculature and thus supporting physical exertion for defensive behaviors such as fleeing (McCorry, 2007); tuning of perceptual and attentional systems toward threat-relevant stimuli, aiding the identification of dangers (Robinson et al., 2012); and a myriad of other adaptive, psychological and physiological alterations (Robinson et al., 2013). Thus, in certain contexts, being in a state of anxiety can help to avoid harm by engaging a range of systems that calibrate perception and peripheral physiology toward survival behaviors such as fighting, fleeing, or freezing.

A common approach for studying adaptive states of anxiety has thus been to present participants with prolonged, unpredictable threats. One of the most frequently used procedures is threat of shock paradigms (Robinson et al., 2013), wherein

participants are instructed that they are either at risk of receiving electrical shocks or are safe from said shocks. Behavioral and biological measures are then contrasted between these conditions to pinpoint mechanisms that may underpin state anxiety. Threat of shock is of course not the only paradigm which has been used. Other approaches include: CO2 procedures, which expose participants to increased levels of carbon dioxide to induce anxiety symptoms, most commonly panic (Hout & Griez, 1984); and public speaking challenges such as the 'Trier Social Stress Test', which involves recording responses prior to participants giving a speech in front of an audience (Kirschbaum et al., 1993). Due to its prominence throughout the literature (especially neuroimaging), findings primarily from threat of shock paradigms form the foundation for much of this thesis.

Pathological Anxiety

States of anxiety come with a significant energy cost, downregulation of digestion processes, and impair some psychological processes such as facets of memory encoding (S. Bolton & Robinson, 2017; McCorry, 2007). When a person's propensity toward anxiety is overly sensitive and chronic, this can cause individuals to experience significant distress, a criterion for a diagnosis of anxiety disorders (Substance Abuse and Mental Health Services Administration, 2016). Thus, oversensitive and chronic engagement of this system is maladaptive and such manifestations of anxiety may give rise to levels of anxiety that interrupt daily functioning.

Such pathological manifestations of anxiety have a powerful impact on public ill health, with global prevalence estimated at around ~5-11% of the population (Baxter et al., 2013). Yet, treatment is lacking. Only around half of those with pathological anxiety will respond to the first treatment they receive (Ansara, 2020; Clark, 2018; NHS Digital, 2022). By investigating how and when anxiety may go awry, we may be better able to guide interventions accordingly. In addition to threat of shock paradigms, research has thus also commonly investigated the underpinnings of anxiety by testing how behavioral and biological responses may vary between individuals as a function of clinical diagnosis.

Trait Anxiety

The distinction between adaptive vs maladaptive manifestations of anxiety is not always obvious. To guide clinical decision-making, pathological anxiety is frequently categorized as being present or absent in an individual (LeBeau et al., 2015). Psychiatry research traditionally studied mental ill health (including anxiety disorders) in such a manner. However, dichotomizing anxiety can impose disadvantages on empirical research, such as increasing the likelihood of false negatives and ignoring important symptom-level heterogeneity (Kraemer, 2007). Hence, dimensional approaches which observe anxiety along a continuum are becoming more widely adopted (Cuthbert & Insel, 2013; Insel et al., 2010; Kotov et al., 2017). Here, I refer to between-subjects, individual differences along this dimension as *trait* anxiety (disposition toward anxious states; Spielberger, 2013), but also consider within-subject variation of *state* anxiety (transient anxiety pertaining to uncertain threats) as belonging to such a continuum.

Investigating the overlapping and distinct mechanisms of *state* and *trait* dimensions may point toward the biological bases of pathological anxiety (Robinson et al., 2013), helping researchers generate clinically-relevant hypotheses and tools for treatment evaluation (Grillon et al., 2019). There is currently no consensus as to definitions/distinctions between state, trait, and pathological anxiety within the field of anxiety research. Throughout the thesis, I operationalize anxiety along both dimensions of *state* anxiety (transient, evoked experiences of anxiety) and between-persons *trait* anxiety (an inherent disposition to anxiety). Moreover, as the line between adaptive and maladaptive anxiety is ambiguous, I do not theoretically distinguish when dimensions are healthy or pathological.

1.2 The Impact of Anxiety on Face Perception

As anxiety appears to promote the detection and avoidance of potential threats in the environment (Marks & Nesse, 1994; Mobbs et al., 2020), its association with alterations in psychological processes are—unsurprisingly—far reaching, from value-based decision-making to spatial navigation (Robinson et al., 2013). Anxiety's impact on perception and cognition is also multifaceted. Some have suggested two separate pathways: influences on 'low-level' threat responding vs 'high-level' processes that

may be more strongly related to the subjective experience of emotion, albeit these two systems interact (Taschereau-Dumouchel et al., 2022). Here, I focus predominantly on one key process, threat vigilance, in the context of face perception due to its prominence throughout the anxiety literature.

Anxiety is known for its impact on vigilant attention toward potential threats in the environment (Robinson et al., 2012). A salient feature of the environment for many animals is conspecifics. The emotional expressions of conspecifics can signal a broad range of information, both about the state of the individual and the environment (Darwin, 1872). For instance, facial expressions of other humans can signal potential threats in the environment (e.g., a fearful face in response to a dangerous animal); or from the expressor themselves (e.g., an angry face directed toward the observer in anticipation of a fight). Accordingly, decades of behavioral and neuroscience research into anxiety have calibrated experimental designs around face perception.

In traditional emotion perception paradigms, static images of faces are presented and participants are asked to categorize them given a selection of emotion categories. Inducing states of anxiety via threat of unpredictable shock appears to increase the accuracy and speed at which faces are categorized, as well as the perceived intensity (Kavcıoğlu et al., 2021; Robinson et al., 2011). Between-subjects differences in trait anxiety seem to affect face processing in similar ways to induced states of anxiety: a perceptual bias, most prominently toward negatively valenced faces, is often observed (Doty et al., 2013; Robinson et al., 2012). These effects are also apparent when explicitly comparing those with anxiety disorders to healthy controls (Arrais et al., 2010; Bradley et al., 1999). Such alterations in face processing are often discussed in relation to fearful faces, but effects are often observed across a range of other emotions, including happy and angry faces, and even a bias toward categorizing neutral faces as fearful (Kavcıoğlu et al., 2021). Moreover, there may well be multiplicative effects of state and trait anxiety, wherein the effects of induced anxiety on attentional biases may be greatest in those scoring higher in trait anxiety (Dyer et al., 2022). However, not all studies have found reliable, generalizable effects. While the threat of shock literature has established a robust effect, it appears other modalities and designs (e.g., CO2 inhalation) have not always found consistent results (Dyer et al., 2022). Therefore, it is worth noting that findings appear sensitive to task

parameters. The source of threat and context of an experiment appear linked to the precise impact of anxiety on face perception.

1.3 Anxiety and the Amygdala

Neuroscience experiments have started to outline the brain structures which give rise to such anxiety-dependent responses to threat. Numerous studies in non-human animals outlined a role of the amygdala in such threat processing. For instance, surgical lesions to mice amygdala appear to decrease defensive behaviors (i.e., freezing) in response to shocks (Slotnick, 1973). Conversely, optogenetic excitation of the mouse amygdala appears to promote defensive behaviors (Ciocchi et al., 2010). Consequently, investigations into human anxiety often focused on this as a primary region of interest. Early human fMRI work utilizing state anxiety inductions indicated activation in the amygdala to be associated with anxiety-relevant biases toward emotional facial expressions (Herry et al., 2007). Moreover, numerous studies demonstrated amygdala responses to faces appeared to scale with individual differences in trait anxiety (Bishop et al., 2004; Dickie & Armony, 2008; Somerville et al., 2004). This led to the view that the amygdala was a key region responsible for instantiating states of anxiety and defensive responding, many referring to it as the 'fear center' (LeDoux, 2020).

1.4 Amygdala-Prefrontal Responding to Threat

Human neuroscience, particularly affective neuroscience, has started to go beyond purely modular views of brain function (i.e., amygdala as the region responsible for anxiety), which may be over-simplistic and unable to sufficiently capture biological dynamics underlying anxiety (Pessoa, 2017). Instead, mental processes, including anxiety, are now often seen as relying on distributed systems across the brain. In the context of anxiety, this is consistent with empirical work demonstrating that circuit-level responses to faces demonstrates better reliability than single region activations (Nord et al., 2017, 2019; Sauder et al., 2013). A more holistic explanation may thus come from studying the wider circuitry associated with anxiety-relevant regions, such as the amygdala. One circuit implicated from the non-human animal literature has been an amygdala-prefrontal circuit which may drive defensive responding.

Inducing state anxiety via threat of shock paradigms in rodents has revealed increased coupling between the amygdala and prelimbic cortex (a homologue to the human dorsomedial prefrontal cortex/anterior cingulate cortex; dmPFC/dACC) using extracellular recordings and pharmacological, optogenetic, and stimulatory manipulations (Karalis et al., 2016; Vidal-Gonzalez et al., 2006). Electrophysiological recordings in non-human primates have also demonstrated increased amygdala-prefrontal coupling whilst under induced states of anxiety (Livneh & Paz, 2012; Taub et al., 2018). Human studies appear consistent with much of this work: inducing states of anxiety via threat of shock increases engagement of an amygdala-dorsomedial prefrontal circuit[1] during face perception (Robinson et al., 2012). Congruent with behavioral studies, increased responsiveness of this circuit is also seen in those scoring high in trait anxiety and with anxiety disorders (Robinson et al., 2014). Anxiolytic medication, such as serotonin re-uptake inhibitors, appear to attenuate the responsiveness of this circuitry, including to faces (Faria et al., 2014; Godlewska et al., 2016). This circuit has thus been hypothesized to underpin attentional amplification of threat-relevant stimuli in the environment. However, the responsiveness of this circuit is not exclusive to face stimuli. When anxiety is induced within-subjects in the absence of explicitly presented visual stimuli, amygdala-prefrontal connectivity increases, the degree of which appears to be modulated as a function of individual differences in trait anxiety (Vytal et al., 2014). This does not discount the role of this circuit in vigilance to threat; rather it suggests that engagement might potentially occur under general states of anxiety, irrespective of environmental cues.

How individual variations in trait anxiety relate to amygdala-prefrontal connectivity remains controversial. By one view, individual differences in *trait* anxiety only surface while under *states* of anxiety (as theorized by the diathesis-stress model, Brozina & Abela, 2006, and as implicated by threat of shock studies, Robinson et al., 2012). An alternative possibility is that impaired amygdala-prefrontal functioning in anxious populations might emerge through stable differences in brain function. That

[1]The functional term dorsomedial prefrontal cortex has been used flexibly across the neurosciences in reference to a variety of anatomically and functionally distinct regions. This is partially due to a lack of consensus on precise regional borders. The region implicated in the anxiety literature point to what could be described, anatomically, as a region close to anterior midcingulate cortex and overlaps with premotor cortex (see figure 1.1). To remain consistent with nomenclature of the anxiety literature, I use the term dorsomedial prefrontal cortex throughout the thesis in reference to this region.

is, high levels of trait anxiety are associated with chronic engagement of amygdala-prefrontal circuitry.

1.5 Anxiety and 'Intrinsic' Amygdala-Prefrontal Connectivity

The use of resting-state paradigms—wherein subjects are instructed to lie task-free in an fMRI scanner—have provided some insight into psychiatric symptoms and chronic 'intrinsic' connectivity in the absence of experimentally-presented stimuli (Canario et al., 2021). Associations between self-reported symptoms of trait anxiety and resting-state connectivity suggest anxious individuals may chronically engage amygdala-prefrontal circuitry (M. J. Kim et al., 2011), even in the absence of experimentally-induced states of anxiety or stimulus presentation. On the other hand, this seems at odds with the non-human primate literature, which suggests anxious temperament is associated with decreased connectivity (Birn et al., 2014). Generally, findings from resting-state studies of anxiety appear particularly inconsistent. A recent systematic review of resting-state anxiety research described studies which found increased, decreased, and no alterations in resting-state amygdala-prefrontal connectivity (Mizzi et al., 2022).

Conceptually, the extent to which resting-state paradigms allow researchers to directly observe 'intrinsic connectivity' also remains controversial as: the scanning environment does not offer a context-free setting for which we can study the brain at 'rest' (Finn, 2021); and is particularly susceptible to influence from confounds arising from physiological signals (Murphy et al., 2013) and fMRI sequence parameters (Cao et al., 2023; Risk et al., 2021). Moreover, this is at odds with prior theoretical models and threat of shock studies which suggest individual differences in trait anxiety only emerge under states of anxiety (Brozina & Abela, 2006; Robinson et al., 2012). In sum, there is *some* tentative evidence to suggest individuals with high trait anxiety chronically engage amygdala-prefrontal circuitry, irrespective of specific environmental cues. However, there is substantially more evidence to suggest individual differences in amygdala-prefrontal responding emerge primarily in response to environmental cues (e.g., induced states of anxiety or emotional facial expressions).

1.6 Anxiety and the 'Defensive Response Network'

So far, I have focused on amygdala-dorsomedial prefrontal circuitry, noted for its involvement in one key aspect of anxiety, threat vigilance. This forms the foundation for much of my hypothesis-testing throughout the thesis. However, a much wider network appears to also be associated with anxiety-relevant processes such as threat vigilance. A broad range of highly connected cortico-subcortical neural circuits, referred to here as the 'defensive response network' (Abend et al., 2022), has been implicated in a range of non-human and human research. I will start by briefly reviewing three subcortical structures, the bed nucleus of the stria terminalis (BNST), hypothalamus, and periaqueductal gray (PAG).

The BNST (sometimes referred to as the 'extended amygdala') is a region dorsal to and highly connected with the amygdala which has been implicated as playing a core role in coordinating adaptive responses to potential dangers. It was previously posited that the amygdala is more strongly related to immediate threats (fear responses), but the BNST was associated with processing chronic, uncertain threats (anxious responding; Davis, 2006). However, this framework remains disputed (Fox & Shackman, 2019). Indeed, human fMRI work has demonstrated indistinguishable activation between the amygdala and BNST for certain (fear) for uncertain threat (state anxiety) responses (Hur et al., 2020); thus, both may contribute to anxiety-relevant processes.

In addition to guiding perception/cognition, regions such as the amygdala and BNST have been seen as playing a key role for instantiating states of anxiety; in other words, they can give rise to the subjective experience of anxiety (Adolphs, 2013). On the other hand, the hypothalamus has previously been thought of as a passive relay to downstream regions in order to engage defensive behaviors and provide signals to peripheral physiology, but does not necessarily instantiate subjective experiences of emotions or provide any specific modulation of these behaviors (Davis, 1992; Wada & Matsuda, 1970). More recent work in mice suggest optogenetic excitation and lesioning of the hypothalamus appears to indicate that the hypothalamus may indeed modulate conditioned responses to electric shocks, so it is not simply a passive relay (Kunwar et al., 2015). There has been some human fMRI work demonstrating associations between hypothalamus activation in response to threats (Mobbs et al.,

2009), but this literature is relatively more scarce and commentary regarding mechanisms has been primarily in relation to animal research (Dalgleish et al., 2009).

Another subcortical region that may play a key role, but has often been overlooked, is the PAG. Electrophysiological recordings and optogenetic manipulation in mice indicate the PAG in both the detection of potential threats in the environment, as well eliciting fleeing behaviors (Deng et al., 2016). Congruent with the animal literature, human fMRI research has demonstrated that, alongside the amygdala and BNST, activation of the PAG can be observed in response to induced anxiety (i.e., threat of shock, Hur et al., 2020). Moreover, such activation has been shown to be modulated as a function of the approach of electrical shocks (Mobbs et al., 2007). The PAG has therefore been posited as being able to track threat imminence (Mobbs et al., 2007) and select appropriate defensive responses accordingly (Faull et al., 2016).

A breadth of research has also demonstrated top-down projections from the cortex to subcortex. In addition to the dorsomedial prefrontal cortex, these include the insula, subgenual anterior cingulate (sgACC), and anterior ventromedial prefrontal cortex (vmPFC). Inducing state anxiety via threat of shock in humans increases activation in the insula (Choi et al., 2012), but this activation has been implicated in a broad array of anxiety-relevant processes. These include (but are not limited to) the insula's role as processing interoceptive information (internal bodily states), anticipating future events, determining controllability over stressors, and acting as integrative hub between emotion and cognition (Choi et al., 2012; Grupe & Nitschke, 2013; Limbachia et al., 2021; Terasawa et al., 2013).

The sgACC has often been implicated in threat learning. Human fMRI research indicates that pairing emotional face stimuli with electric shocks can result in increased functional connectivity between the sgACC and amygdala (Hakamata et al., 2020), suggesting this region may provide input regarding learned threat. Moreover, this connection may be significantly stronger in individuals with high trait anxiety levels (Hakamata et al., 2020). This also indicates a role of the sgACC in perceived intensity of threats. Indeed, work which has modulated the intensity of electric shocks suggests parametric modulation of activation in the sgACC as a function of perceived intensity of threat (Straube et al., 2009). Again, this representation of threat intensity appears to also correlate with individual differences in trait anxiety (Straube et al., 2009).

Contributions of a region in anterior vmPFC/medial orbitofrontal cortex, just anterior to the sgACC, to anxiety has been evidenced in a small number of studies. The literature in this domain is relatively far scarcer than the aforementioned regions. Unlike most other regions in the 'defensive response network', anterior vmPFC appears to demonstrate reduced activation following threat of shock (Kirlic et al., 2017). A study lesioning this region in macaques demonstrated that such lesions *increase* defensive behaviors (Pujara et al., 2019), an effect that would not be expected in other regions, such as the amygdala (Kalin et al., 2001). Some have posited that this region may therefore be more strongly related to representations of positive affect and/or safety signal integration (Myers-Schulz & Koenigs, 2012; Tashjian et al., 2021).

To summarize, associations between state and trait anxiety and an amygdala-dorsomedial prefrontal circuit has been one of the most consistent findings in the threat of shock literature, as demonstrated via meta-analytic evidence (Chavanne & Robinson, 2021). When in a state of anxiety, this circuit is thought to tune attentional resources toward threat-relevant stimuli in the environment (threat vigilance). However, a much wider grouping of cortical and subcortical regions, referred to here as the 'defensive response network', is thought to be recruited to engage a range of anxiety-relevant processes, from threat conditioning to safety signaling. In addition to the amygdala and dmPFC, this includes the BNST, hypothalamus, PAG, insula, sgACC, and anterior vmPFC. Broadly speaking, projections from the cortex have traditionally been thought of as providing regulatory, evaluative, and contextual inputs to more fundamental threat processes in subcortical regions (Tillman et al., 2018). However, while I have discussed the aforementioned regions in relation to specific perceptual and cognitive processes, these likely operate as a broad, interactive network to instantiate states of anxiety and orchestrate defensive behaviors (Chavanne & Robinson, 2021; Gorka et al., 2018). Moreover, by no means does this 'defensive response network' necessarily constitute the sole circuitry responsible for processing threat and generating anxious responses, but is comprised of fundamental structures that underpin states of anxiety, reported consistently across studies (Chavanne & Robinson, 2021; Shackman & Fox, 2021).

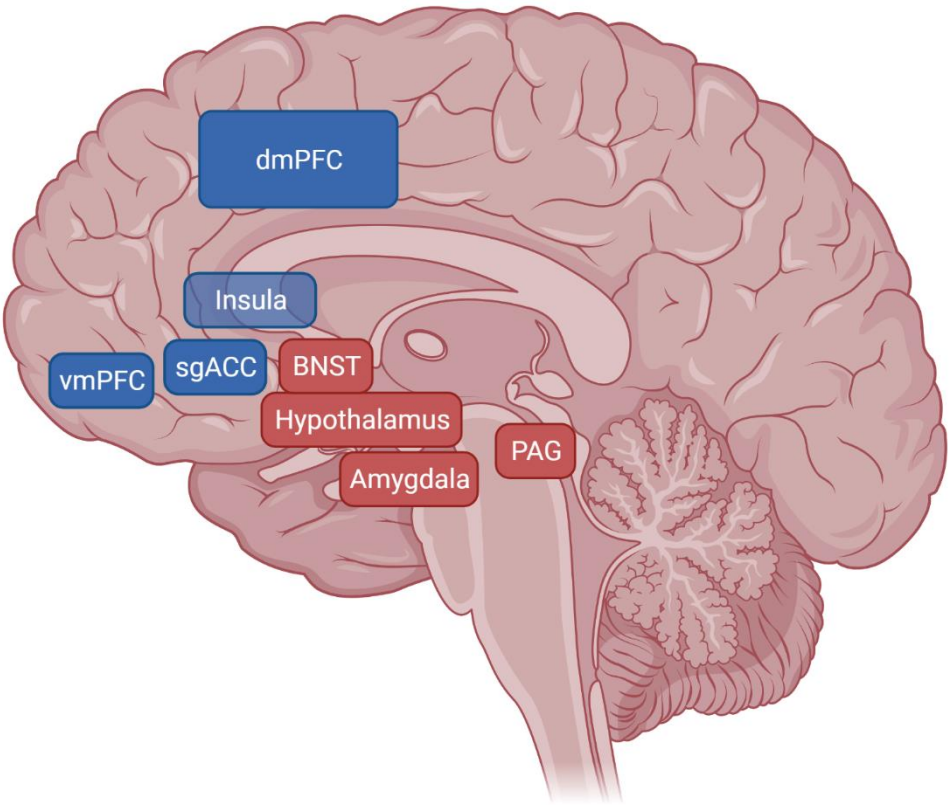

Figure 1.1. *A set of regions referred to here as a 'defensive response network' which forms fundamental circuitry recruited during states of anxiety. Subcortical regions (red) include amygdala, bed nucleus of the stria terminalis (BNST), hypothalamus, and periaqueductal gray (PAG). Cortical regions (blue) include dorsomedial prefrontal cortex (dmPFC), insula, subgenual anterior cingulate cortex (sgACC), and an anterior section of the ventromedial prefrontal cortex (vmPFC).*

1.7 Anxiety and Peripheral Physiology

The Autonomic Nervous System

An overarching purpose of the 'defensive response network' is to facilitate the detection, identification, and avoidance of dangers in the environment (Mobbs et al., 2015). The detection and identification of threat can be aided by tuning perceptual and cognitive systems to seek out sources of harm. In order to avoid these threats, survival behaviors such as fighting or fleeing may be needed. States of anxiety may serve to pre-empt such physical exertion and thus—in addition to alterations in the brain—also impact the peripheral nervous system.

Early theories did not often emphasize the brain as a central mechanism for anxiety, but posited peripheral physiology to be the biological basis (Dewey, 1894; James, 1884). There were theorizations with regards to both top-down and bottom-up influences of affective states on the body. Put simply, some suggested that altered bodily states arose in response to emotions, while others argued emotions arose in response to bodily states (James, 1894). Since such early postulations, there have been major developments in our understanding of peripheral physiology, as well as empirical work advancing our theories of how bodily states may be associated with anxiety. This has led us to an understanding that anxiety is associated with signals from the brain to the body *and* from the body to the brain, which I review below.

Anxiety has been associated with a range of changes to peripheral physiology, including increased heart rate, respiration, perspiration, and pupil dilation (e.g., increased heart rate; McCorry, 2007). This impact on peripheral physiology arises primarily via the autonomic nervous system. Activity in two, prominent components of this system, the sympathetic and parasympathetic branches, are constantly balanced to promote homeostasis dependent on context (Buijs, 2013). Conceptually, the sympathetic branch is traditionally associated with adaptive 'fight-or-flight'-like responses that are integral to anxiety, while dominance of the parasympathetic branch is associated with 'rest-and-digest'-like behaviors (McCorry, 2007). This is a somewhat crude simplification, as different affective states, contexts, and goals may uniquely act across these systems in discrete manners (Critchley, 2005, 2009), but it provides a useful basic framework for approaching the study of anxiety. Broadly speaking, the balance in activity of these branches is impacted across a range of spectral frequencies ranging from respiration modulating balance within seconds (Stein et al., 1994) to influences of sleep and circadian rhythms throughout the day (H. J. Burgess et al., 1997). Acute mental stressors appear to impact autonomic balance in the order of seconds to minutes (Salahuddin et al., 2007). Specifically, anxiety elicits increases in activity in of the sympathetic branch of the autonomic nervous system, as well as (vagally-mediated) parasympathetic withdrawal (Friedman & Thayer, 1998a).

Popular proxies for autonomic balance are cardiac measures (heart rate and heart rate variability). This is due to the autonomic nervous system's impact on pacemaker cells in the sinoatrial node of the heart (Zaza & Lombardi, 2001). When sympathetic activity is high (and/or parasympathetic activity is low), the rate of cardiac

action potentials is increased, resulting in increased heart rate (Grassi et al., 1998). Moreover, the heart is not a regular metronome: variability is natural, arising from continual fluctuations in autonomic balance (the ratio of sympathetic to parasympathetic activity) and baroreceptor reflex (the increasing/decreasing of intervals between beats to regulate blood pressure). Sympathetic dominance (and consequently, anxiety) is associated with lower variability in heart rate (Elghozi & Julien, 2007). While it would be ideal to not rely on such proxies and measure activity within the autonomic nervous system directly, this is difficult to achieve due to the invasiveness of required procedures giving rise to issues concerning safety (Mano et al., 2006). Heart rate and heart rate variability thus offer a useful proxy for researchers to study the impact of anxiety on the autonomic nervous system.

Anxiety is known for its top-down influence on the autonomic nervous system. That is, anxiety causes alterations in autonomic balance. For instance, when state anxiety is induced within-subjects, increases in heart rate and decreases in heart rate variability can be observed (Battaglia et al., 2022; de Groot et al., 2020). Following induced anxiety, individual differences in trait anxiety appear to show associations with cardiac responding (Beatty & Behnke, 1991; Kantor et al., 2001; Levine et al., 2016). Moreover, patients with pathological levels of anxiety appear to show chronically reduced heart rate variability in the absence of tasks or state anxiety inductions (Chalmers et al., 2014; Pittig et al., 2013). Therefore, there is clear evidence that anxiety engages peripheral physiological systems. This may (in some instances) serve as an adaptive function to prepare the body for physical exertion.

There is emerging evidence that, in addition to influencing peripheral physiology, anxiety (and its associated perceptual/cognitive processes) is reciprocally influenced by the body. Theories of interoception highlight the role of bottom-up input; the perception of internal, visceral sensations also impact anxiety-relevant processes. This includes signals from the stomach, lungs, and—pertinent to this thesis—the heart (Craig, 2003; Critchley & Harrison, 2013).

The effects of cardiac signals are seen as modulators of state anxiety-relevant processes within individuals. For instance, the perceived intensity of threat-relevant stimuli (i.e., fearful faces) appears to be modulated as a function of the cardiac cycle (Garfinkel et al., 2014). Moreover, researchers have derived behavioral measures

gauging the degree to which individuals are attending to their own cardiac signals (referred to as interoceptive awareness), such as tasks which test the ability of participants to count their own heartbeats (Garfinkel et al., 2015). When these tasks are delivered alongside state anxiety inductions (i.e., public speaking challenge), increased awareness of cardiac signals has been observed (Durlik et al., 2014). Moreover, the degree to which people attend to such internal bodily signals appears to vary between individuals. There is converging evidence to suggest trait anxiety and pathological anxiety are associated with increased sensitivity to the perception of heartbeats (Domschke et al., 2010).

However, the methodologies in much of the human interoception literature are predominantly correlational in nature, limiting the ability to infer a causal relationship between visceral signals and anxiety. One recent study in non-human animals has provided causal evidence for cardiogenic signals attenuating anxiety. Optogenetically-induced tachycardia in mice resulted in anxiety-like behaviors such as an aversion to exploration of open spaces and suppression of reward seeking (Hsueh et al., 2023). Pharmacological medication provide some evidence for a causal role in humans. For example, propranolol is a sympatholytic beta-blocker that acts by directly downregulating cardiac activity (e.g., heart rate). It is used regularly to reduce the severity of anxiety, particularly due to its impact on somatic symptoms (Suzman, 1976). Given that non-somatic anxiety symptoms are lessened by downregulation of sympathetic activity indicates a role of visceral signals in shaping subjectively-experienced anxiety in humans. However, propranolol's efficacy varies largely (Papadopoulos et al., 2010; Protopopescu et al., 2005) and is able to cross the blood-brain barrier (Pardridge et al., 1983), ruling out selective effects on peripheral physiology alone. Therefore, evidence for visceral signals shaping anxiety in humans remains primarily correlation.

Taken together, there is substantial evidence that states of anxiety induce and are influenced by internal bodily states. The strength of this reciprocal communication appears to shift alongside measures of trait anxiety. Anxiety appears to increase activity in the sympathetic branch of the autonomic nervous system and decrease activity in the parasympathetic branch. This manifests in bodily changes such as increases in heart rate and decreases in heart rate variability. Moreover, bodily signals, such as those from the heart, may in turn shape anxiety-relevant processes.

Specifically, anxiety may be associated with increased sensitivity and awareness towards these internal signals.

Neural-Autonomic Communication in Anxiety

While scarce, some studies have begun to elucidate the reciprocal communication between central neural structures and the autonomic nervous system. Regions across the 'defensive response network' appear associated with neural-autonomic communication in the context of anxiety. This includes amygdala, dorsal and ventral medial prefrontal regions, sgACC, and insula, the last of which has received particular attention.

One approach for studying communication between the brain and peripheral physiology is by correlating the degree to which activation or connectivity in the brain is associated with measures of autonomic balance. One study found that the degree to which the heart rate increases in response to a public speaking challenge was positively correlated with activation in subgenual/perigenual ACC and medial OFC/vmPFC (Wager et al., 2009). Another study investigated individual differences in resting-state 'intrinsic' connectivity and its associations with state anxiety (i.e., threat of painful thermal stimulation) evoked skin conductance responses (Abend et al., 2022). Here, the authors found amygdala-vmPFC connectivity to be positively correlated with anxiety-evoked changes in skin conductance. Likewise, a study which induced worry (asking participants to ruminate about a previous sad, anxious, or stressful emotional event), found the degree to which amygdala-dmPFC and amygdala-vmPFC circuitry responds to the worry-induction to be correlated with individual differences in heart rate variability responses (though this may vary as a function of pathological anxiety; Makovac, Meeten, Watson, Herman, et al., 2016). These studies indicate anxiety-relevant correlations between brain activity/connectivity and autonomic responses. However, it is difficult to infer what function these brain responses are serving; are they causing increases in heart rate or reflecting an increase in interoceptive attention to visceral signals, or even both?

One approach to disentangle bottom-up interoceptive influences from top-down signaling to the autonomic nervous system is through active manipulation. Evidence in rodents using postmortem tissue staining, electrophysiological recordings, and

optogenetic manipulation of cardiac activity point to a causal role of the insula as a key hub for the integration of visceral signals and anxiety-relevant behaviors (Hsueh et al., 2023). A technique applied within the human literature has been to instruct participants to explicitly attend to their own bodily signals (e.g., heartbeats or breathing). Neural responses can then be contrasted between the timepoints when participants are paying more or less attention to their own bodily signals, even in the absence of altered autonomic balance (e.g., increased heart rate). fMRI studies using this approach have demonstrated that increasing interoceptive awareness is correlated with increased activation in the insula, as well as amygdala and sgACC (Doll et al., 2016; Terasawa et al., 2013).

Taken together, we have seen some evidence for regions within the 'defensive response network' in the brain being recruited for communication with the autonomic nervous system. This evidence suggests that anxiety-evoked increases in activity across the amygdala, dmPFC, insula, and sgACC are associated with increased autonomic responses and interoceptive awareness (figure 1.2).

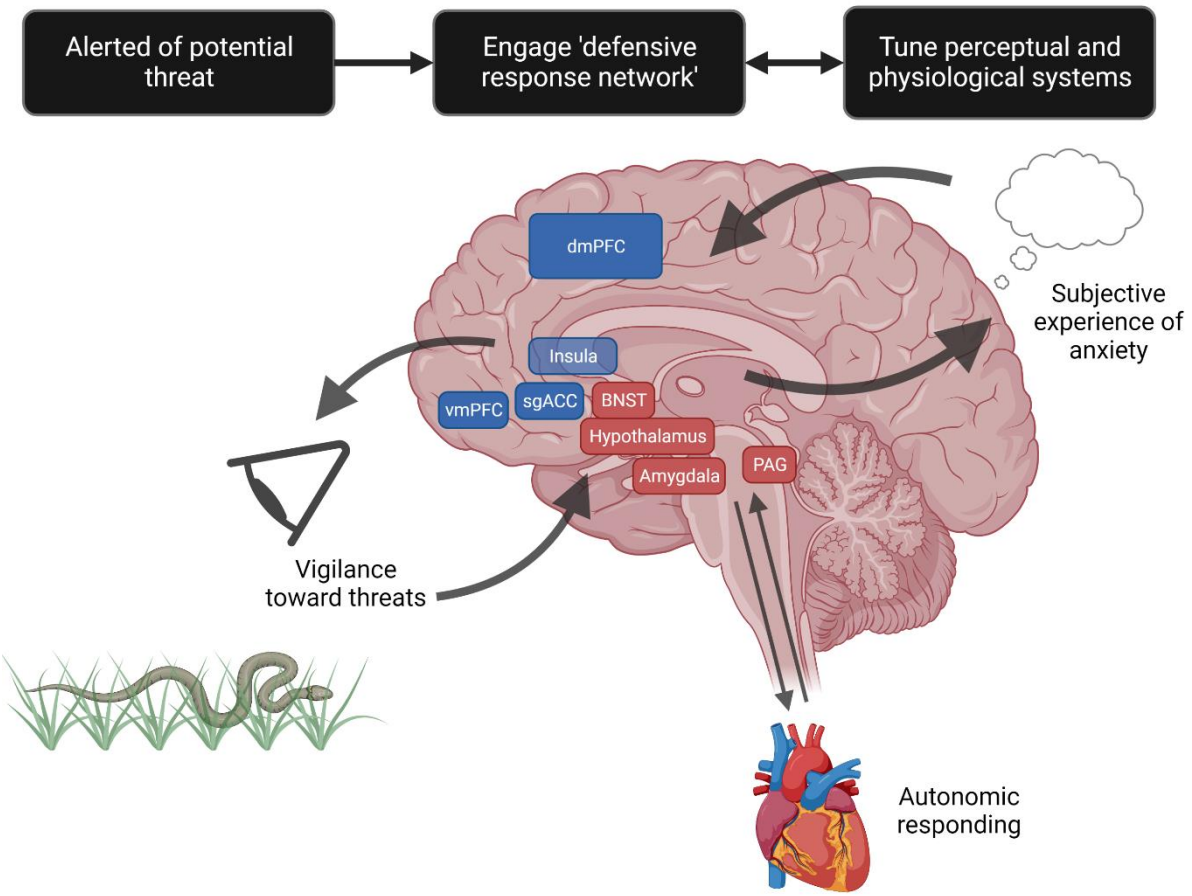

Figure 1.2. *An illustrative overview of fundamental neurobiological mechanisms underlying threat responding. When alerted to potential threats in the environment, the brain engages a 'defensive response network'. This can promote attentional vigilance toward threats, communication with the autonomic nervous system, and can give rise to subjective experiences of anxiety.*

1.8 Moving Beyond Traditional Task Paradigms: the Case for Movies

The Need for Ecological Validity

From the literature, we have learnt that anxiety appears to be associated with a distributed cortico-subcortical-autonomic network that drives anxiety-relevant processes. Specifically, anxiety may be associated with 1) altered amygdala-prefrontal responsivity to affective environmental cues, such as faces; 2) increased amygdala-prefrontal connectivity whilst under uncertain threat anticipation (threat of shock); 3) chronic amygdala-prefrontal connectivity; 4) altered autonomic responding; and 5) increased communication between the brain and the autonomic nervous system. Our understanding of the neurobiology of anxiety has been built on the foundation of

tightly-controlled experimental paradigms. This has been done so as to allow researchers to mitigate the impact of confounds such as low-level perceptual features which might bias results. The downside of this approach however, is that it comes at a cost to ecological validity (Vigliocco et al., 2023). More specifically, tasks employed in anxiety research do not typically present “rich, multimodal dynamic stimuli that represent our daily lived experience” and cannot be assumed as unquestionably providing “a reasonable approximation of how we encounter stimuli in everyday life” (Sonkusare et al., 2019).

The degree to which results generalize from the anxiety literature to more naturalistic settings outside of specific laboratory conditions remains unresolved. This is critical for anxiety research because a key goal is to aid those suffering from anxiety disorders. Firstly, the lack of generalizability to naturalistic settings has implications for the assessment of how anxiety impacts people’s daily functioning. There is currently a call to move assessments of anxiety and mood disorders toward measures of underlying constructs, such as attentional bias to threat, as opposed to clustering self-report symptoms into discretized diagnoses (Cuthbert & Insel, 2013; Insel et al., 2010). This could be achieved through a battery of self-report, behavioral, and biological measures. In other areas of psychiatry, this is already implemented. For instance, clinical neuropsychologists will evaluate brain images from patients with brain lesions and evaluate underlying facets of cognition accordingly. However, in these cases, it has been demonstrated that using ecologically-valid assessments of cognition may be better able to encapsulate how such lesions impact a patients daily functioning (P. W. Burgess et al., 2006). If there is an eventual move toward using behavioral or biological measures in the assessment of anxiety and mood disorders, there needs to be validation that these measures capture how anxiety impacts a person’s daily functioning outside of the specific assessment method.

The motivation for validating findings from the anxiety literature in ecologically-rich settings also extends to the development of treatments. Therapeutic interventions are designed to alleviate anxiety in people’s daily lives. Yet, only approximately half of those with anxiety disorders will respond to initial psychological or pharmacological treatment (Ansara, 2020; Clark, 2018; NHS Digital, 2022). These interventions are selected and developed based on our current evidence base; for instance, by synthesizing pharmacological agents (i.e., anxiolytic medication) in relation to specific

biological targets evidenced in basic research (Sartori & Singewald, 2019). Traditional methods, such as fMRI-measured brain responses to static faces, are then employed as a test of the efficacy of anxiolytic medication in randomized controlled trials (Gingnell et al., 2016). If results from traditional paradigms (e.g., brain responses to static faces) do not encapsulate how anxiety impacts people outside of such settings, this may necessitate re-evaluations in the development and testing of clinical interventions for anxiety.

Public speaking challenges are one type of procedure used in anxiety research that may constitute a more ecologically-rich setting (Kirschbaum et al., 1993). These operate by asking participants to prepare a presentation to deliver to an audience and recordings (e.g., physiological monitoring) occur prior to, during, and after the presentation. This, arguably, constitutes a more naturalistic paradigm compared to other procedures, like threat of shock, as this is representative of a setting that many naturally experience outside of laboratory settings. However, public speaking challenges induce a specific type of social anxiety related to public speaking that is arguably distinct from direct threat-oriented models of anxiety. Responses arise partially as a product of sociocultural attitudes toward such settings and is not always effective at eliciting states of anxiety (Miller & Kirschbaum, 2019). Moreover, the rigidness for the procedure means there are limitations as to which measures can be acquired. For instance, stimulus testing (e.g., face perception) and neuroimaging are particularly difficult to smoothly implement as part of public speaking challenges, due to issues such as movement-related artifacts (Rosenbaum et al., 2018). These drawbacks do not discount the contribution of public speaking challenges to our understanding of anxiety; rather, highlights that public speaking challenges do not on their own solve the field's constraints on generalizing to more naturalistic contexts.

One hybrid method which could allow researchers to study anxiety in more naturalistic settings is movie-watching. That is, presenting video clips to participants while recording behavioral, physiological, and neuroimaging measures. Unlike traditional task-based designs, which present static, decontextualized stimuli (e.g., grayscale cropped faces presented for < 1 second), movies constitute dynamic, multisensory, contextualized content that may be representative of everyday experiences (Aliko et al., 2020).

Using movies as a platform for studying mental phenomena is not entirely new to cognitive neuroscience. In the neuroimaging domain, this was first introduced in the mid-2000s, where a study was able to demonstrate activation of the fusiform gyrus to faces during movie-watching (Hasson et al., 2004). Earlier work emphasized the fusiform gyrus as a core node for face processing, but these findings were typically based on paradigms which presented grayscale, cropped faces statically and with minimal context (Kanwisher et al., 1997). By detecting the same effects during movie-watching, there was evidence for generalizability of fusiform activation to faces in a relatively more naturalistic context, which may more accurately capture the processes underlying the day-to-day perception of faces.

In addition to studying mental processes in relatively more naturalistic settings, there are numerous technical benefits to movie paradigms. Traditional task-based paradigms can induce drifts in mood over the course of the task (participants become less happy throughout the experiment) which results in desensitized responses to stimuli (i.e., reward; Jangraw et al., 2023). Meanwhile in completely task-free designs (resting-state), participants often report feeling drowsy, which can confound neuroimaging measures (Joliot et al., 2023). Movies, on the other hand, were created to engage audiences. We typically do not spend our free time completing traditional cognitive tasks, whereas we do watch movies frequently and voluntarily (we even pay to see them). The average person in the UK spends over 5 hours consuming television and video content per day (Ofcom, 2022). In addition to being able to probe mental processes of interest, such engagement can promote in-study compliance, resulting in reduced head movements, gains in signal to noise ratio, and improved reliability (Byrge et al., 2022; Eickhoff et al., 2020; Frew et al., 2022).

Analytical Approaches to Movie-Watching

Movie paradigms confer several analytical benefits, such as the ability to examine data in numerous ways. Here, I will briefly discuss 3 methods, namely feature-based general linear modelling (GLM), seed-based functional connectivity, and intersubject correlation, which I apply throughout the thesis. Feature-based GLM is a common approach inspired by the task-based literature. As with task designs, the onsets and durations of features within movies are coded and convolved with the hemodynamic

response function. These time series are inserted as regressors into a general linear model wherein brain activation or connectivity can be estimated in response to components of the movie stimulus. This approach has been used successfully across numerous studies (for instance, investigations into the encoding of spatial information during movie-watching; Häusler et al., 2022). An advantage of feature-based GLM is that the models can be interpreted in the context they were specified. That is, understanding which features of the movie are evoking responses in the brain. However, these approaches rely on numerous assumptions (e.g., shape of the hemodynamic response function and fluctuations in temporal dynamics), which may come at the cost of statistical sensitivity.

Data-driven analyses inspired by the resting-state literature have also been implemented within movie-watching paradigms. A classic approach within this literature is seed-based functional connectivity. Here, the time series of regions within the brain are extracted and then correlated with one another to produce functional connectivity matrices. These matrices can then be inputted into group-level models to test for the effects of different movies and/or individual differences in traits on brain connectivity. This is entirely data-driven, as no specific features of the movie are explicitly built into the model. Using such an approach, researchers have been able to demonstrate connectivity measures derived from movies may outperform those of rest in predicting individual differences in cognitive and emotional traits (Finn & Bandettini, 2021). This approach may offer improved sensitivity for detecting phenotypic variation and clustering, as it does not rely on as many assumptions as feature-based GLM. However, seed-based functional connectivity measures are typically time-invariant. Metrics are derived from movie-wide averages, so minimal information regarding stimulus features are driving connectivity measures. For example, two participants could demonstrate different connectivity profiles across time, but retain the same movie-wide connectivity measure (e.g., if one subject had high connectivity in the first half, but not second, their connectivity measure would be the same as another participant who had high connectivity in the second half, but not first). This can result in connectivity measures being less sensitive in the detection responses to specific features within a movie.

Finally, a hybrid approach which is more unique to movie-watching paradigms is intersubject correlation. Here, each subjects' dependent measure (e.g., neural time

series) is correlated with every other subject to produce pairwise metrics, measures of ‘intersubject similarity’ (instead of within-person measures, as is the case with feature-based GLM and seed-based functional connectivity). This can be implemented with movies as participants’ measures are recorded in response to the exact same stimulus timings. Put simply, each subject typically watches the same video, which elicits a generally shared experience, and so their time series can be directly compared. Intersubject correlation is unconstrained in that it does not rely on assumptions about time series (e.g., shape of fMRI responses) but is also time-locked and driven by features within the movie, such as fluctuations in emotional context. However, despite being time-locked and sensitive to the content within a movie, results do not allow for temporal specificity (inferring which time points in the movie are driving effects) because similarity measures are typically based on comparisons across the entirety of movie-viewing.

There has now been an extension of intersubject correlation calibrated primarily for the study of individual differences during movies, coined intersubject representational similarity analysis (inspired from previous implementations of representational similarity analysis, Kriegeskorte et al., 2008). Here, intersubject similarity measures are generated both for the dependent measure (e.g., neural time series), as well as the measure of individual differences (e.g., trait anxiety scores). The intersubject similarities can then be compared. For example, pairwise similarities in neural responding can be correlated with pairwise similarities in trait anxiety. If a significant relationship is observed, we can infer that activity in brain regions varies as a function of trait anxiety during movie-watching. This technique has been demonstrated as providing sensitivity to detecting shared and idiosyncratic representations relevant to affective systems (G. Chen et al., 2020; P.-H. A. Chen et al., 2020; Finn et al., 2020; Finn & Bandettini, 2021). For predicting individual differences in self-reported trait measures, there is evidence demonstrating gains in sensitivity compared to traditional resting-state style approaches (G. Chen et al., 2020).

Movies elicit rich, complex psychological and biological responses. Accordingly, selecting appropriate analyses to apply to such data can be a challenge. Here, I have outlined three key approaches: feature-based GLM, seed-based functional connectivity, and intersubject correlation (figure 1.3). Each of these have

their relative advantages and disadvantages. Broadly speaking, each approach requires some compromise between statistical sensitivity vs theoretical precision. Given the complexity of movies and range of questions that can be addressed with such data, there is no one approach which fits all. Using these approaches in parallel provides the possibility of being able to carve out a better understanding of mental phenomena through differing analytical perspectives. Accordingly, throughout the present thesis, I make use of all three of these techniques for studying anxiety in the context of movie-watching.

Figure 1.3. *Three key analytical approaches for movie-watching analyses, which I use throughout the thesis. Inspired by traditional task paradigms, is feature-based general linear modelling, which can tests activation and connectivity to specific stimulus onsets. From the resting-state literature, I employ seed-based functional connectivity, which derives connectivity measures across entire movies. A hybrid approach, more specific to movie-watching, is intersubject correlation which calculates measures of similarity between subjects as they watch movie stimuli. Inspired from (Vanderwal et al., 2019).*

Applying Movie-Watching to Anxiety Research

Movies could offer a broad array of benefits to neuroscience and psychology research in general, but anxiety may particularly benefit. Using movies as a stimulus could allow us to explore anxiety's impact on specific cognitive processes in a more naturalistic manner. They could allow us to test how trait anxiety shapes responses to dynamic, contextually rich facial expressions (beyond static, grayscale faces). Moreover, movies can serve as novel and more naturalistic means of inducing anxiety. There is no doubt that threat of shock elicits states of anxiety, as evidenced by self-reported feelings of anxiety (Robinson et al., 2011). However, threat of shock dichotomously probes states of anxiety or safety and is not typically experienced outside of laboratory settings. On the other hand, movies can elicit continual fluctuations in suspenseful uncertainty (e.g., by posing potential dangers to characters) that engage audiences and elicits anxiety (Schmälzle & Grall, 2020). This could allow us to test ongoing dynamics of state anxiety through a source that may be experienced outside of laboratory settings.

Despite methodological and theoretical advantages offered by movie-watching paradigms, there is scarce work investigating fear/anxiety in the context of movies. One study demonstrated that increases in the subject experience of fear during horror movies was associated with increased amygdala-dmPFC responding (Kinreich et al., 2011). Another study investigated acute fear ('jump-scares') and sustained fear (arguably, states of anxiety) during horror movies (Hudson et al., 2020). This found increased activation in amygdala, dmPFC, and periaqueductal gray in response to acute fear, but these regions were not as responsive to sustained fear (sgACC and precuneus were responsive to sustained fear; note: this study did not provide any direct contrast between fear vs anxiety). Thus, two studies have indicated state anxiety-dependent recruitment of amygdala-dmPFC circuitry during movie-watching.

To my knowledge, no studies had directly explored anxiety-dependent responses to movies further than this (prior to the present thesis). Most prominently, no work had directly investigated individual differences in trait anxiety and responses to movies, which forms the basis for the majority of studies in the present thesis. Specifically, no work had looked at the impact of trait anxiety on the perception of faces during movies; the response to suspense (in contrast to threat of shock); and

autonomic responses to movies. Moreover, no work had directly investigated the impact of anxiety on neural-autonomic communication during movies.

1.9 Thesis Aims and Research Questions

I have outlined key findings from the literature which have given us a fundamental understanding of a cortico-subcortical-autonomic network that underpins anxiety, and how this system pertains to anxiety's impact on perception and cognition. Specifically, anxiety appears to be associated with a 'defensive response network' in the brain. At the core of this network is an amygdala-dmPFC circuit which is thought to drive attentional amplification of threat-relevant stimuli in the environment. This 'defensive response network' also includes other regions implicated in anxiety-relevant processes, namely the BNST, hypothalamus, PAG, insula, subgenual anterior cingulate (sgACC), and anterior ventromedial prefrontal cortex (vmPFC). Activity across this network appears to drive anxiety-relevant processes such as threat conditioning and safety signaling. Moreover, anxiety also manifests in—and is influenced by—altered bodily states. In particular, there appears to be an intrinsic link between anxiety-relevant processes in the brain and activity within the autonomic nervous system.

Traditional paradigms have induced anxiety (e.g., threat of shock) and tested responses to specific stimuli (e.g., grayscale, statically-presented faces) in tightly-controlled experimental settings which may not resemble the rich, complex, and multisensory contexts that we experience in our everyday lives. Therefore, movie-watching offers one potential platform in which we can start to bridge this gap toward ecological validity. In the present thesis, I aimed to explore the questions of whether—during movie-watching—anxiety was associated with altered neural, subjective, and autonomic responses to movies. The biological measures (i.e., 'defensive response network' in the brain and cardiac responding) driving the hypotheses throughout this thesis were motivated by the need to determine if findings from traditional task-based paradigms generalize to more naturalistic settings. Specifically, I focus on testing whether threat of shock-elicited neurobiological responses to specific salient stimuli (i.e., faces) and ongoing states of anxiety are also present during movie-watching.

In chapters 2-4, I investigate associations between anxiety and brain responses during movies. Specifically, in chapter 2, I use feature-based general linear modelling to explore the extent to which trait anxiety is correlated with amygdala-dmPFC responses to faces in movies. In chapter 3, I test whether trait anxiety is associated with altered amygdala-prefrontal responding to suspense (a potentially more naturalistic induction of state anxiety compared to threat of shock). In chapter 4, I test whether the findings from chapter 3 can be explained through measures of resting-state connectivity. In chapter 5, I outline the development of an analytical pipeline for the processing of cardiac data, which I then use in chapter 6 to test whether individual differences in trait anxiety are associated with autonomic responses to movies. Finally, in chapter 7, I tie the previous chapters together by investigating the extent to which states of anxiety during movies may impact communication between the brain and the autonomic nervous system. Finally I provide a general discussion of these investigations.

1.10 Movie Databases

An advantage of movie-watching studies is that, by making data openly available data, they can be flexibly analyzed to answer different theoretical questions. In my experimental chapters, I make use of multiple openly available databases which I performed secondary analyses on, as well as a primary dataset I collected. To my knowledge, none of these had previously been analyzed in the context of anxiety. Table 1.1 lists a brief summary of these databases.

Table 1.1. *Summary of datasets used in the thesis.*

Database	Secondary analysis	Full sample size	Conditions	Scanner strength
Naturalistic Neuroimaging Database	Yes	86	Full length movies	1.5T
Human Connectome Project	Yes	178	Series of 1-5 minute video clips	7T
Cambridge Center for Aging and Neuroscience	Yes	652	8 Minute video clip + resting-state	3T
Internal dataset	No	150	2 x 8 minute video clips	N/a
Caltech Conte Center	Yes	55	8 minute video + 5.5 minute video	3T

1.11 Chapter Prefaces

Throughout the thesis, I present results from work that has been published in peer-reviewed journal or uploaded as preprints. The introductions have been written for readers with minimal context (i.e., without having to read the other chapters). For readers of this thesis to be able to go through chapters not only chronologically but also in isolation, I have edited the chapters throughout where necessary, added prefaces to chapters linking them to the overarching motivation of the thesis, and included references to the published versions of the studies.

1.12 General Introduction Summary

In this general introduction I have outlined that there are three key conceptualization of anxiety: state, pathological, and trait. For the purpose of the present thesis, I operationalize anxiety along continuous dimensions of state anxiety (induced, transient anxiety) and trait anxiety (a person's disposition to states of anxiety).

Evidence from traditional behavioral paradigms have demonstrated that state and trait anxiety are associated with biased processing of threat-relevant information in the environment. A common finding has been that anxiety makes individuals more vigilant in detecting and identifying facial expressions. An amygdala-dorsomedial prefrontal circuit is thought to underpin the attentional amplification of such stimuli. A wider array of regions in the brain forming a 'defensive response network' is also implicated in orchestrating such anxiety-relevant processes and defensive behaviors. Core to these processes is also altered physiological responding, namely changes to autonomic activity.

Despite such a wealth of literature exploring the impact of anxiety on the brain and cognition, there is much left to be explored. Studies in anxiety have typically been conducted in highly controlled task-based paradigms that may not be representative of responses outsides such settings. Addressing this gap is critical to advance anxiety research because current interventions, which aim to alleviate peoples' daily experience of anxiety, are built on the foundation of these experimental paradigms. Using movies as stimuli in experiments offers a potential platform in which we can bring research closer to more naturalistic settings, which comes with a range of theoretical and methodological advantages. In the present thesis, I aim to extend the research focus on anxiety to movie-watching paradigms. Throughout my experiments, I therefore test a variety of hypotheses on how anxiety may be associated with neural and autonomic responses to movie stimuli.

2. Face-Dependent Amygdala Connectivity during Movie-Watching

2.0 Preface

A wealth of studies using threat of shock to induce states of anxiety have demonstrated that states of anxiety bias the processing faces, the degree to which varies with individual differences in trait anxiety. Specifically, there is evidence to suggest anxiety results in quicker and more accurate detection/identification of facial expressions. An amygdala-dmPFC circuit is thought to drive this attentional amplification. In chapter 2, I set out to test the extent to which anxiety would be associated with neural responses to faces during movies.

For the final published versions of this study, please refer to the below reference:

Kirk, P. A., Robinson, O. J., & Skipper, J. I. (2022). Anxiety and amygdala connectivity during movie-watching. *Neuropsychologia*, *169*, 108194. https://doi.org/10.1016/j.neuropsychologia.2022.108194

2.1 Abstract

Rodent and human studies have implicated an amygdala-prefrontal circuit that drives threat vigilance. To date, this has been established in tightly controlled paradigms (predominantly using static face perception tasks) but has not been extended to more naturalistic settings. Consequently, using 'movie fMRI'—in which participants watch ecologically-rich movie stimuli rather than constrained cognitive tasks—I sought to test whether individual differences in anxiety correlate with the degree of face-dependent amygdala-prefrontal coupling in two independent samples. Analyses suggested increased face-dependent superior parietal activation and decreased speech-dependent auditory cortex activation as a function of anxiety. However, I failed to find evidence for anxiety-dependent increases in face-dependent amygdala-prefrontal connectivity. My findings suggest that work using experimentally constrained tasks may not replicate in more ecologically valid settings and, moreover, highlight the importance of testing the generalizability of neuroimaging findings outside of the original context.

2.2 Introduction

As highlighted in chapter 1, a key function of anxiety is to promote vigilance toward potential threats in the environment, but chronic engagement of this system may underlie pathology (Robinson et al., 2012). Research has reliably shown anxiety biases the processing of faces (Robinson et al., 2011; Surcinelli et al., 2006), a highly salient feature of the environment for (highly social) humans. Consequently, neuroimaging experiments of anxiety have predominantly utilized face-perception tasks, often focussing on amygdala activation. There are numerous studies demonstrating increases in amygdala response to faces parametrically scales with affective bias (i.e., fear/anxiety; de Groot et al., 2020; Killgore & Yurgelun-Todd, 2005; Somerville et al., 2004) and is seen in the presence of anxiety disorders (Cooney et al., 2006). Subsequent research has demonstrated, however, that within-subject amygdala response across time holds moderate-to-poor reliability (Nord et al., 2017; Sauder et al., 2013). Taking a modular, amygdala-centric view may indeed be over-simplistic, and unable to sufficiently capture biological dynamics underlying anxiety. Instead, a more holistic explanation may come from studying the wider circuitry associated with the amygdala.

There is now substantial evidence from the animal literature implicating amygdala-prefrontal circuitry in threat processing (for a review, see Robinson et al., 2019), wherein dorsomedial prefrontal/anterior cingulate cortex (dmPFC/ACC) provides top-down entrainment of amygdala reactivity, and this bears importance for responding to potential threat (Karalis et al., 2016). Recruitment of this circuit has also been demonstrated in human subjects: increased amygdala-dmPFC/ACC coupling during the processing of fearful faces has been demonstrated in humans undergoing induced anxiety (Robinson et al., 2012). Notably, this coupling positively correlates with self-report measures of anxiety symptoms and may constitute a more temporally stable signal than amygdala reactivity alone (Nord et al., 2019). This circuitry is posited to drive anxiety-induced amplification of salient stimuli; thus, excessive recruitment of this circuitry could result in chronic attentional biases for threat (Robinson et al., 2012). The implication of this ‘aversive amplification’ circuit in humans has been replicated elsewhere, such as in: clinical samples (Demenescu et al., 2013; Robinson et al., 2014), resting-state analyses (Vytal et al., 2014), emotion regulation tasks (Zotev et al., 2013), and predator-prey paradigms (Gold et al., 2015). Of course, other fMRI

paradigms have demonstrated anxiety-dependent amygdala connectivity to regions such as ventromedial prefrontal cortex (M. J. Kim et al., 2011) and insula (Roy et al., 2013). Nonetheless, increased amygdala-dmPFC/ACC coupling is a consistent finding, and as such, is a commonly adopted model for biomarker-focused anxiety research (Brehl et al., 2020; Grillon et al., 2019; Yuan et al., 2016).

Despite a multitude of fMRI studies investigating the neural substrates of anxiety, a methodological gap remains in the literature. Research has predominantly relied on static, unnatural face stimuli presented without any context. These paradigms deviate from the natural perception of faces in day-to-day settings (Barrett et al., 2007) and may lead to misclassification of expressions, particularly those of fearful/sad faces (Carlisi et al., 2021). Such tightly-controlled experiments could lead to theory that may overlook dynamic, context-dependent networks in the brain (Skipper, 2014; Sonkusare et al., 2019; Spiers & Maguire, 2008). Previous studies have built a fundamental understanding of core threat circuitry, but whether anxiety-related brain activity in less constrained settings can be explained by current theory has yet to be established.

The recent uptake in 'movie fMRI' paradigms—where participants watch real movies whilst in the fMRI scanner—allows the opportunity to address some of these concerns. This method may help validate and extend current models of anxiety, improve data quality, and inform biomarker-based research (Eickhoff et al., 2020; Finn & Bandettini, 2021; Hasson et al., 2010; Vanderwal et al., 2019). Indeed, two studies so far have demonstrated within-subject amygdala-prefrontal coupling during anxiety-inducing movie scenes (Hudson et al., 2020; Kinreich et al., 2011). To my knowledge however, there exists no study investigating whether between-subject differences (i.e., self-reported symptoms of anxiety) in amygdala-prefrontal circuitry are seen in ecologically-richer contexts. Therefore, in the present preregistered two-experiment study, I investigated the relationship between self-reported anxiety and amygdala-connectivity in two independent movie-watching fMRI datasets.

Database Summary

In the present project, I used two openly available databases which include movie fMRI, the Naturalistic Neuroimaging Database (Aliko et al., 2020; experiment 1) and

Human Connectome Project (Van Essen et al., 2013; experiment 2). A table describing participants and fMRI sequences is provided for comparisons (table 2.1). Both databases required participants to have no history of psychiatric or neurological illness. This information is elaborated on within experiment-specific reporting. Distributions of anxiety scores (from the NIH Toolbox's Fear-Affect CAT Age 18+; *NIH Toolbox*, n.d.) are also provided (figure 2.1).

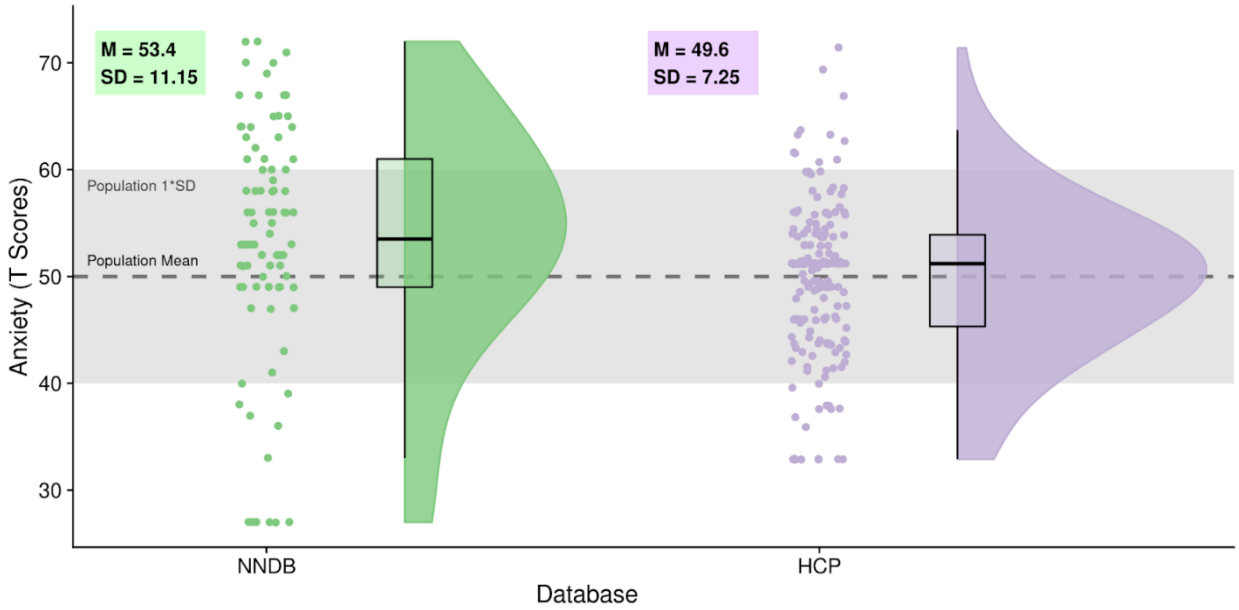

Figure 2.1. *Raincloud Plots (M. Allen et al., 2019) of anxiety scores for the Naturalistic Neuroimaging Database (NNDB) and Human Connectome Project (HCP): jittered data points represent individual participants, box plot hinges mark 25th/50th/75th percentiles, box whiskers indicate 1.5*interquartile range, and density plots represent smoothed distribution.*

Table 2.1. *Key cross-experiment comparisons. Columns (left to right) refer to: databases used; participant N (including gender and age-range); MRI magnet strength; repetition time; echo time; flip angle; voxel size; multiband acceleration; phase encoding direction.*

Database	N	Magnet	TR	TE	FA	Voxels	MB	Phase
NNDB	86 (42 F/44 M; 18–58 years)	1.5T	1000ms	54.8ms	75°	3.2mm^3	4	A->P
HCP	178 (108 F/70 M; 22-31+ years)	7T	1000ms	22.2ms	45°	1.6mm^3	5	Variable

Experiment 1 Hypotheses (Naturalistic Neuroimaging Database)

Based on the 'aversive amplification' circuitry hypothesis (Robinson et al., 2014), I preregistered the following predictions in regard to my analyses of the Naturalistic Neuroimaging Database:

1. Self-reported symptoms of anxiety will positively correlate with face-dependent dmPFC-left amygdala functional connectivity.
2. Self-reported symptoms of anxiety will positively correlate with face-dependent dmPFC-right amygdala functional connectivity.

Experiment 2 Hypotheses (Human Connectome Project)

Prior to reanalysis in the updated naturalistic neuroimaging database (see *Methods*), I previously observed depleted amygdala-cingulate and -middle frontal gyrus connectivity as a function of anxiety. This effect was no longer apparent following reanalysis with updated preprocessing (reported below). As such, I hypothesized a similar effect on an independent dataset to provide out-of-sample validation. Specifically, I predicted:

1. Self-reported symptoms of anxiety will negatively correlate with seed-based amygdala-dmPFC functional connectivity during movie-watching.
2. Self-reported symptoms of anxiety will negatively correlate with seed-based amygdala-middle frontal gyrus functional connectivity during movie-watching.

2.3 Methods

Preregistration

My planned analyses were preregistered. Along with my code, these are available on the Open Science Foundation (https://osf.io/345nj/).

Datasets

Naturalistic Neuroimaging Database (Experiment 1)

I conducted analyses on the Naturalistic Neuroimaging Database (Aliko et al., 2020). In brief, the database contains a set of 86 right-handed participants (42 females; aged 18–58 years, M = 26.81, SD = 10.09) viewing entire movies whilst under functional MRI. Participants watched one movie during scanning, and the movie varied between participants (10 movies in total; minimum length = 92 min; maximum length = 148 min; table 2.2). Scanning was conducted on a 1.5 T Siemens MAGNETOM Avanto (T2*-weighted images: TR = 1000ms, TE = 54.8ms, Slices = 40; FA = 75°, voxel size = 3.2mm^3, MB = 4). The functional data had already been preprocessed using the following steps: slice-time correction; volume registration; registration of functional images to warped anatomical scan; spatial smoothing to 6 mm FWHM; normalization; and manual ICA artifact rejection. The use of ICA-denoising is particularly relevant to my analyses in addressing physiological confounds (e.g., respiration) that would otherwise be of relevant concern (Chang & Glover, 2009; Glasser et al., 2019). For a full overview of database details, see (Aliko et al., 2020).

Since preregistering my analyses, the naturalistic neuroimaging database released a new version (v2.0), which contains a fix for an issue with timeseries scaling for runs of different lengths as well as the implementation of a standardized preprocessing pipeline, ‘afni_proc.py’. In the present manuscript, I summarize my

original findings under results and report the updated analyses. The full reporting of my original results can be found in the preprint (Kirk et al., 2021, version 1).

Stimulus Onsets. Word onset and face data from the movies was extracted using Amazon Web Services' Transcribe (https://aws.amazon.com/transcribe/) and Rekognition (https://aws.amazon.com/rekognition/). Detected face and word onsets had an associated confidence score for being correct (0–100%). For this, I selected an arbitrary threshold of 90%. Across movies, an average of 92.7% (SD = 2.63%) of faces detected fell within this threshold (table 2.2). Rekognition has been shown to perform well in naturalistic detection of faces (Hsu & Chen, 2017). Transcribe word information was matched and aligned with subtitle information (see Aliko et al., 2020). To further validate the accuracy of the face and word detection algorithms, I specified confirmatory contrasts, wherein I saw expected fusiform and auditory cortex activation respectively (figure 2.2). Face and word onsets had variable durations. For the purposes of obtaining psychophysiological interaction terms, onsets were resampled into stable 200 ms windows (5 Hz).

Behavioural Data. Approximately two weeks prior to scanning, participants completed questionnaires from the NIH Toolbox (*NIH Toolbox*, n.d.). Of relevance to the present study, this included an emotion battery (Salsman et al., 2013). Here, I used the Fear-Affect CAT Age 18+ uncorrected T-scores. The questionnaire measures "symptoms of anxiety that reflect autonomic arousal and perceptions of threat" (*NIH Toolbox*, n.d.). This holds convergent validity with other, commonly used anxiety questionnaires (Salsman et al., 2013; Schalet et al., 2014).

Table 2.2. *Naturalistic Neuroimaging Database summary: movie watched, number of subjects, movie length, and the proportion of detected faces that fell within my confidence threshold.*

Movie	N	Duration (mins)	Proportion of faces over 90% confidence
500 Days of Summer	20	91.17	95%
Citizenfour	18	113.40	94%
12 Years a Slave	6	128.53	91%
Back to the Future	6	111.23	92%
Little Miss Sunshine	6	98.33	91%
The Prestige	6	125.25	91%
Pulp Fiction	6	148.03	91%
The Shawshank Redemption	6	136.35	89%
Split	6	112.32	97%
The Usual Suspects	6	101.70	96%

Human Connectome Project 7T Dataset (Experiment 2)

The Human Connectome Project is a large-scale database of multimodal MRI data (Van Essen et al., 2013). Within the database is a subset of functional scans (n = 184; runs = 4) collected with a 7T Siemens MAGNETOM whilst participants watched movie scenes across 4 sessions/2 days (TR = 1000ms, TE = 22.2 ms, slices = 85; FA = 45°, voxel size = 1.6mm^3, MB = 5). Participants watched 14 movie clips (duration range = 65-255s) interspersed with 22 rests (20s) and 4 repeated video validation clips (83s). I provide a summary below (table 2.3; for full details, see https://protocols.humanconnectome.org/HCP/7T/). This constituted the dataset for experiment 2. Six subjects had at least 1 run of movie data missing. These were excluded, leaving a final n = 178.

Table 2.3. *Presentation order for Human Connectome Project movie-watching data. All clips were preceded and followed by 20s rest.*

Day 1		**Day 2**	
Block 1	**Block 2**	**Block 3**	**Block 4**
Two Men (244s)	Inception (227s)	Off The Shelf (181s)	Home Alone (232s)
Welcome To Bridgeville (222s)	Social Network (259s)	1212 (185s)	Erin Brockovich (230s)
Pockets (188s)	Ocean's Eleven (249s)	Mrs. Meyer's Clean Day (204s)	The Empire Strikes Back (255s)
Inside The Human Body (64s)	Validation clip (83s)	Northwest Passage (143s)	Validation clip (83s)
Validation clip (83s)		Validation clip (83s)	

Preprocessing. The data available was already preprocessed using a minimal pipeline (*fMRIVolume*: gradient-distortion correction, FLIRT-based motion correction, TOPUP-based unwarping, coregistration, transformation to MNI, intensity normalization & bias field removal; (Glasser et al., 2013). In addition to these steps, I smoothed the data to 6mm FWHM ('3dBlurToFWHM'; masked in subject-specific grey matter) to match the smoothness of the data in experiment 1. Key differences to the preprocessing performed on the Naturalistic Neuroimaging Database are: the use of TOPUP-based unwarping; lack of ICA-denoising (in volume-based data); and lack of slice-time correction.

Behavioral and Demographic Data. The Human Connectome Project also used the NIH Toolbox, and thus contains the *Fear-affect CAT 18+* uncorrected T-scores which I used for my analyses. Human Connectome Project age data is provided in pseudonymized brackets (22-25; 26-30; 31-35; 36+). For the purposes of my regressions, these were coded as categorical factors. As certain age by gender cells did not have sufficient N to run my group-level model (relevant N's: 22-25 years

females = 1; 36+ years females = 2; 36+ years males = 0), ages were re-coded into two brackets (22-30 years, M = 56, F = 51; 31+ years, M = 14, F = 57).

Analyses

fMRI time series extraction and modelling were conducted in AFNI (Cox, 1996). Relevant AFNI functions are denoted in parentheses. Due to memory constraints, within-subjects analyses were conducted on sections of slices at a time ('3dZcutup'). Beta-weight outputs were then concatenated back into whole-brain maps ('3dZcat') before group-level analysis. All analyses used two-sided tests thresholded at α=.05.

Regions of Interest Masks

My key regions of interest include the amygdala and dorsomedial prefrontal cortex (dmPFC). My amygdala ROIs were selected through individual anatomical parcellations of T1 images in Freesurfer (Fischl, 2012). ROI masks were visually inspected for successful segmentation. My dmPFC ROI was a functional mask from a recent meta-analysis of anxiety (Chavanne & Robinson, 2021; 'patients>controls 20mm' at ~[0 25 40]).

Naturalistic Neuroimaging Database Modelling

My within-subjects models were constructed using generalized psychophysiological interactions (McLaren et al., 2012). This enabled me to test context-dependent connectivity with amygdala above and beyond task-related activation and covariation with the raw amygdala time series. In line with AFNI recommendations (https://afni.nimh.nih.gov/CD-CorrAna), I conducted the following preparatory pipeline for each subject: 1) extract time series of amygdala ('3dmaskave'); 2) upsample to resolution of stimuli onsets ('1dUpsample'); 3) deconvolution of seed time series ('waver', basis function = BLOCK, then '3dTfitter'); 4) obtain and convolve interaction terms for stimuli onsets ('1deval', then 'waver', basis function = BLOCK); and 5) downsample interaction terms to resolution of TR ('1dcat'). I built my 1st level design matrices ('3dDeconvolve', -mask "sub-*_T1w_mask") inputting 9 regressors: face onsets convolved with a hemodynamic response function (HRF; basis function = dmBLOCK), HRF-convolved word onsets (basis function = dmBLOCK), left amygdala seed time series, right amygdala seed time series, left amygdala face interaction term,

right amygdala face interaction term, left amygdala word interaction term, right amygdala word interaction term, and a constant (-polort 0).

I constructed a group-level matrix using AFNIs multivariate modelling ('3dMVM') with 1st-level beta-weight maps inserted as within-subject variables ('-wsVars'). Anxiety, gender, age, and movie watched were inputted as between-subject regressors ('-bsVars'). The inclusion of the latter regressors in my model allowed me to test differences above and beyond those induced (linearly) by specific movies, age, and/or gender. All analyses were coded as general linear tests ('-gltCode'). My whole-brain analyses used t-tests with an initial cluster-defining threshold of $p_{uncorr.}<.001$ before whole-brain cluster correction ('3dFWHMx' with group residuals, '3dClustSim'; i.e., k >= 10.4). 3dMVM and 3dClustSim were constrained using subject-wide averaged masks ("sub-*_T1w_mask"; '3dMean'). Whole-brain results are reported in MNI space.

Given that my hypotheses sought to test a specific functional landmark within the medial prefrontal cortex, whole-brain statistical correction could have been overly conservative. As such, I also conducted ANCOVAs of dmPFC-averaged betas for my main hypothesis-testing in the Naturalistic Neuroimaging Database.

Human Connectome Project Modelling

I first removed effects of no interest from raw time series for each run using '3dDeconvolve' by including baseline terms with drift (-polort A) and 12 motion parameters (raw + temporal derivatives) as regressors to produce a cleaned, error time series. I then extracted amygdala seeds ('3dmaskave') from the cleaned time series before computing left and right amygdala-whole brain beta-weights and correlation maps ('3dDeconvolve'; '3dcalc'; r maps were Fisher z-transformed). Volumes which included majority rest or validation clips (i.e., assigning 0 to TRs in seed regressors). The first 10 seconds of movie volumes were also excluded to rule out influence from rests.

I took within-subjects amygdala time series beta-weight maps and whole-brain correlations forward to a group-level model ('3dMVM'; Chen et al., 2014) with anxiety scores, age, gender, and run as regressors. This was again masked within a grey matter mask (average Freesurfer-derived grey matter segmentations). Whole-brain analyses employed cluster-level correction ('3dClustSim') using a spatial autocorrelation function estimated from group-level residuals ('3dFWHMx'). I

inspected results using contrast-specific two-sided t-tests at two levels of voxel-wise correction: $p_{uncorr.}<.01$, and $p_{uncorr.}<.05$, which resulted in cluster thresholds of k >= 431.7 and k >= 1913.7 respectively.

For post-hoc exploratory-testing, I also made use of a canonical 400 parcel-level segmentation (Schaefer et al., 2018). Linear models including anxiety scores, age, and gender and regressors were conducted for each movie clip (14) by amygdala connection (2) by parcel (400) combination (total = 11200). Beyond this, I did not submit these to formal hypothesis-testing; rather, I visualized amygdala connectivity x anxiety t scores on a per clip basis to aid in interpretation of my results.

Control Analyses. I additionally included post-hoc control analyses to test whether observed connectivity results were driven by anxiety-correlated noise across both datasets. I reconducted my analyses using calcarine sulcus as a seed (instead of amygdala). This was to test whether any of my anxiety-dependent psychophysiological or seed results may be a product of global signal correlations, rather than an effect specific to amygdala connectivity.

Deviations from Preregistration

I note the following deviations from preregistration for the Naturalistic Neuroimaging Database:

- I did not preregister a plan to handle centering for the purposes of my group-level intercepts. Anxiety scores were mean-centered. As age showed a strong positive skew this was median-centered for the purposes of group-level intercepts.
- I preregistered to construct multiple group-level models using F tests. However, I streamlined this by having a single coherent group-level model, coding two-sided t-tests for planned analyses whilst retaining the same statistical thresholding. This was done to provide directionality (e.g., increased vs decreased connectivity).
- I decided to re-inspect my results at more liberal voxel-wise thresholds in order to investigate relatively more diffuse effects (Cox, 1996). I also included a word by anxiety correlation in the analysis. Post-hoc tests are noted within text.

- As the intersection of between-subjects grey matter resulted in an overly thin mask, I changed required overlap from 100% to 95% of participants.
- In the present manuscript, I summarize my original findings and report the updated analyses for version 2 of the naturalistic neuroimaging database. The full reporting of my original results can be found in the preprint (Kirk et al., 2021, version 1).
- The control analysis was conducted post-hoc.

I note the following deviations from preregistration for the Human Connectome Project dataset:

- I preregistered to code ages into four categories. However, as certain gender by age cells did not have sufficient N to run my model, I collapsed ages into two categories (see *behavioral/demographic data*).
- As the intersection of between-subjects grey matter resulted in an overly thin mask I changed required overlap from 100% to 95% of participants.
- ROI and control analyses were conducted post-hoc.

2.4 Results

Naturalistic Neuroimaging Database Version 1 Results

I originally conducted my analyses on the Naturalistic Neuroimaging Database using an earlier version of the dataset (V1). As my updated analyses (on NNDb V2) altered my inference, I report here a brief summary of the relevant original findings. Firstly, I saw no correlations with anxiety scores for face onsets. For my hypothesized seed-based functional connectivity analyses, I did not observe any correlations between psychophysiological interactions and anxiety scores. I did not observe effects of anxiety on seed-based functional connectivity at my initial voxel-wise threshold. I then re-inspected results with more liberal voxel-wise thresholding ($p < .01$, $p < .05$; cluster-corrected). For my seed regressors, I observed correlations between anxiety and: right amygdala-anterior/mid cingulate (voxel-wise $p < .05$, peak = [1 43 13], 273 voxels) and left amygdala-right anterior middle frontal gyrus connectivity (voxel-wise $p < .05$, peak = [31 58 22], 175 voxels; lateral Brodmann area 10). Contrasting these results with

main effects suggested these were functionally excitatory connections independent of faces/words, but were depleted as a function of anxiety.

Activation-based Analyses (Naturalistic Neuroimaging Database Only)

To provide a basic characterization of the naturalistic neuroimaging dataset and validate the use of my onset regressors, I ran two-tailed t-tests for altered activation to 1) faces and 2) words. As expected, I saw increased activation to faces in fusiform gyri (peak = [40 -89 -17], 1133 voxels), notably overlapping with meta-analytic fusiform face area activation (peaks = [39 -53 -22; -40 -54 -23]; (Aliko et al., 2020; Berman et al., 2010). I did observe separate clusters of reduced lingual/fusiform gyri activation to faces (left peak = [-29 -50 -8], 634 voxels; right peak = [28 -56 -8], 676 voxels), though these were more distal to typical face-selective activation. In regard to word onsets, I saw increased activation in primary auditory cortices/superior temporal gyrus (left peak = [-68 -11 4], 2092 voxels; right peak = [67 -5 1], 1379 voxels; figure 2.1.2).

I saw two cluster-corrected positive correlations with anxiety scores for faces in superior parietal lobe (left peak = [-29 -68 64], 18 voxels; right peak = [34 -65 58], 23 voxels). For my post-hoc word onset analysis, I observed a cluster in left auditory cortex to negatively correlate with anxiety scores (peak = [-65 -32 16], 34 voxels).

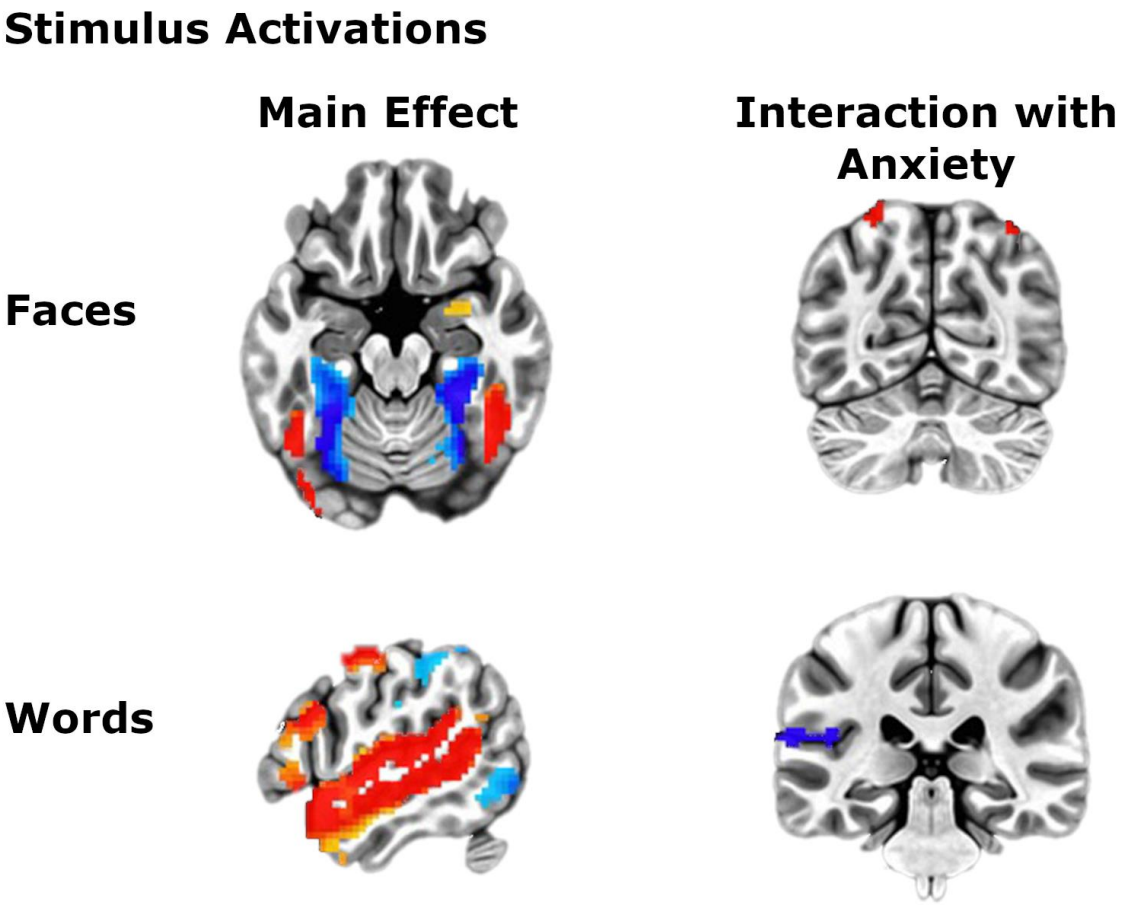

Figure 2.2. *Whole-brain results ($p_{uncorr.}$<.001, cluster-corrected at k >= 11; red voxels = increased activation, blue voxels = reduced activation) demonstrating brain activations to faces and words and how activation to these stimuli correlate with self-reported anxiety.*

Face-Dependent Amygdala Connectivity (Naturalistic Neuroimaging Database Only)

For PPI main effects, I observed increased connectivity as a function of faces, notably increased connectivity between amygdala and dmPFC (figure 2.3). I also observed effects of increased amygdala connectivity as a function of words in medial prefrontal cortex (left and right amygdala terms) and auditory cortex/superior temporal gyrus (right amygdala only).

For my hypothesized connectivity analyses, I did not observe any cluster-corrected correlations with anxiety scores. As whole-brain statistical correction could be overly conservative, I conducted ROI analyses to test my hypotheses. Congruent with the whole-brain tests, ROI ANCOVAs also failed to demonstrate a significant effect of anxiety. I repeated analyses post-hoc with more liberal voxel-wise threshold ($p < .01$ & $p < .05$; cluster-correction thresholds = 35.8 & 144.9 respectively. I observed a positive correlation between anxiety scores and face-dependent amygdala-superior temporal sulcus connectivity (voxel-wise $p < .05$; left peak = [-68 -11 4], 250 voxels; right peak = [61 -20 16], 223 voxels).

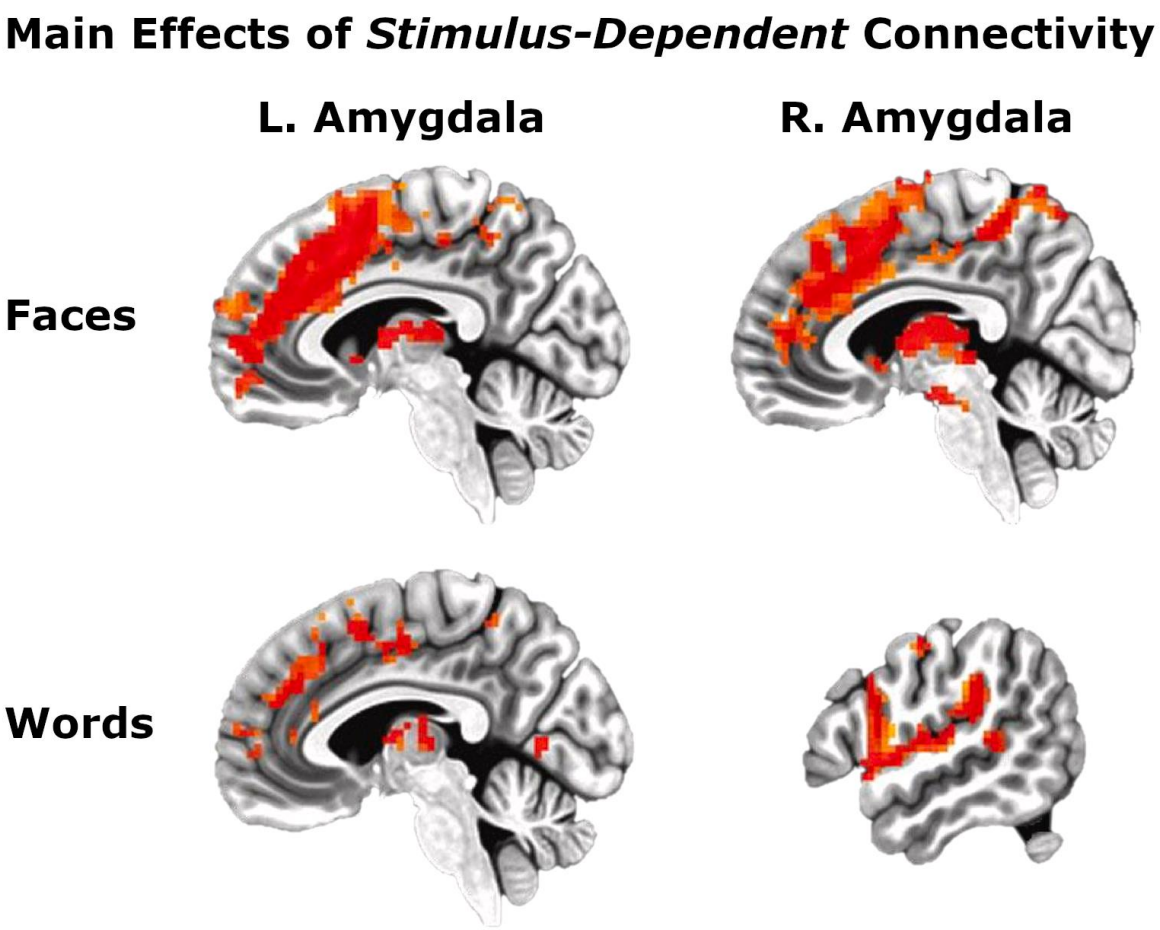

Figure 2.3. *Whole-brain results ($p_{uncorr.}$<.001, cluster-corrected at k >= 11) demonstrating increased amygdala connectivity as a function of faces and spoken words.*

Seed-Based Functional Connectivity

Naturalistic Neuroimaging Database

I conducted two preregistered, exploratory left and right amygdala seed-whole brain correlations. This tested for effects of amygdala connectivity independent of specific stimuli (i.e., faces and words) within movies. For the left amygdala seed term, I saw increased left amygdala-inferior occipital gyrus connectivity as a function of anxiety (peak = [-53 -77 -5], 12 voxels). Following more liberal, post-hoc thresholding, I also saw increased left amygdala-middle frontal gyrus connectivity (voxel-wise p < .01, peak = [-41 37 43], 40 voxels) and right amygdala-middle temporal gyrus connectivity (voxel-wise p < .05, peak = [58 -71 10], 195 voxels) as a function of anxiety.

Human Connectome Project

I observed main effects of amygdala seeds partially consistent with the previous experiment (figure 2.4), including positive connectivity to fusiform face area, prefrontal cortex, and cingulate gyrus. However, I did not observe any corrected correlations between anxiety scores and seed connectivity in whole-brain analyses.

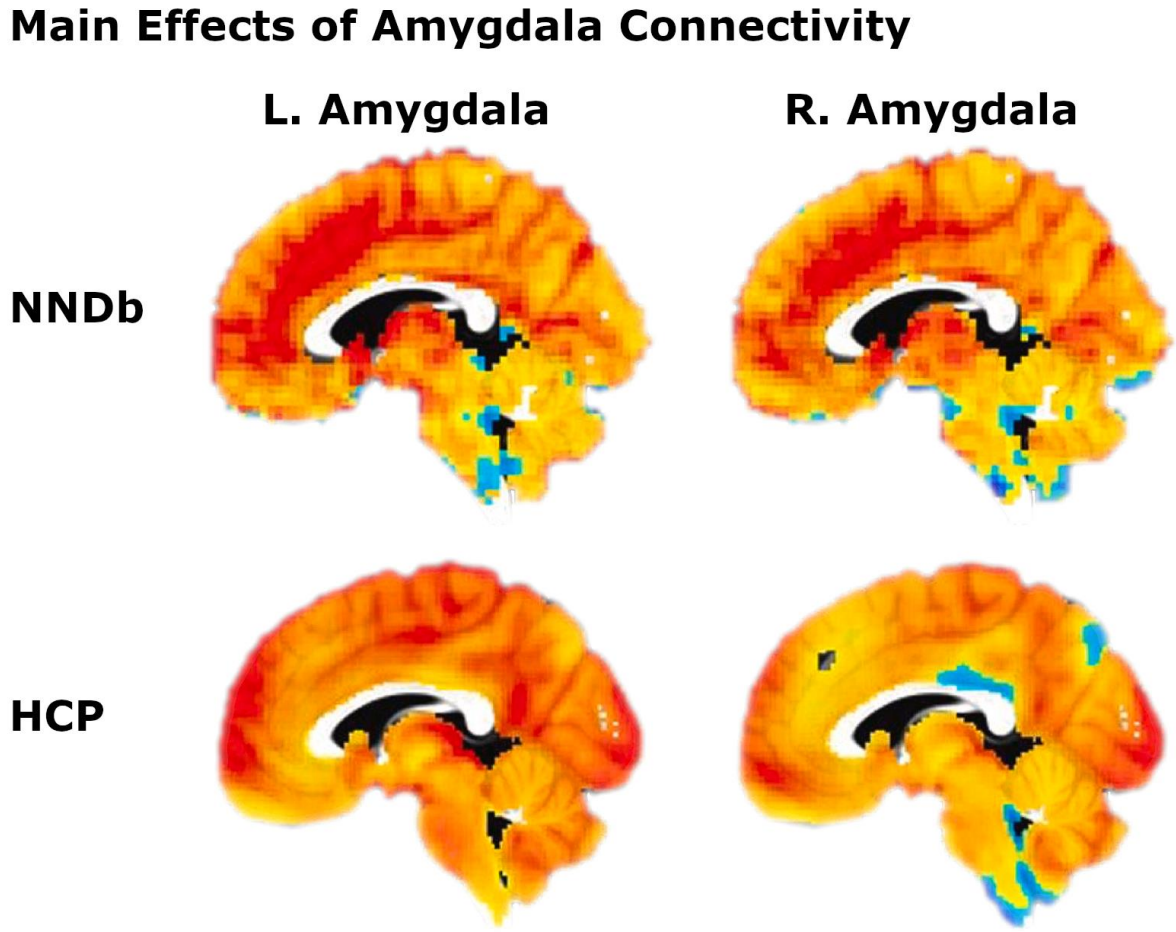

Figure 2.4. *Whole-brain results ($p_{uncorr.}$<.001, cluster-corrected; red voxels = increased activation, blue voxels = reduced activation) demonstrating main effects of amygdala-whole brain seed connectivity.*

I reconducted the above analyses using Fisher z-transformed correlation coefficients instead of beta-weights. This did not alter inference. Another property of the HCP dataset was that the runs used different phase encoding directions (runs 1/4

= Anterior-Poster, runs 2/3 = Posterior-Anterior). As phase encoding direction is known to have an impact on distortions and signal dropout around the amygdala (De Panfilis & Schwarzbauer, 2005), the variable phase encoding employed in the present dataset could mask results collapsed across runs. As such, I preregistered additional analyses to test effects of anxiety on runs which used Anterior-Posterior and Posterior-Anterior phase encoding separately. For runs with AP phase only (congruent with the Naturalistic Neuroimaging Database) I observed two cluster-corrected (i.e., voxel-wise $p < .05$, $k < 1913.7$), anxiety-relevant results: a heightening of left amygdala-right fusiform/cerebellum connectivity (peak = [36 -59 -46], 2043 voxels) and a degradation of right amygdala-right fusiform/cerebellum connectivity (peak = [37 -78 -19.2], 1950 voxels). Neither of these clusters were apparent in runs which used PA phase.

Control Analyses

For my calcarine connectivity control analysis, I did not find any correlations with anxiety across all voxel-wise thresholds (.001, .01, .05) in both the Naturalistic Neuroimaging Database and Human Connectome Project. This suggested my previous anxiety-relevant connectivity results were not driven by global noise (e.g., motion; though this assumes between-subject differences in BOLD artifacts are consistent across the whole brain).

Clip-Level Analysis

For my clip-level analysis, I did not submit clip by parcel model outputs to any formal statistical testing. For descriptive, exploratory purposes only, I note variability in the number of parcels surpassing uncorrected significant thresholds across the clips (range = 7:72; table 2.4; figure 2.5).

Table 2.4. *Movie clips and the number of amygdala-parcel x anxiety correlations surpassing uncorrected p < .05.*

Clip	No. Parcels < .05	Clip	No. Parcels < .05
1212	7	Empire Strikes Back	28
Mrs Meyers Clean Day	7	Erin Brockovich	34
Social Network	14	Pockets	43
Welcome to Bridgeville	16	Two Men	44
Northwest Passage	24	Ocean's Eleven	49
Home Alone	25	Inception	67
Inside the Human Body	27	Off the Shelf	72

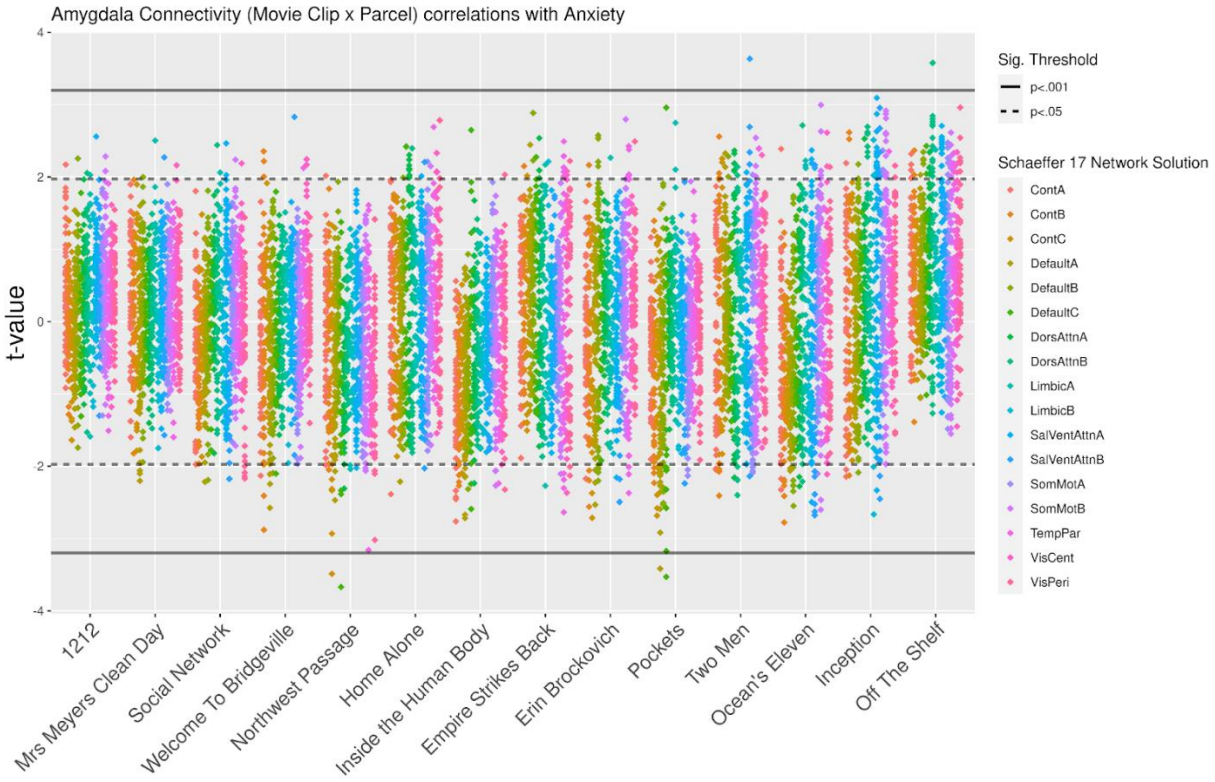

Figure 2.5. *Left and right amygdala connectivity (2 x 400 parcels) x anxiety t-scores per movie clip. Clips (x axis) ordered by number of amygdala-parcels demonstrating uncorrected significance (p < .05).*

2.5 Discussion

This project was motivated by an amygdala-prefrontal model of threat-processing. Initially evidenced by the rodent literature (see Robinson et al., 2019), this model outlines an excitatory amygdala-prefrontal circuit which drives harm avoidance (Robinson et al., 2014). fMRI work has implicated a homologous circuit in humans: experiments have demonstrated amygdala-prefrontal coupling to faces appears increased whilst under threat of shock, the degree of which correlates with self-reported anxiety (Robinson et al., 2012). In the present preregistered two-experiment study, I sought to extend this model of anxiety to naturalistic settings through means of movie fMRI. To this end, I correlated face-dependent connectivity with self-reported anxiety symptoms in a movie-watching database. In my original analyses, seed-based functional connectivity analyses suggested self-reported anxiety to correlate with degraded amygdala-dmPFC coupling, but only when using post-hoc thresholding. However, I failed to replicate this effect in a second dataset. Moreover, this effect dissipated when using an updated version of the database with improved preprocessing, thus I do not infer this as a stable finding. Following reanalysis in the updated database, I observed anxiety-relevant correlations with stimulus-onset activation, but did not observe robust alterations in connectivity.

Within the main effects tests of whole-brain activation (experiment 1), I report expected engagement of fusiform gyrus and auditory cortex to faces and spoken words respectively. In addition to replicating previously observed effects, this mitigated concerns regarding the accuracy of the stimulus-detection algorithm to adequately detect face and spoken word onset information. Moreover, I noted two correlations with anxiety. As a function of anxiety, I saw greater face-dependent bilateral superior parietal activation and reduced spoken word-dependent activation in left auditory cortex. As these were not hypothesized clusters, I do not comment on these further, but—given that these effects passed a priori thresholding—future work should seek to test whether these effects are apparent in an independent sample.

For my psychophysiological interaction analyses, I observed widespread main effects. This included increased face-dependent connectivity to inferior frontal gyri, medial prefrontal cortex, and superior temporal gyri. I did not see correlations between anxiety and connectivity in my hypothesized regions, though inspection of results with post-hoc thresholding implicated increased face-dependent amygdala-superior

temporal sulcus connectivity as a function of anxiety. I also conducted seed-based functional connectivity analyses across two datasets. I did not observe any cluster-corrected results at my preregistered voxel-wise threshold. Using post-hoc thresholds, I noted anxiety-relevant amygdala-middle frontal and -middle temporal connectivity in experiment one, and amygdala-fusiform connectivity in experiment two. Given the lack of overlap between these studies and the use of post-hoc thresholds, I do not make a strong inference regarding these results. I emphasize however that differences in movie content and length between these two datasets should be considered for future studies wishing to provide replications and/or out-of-sample validation.

Across the psychological sciences, our theories and models are built on the foundations of highly controlled studies (Yarkoni & Westfall, 2017). Said experimental designs were driven by the need for adequately controlling potential confounds. However, this comes with the cost of limited contextual generalizability. Indeed, the present results highlight a discrepancy when utilizing relatively more naturalistic stimuli. It may be that harm avoidance circuitry is not maximally engaged during face perception. Instead, said processing may occur more broadly for generally salient information in the environment (though the seed-based functional connectivity analyses did not evidence this). My work has further emphasized the need within affective neuroscience to scrutinize what components of theories do and don't extend to ecologically-richer settings.

While it has become apparent that movie fMRI can evoke relatively more stable, richer, and clinically insightful functional networks (Eickhoff et al., 2020; Finn & Bandettini, 2021; Meer et al., 2020), the present study highlights the need for careful consideration of stimulus complexity when modelling dynamic movie fMRI data. I was unable to explore the temporal properties (e.g., emotional content) with the data available. However, when I re-analyzed data on a scene-by-scene basis, the results implied that differences may occur as a function of movie stimulus. Given that individual differences in anxiety may be most prominent within threatening environments, directly modelling dynamic, canonical valence/arousal ratings may increase sensitivity to these effects (as has been demonstrated within the depression literature: Gruskin et al., 2020). Moreover, said dynamics may be nested throughout multiple features of the movies, ranging from overall emotional tension to specific content within faces (e.g., novelty, expression).

Alternatively, traditional approaches to fMRI analyses (i.e., feature-based GLM) may be particularly constrained when attempting to capture anxiety-relevant neural systems during movie-watching. One possible avenue for future work would be to bridge data- and hypothesis-driven approaches through the use of techniques such as intersubject representational similarity analysis (P.-H. A. Chen et al., 2020). This may help implicate whether previously reported anxiety-relevant brain circuitry is engaged during movie-watching without the need for assumptions regarding stimulus features or hemodynamic response.

I also highlight here the tools used to assess anxiety in the present project. Though the NIH toolbox offers a useful battery for a wide assessment of cognitive/affective domains, this was a computerized adaptive questionnaire that typically administers far fewer questions than more standardized anxiety questionnaires, such as the state-trait anxiety inventory (Spielberger, 1983), which may be more appropriate for detecting subtle differences along the continuum of anxiety severity. It may also be plausible that the two dimensions of state vs trait anxiety may reveal dissociable effects, though I have previously noted these two measures (as assessed by questionnaires) to correlate very highly (r = .83; see Kirk, Robinson, & Gilbert, 2021). Consequently, the dissociation of these may be further elucidated through correlations with both questionnaires and regressors marking tonal shifts throughout movie stimuli. I also note the non-clinical nature of the present project. Given that individuals demonstrating particularly high anxiety may avoid volunteering for fMRI studies (Charpentier et al., 2021), explicit comparisons between individuals with anxiety disorders and healthy controls may reveal differences not apparent here.

Finally, I highlight that several of my results presented were detected using post-hoc voxel-wise thresholds. As such, conclusions regarding these effects should be tentative. Furthermore, I also note that my results indicate preprocessing steps (experiment 1 v1 vs v2) and scanning parameters (experiment 2) likely impact the sensitivity of detecting effects of anxiety. Future work interested in investigating amygdala-prefrontal connectivity in movie fMRI should pay particular attention to how the sensitivity of the BOLD signal in medial temporal lobe and prefrontal cortices may be impacted by preprocessing and sequence parameters. Given this limitation, it is not possible within the constraints of the present project to rule out the role of this circuitry

in anxiety-related face processing. Nonetheless, I believe the present work has laid foundations to help guide future movie fMRI work into anxiety.

Conclusion

My project aimed to test whether an amygdala-prefrontal threat-processing model of anxiety could extend to naturalistic stimuli. I noted effects of anxiety on face-dependent superior parietal activation and word-dependent auditory cortex activation. However, I failed to find a correlation between face-dependent amygdala-prefrontal coupling during movie-watching and self-reported anxiety. Seed analyses also did not reveal robust effects of anxiety-relevant amygdala-cingulate connectivity. Overall, this work tempers the proposed role of this circuitry in anxiety and highlights the importance of testing predictions derived from experimentally constrained contexts in more naturalistic settings to ensure generalizability.

3. Amygdala-Prefrontal Dynamics and Suspense

3.0 Preface

In chapter 2, I failed to find evidence of an association between trait anxiety and amygdala-prefrontal responses to faces during movies. One potential explanation is that trait anxiety may be primarily associated with altered brain responses only following state anxiety induction. As such, in chapter 3, I sought to use a more dynamic approach which incorporated ongoing emotional dynamics within movies. I focus on suspense as a naturalistic mode of anxiety induction. In this chapter, I test whether the association between individual differences in trait anxiety and amygdala-dmPFC responses is emerge primarily during highly suspenseful scenes in movies.

For the final published versions of this study, please refer to the below reference:

Kirk, P. A., Holmes, A. J., & Robinson, O. J. (2022). Anxiety Shapes Amygdala-Prefrontal Dynamics During Movie-Watching. *Biological Psychiatry Global Open Science*. https://doi.org/10.1016/j.bpsgos.2022.03.009

3.1 Abstract

A well-characterized amygdala–dorsomedial prefrontal circuit is thought to be crucial for threat vigilance during anxiety. However, engagement of this circuitry within relatively naturalistic paradigms remains unresolved. Using an open functional magnetic resonance imaging dataset (Cambridge Centre for Ageing Neuroscience; n = 630), I sought to investigate whether anxiety correlates with dynamic connectivity between the amygdala and dorsomedial prefrontal cortex during movie-watching. Using an intersubject representational similarity approach, I saw no effect of anxiety when comparing pairwise similarities of dynamic connectivity across the entire movie. However, preregistered analyses demonstrated a relationship between anxiety, amygdala-prefrontal dynamics, and anxiogenic features of the movie ('canonical' suspense ratings). My results indicated that amygdala-prefrontal circuitry was modulated by suspense in low-anxiety individuals but was less sensitive to suspense in high-anxiety individuals. I suggest that this could also be related to earlier anticipation or slowed disengagement to suspense. Moreover, a measure of threat-relevant attentional bias (accuracy/reaction time to fearful faces) demonstrated an association with connectivity and suspense. Overall, this study demonstrated the presence of anxiety-relevant differences in connectivity during movie-watching, varying with anxiogenic features of the movie. Mechanistically, exactly how and when these differences arise remains an opportunity for future research.

3.2 Introduction

Studies to date have outlined an amygdala-dorsomedial prefrontal circuit that may underlie threat processing (Milad et al., 2007; Robinson et al., 2012). Conditioning paradigms have demonstrated this in vitro in both rodents (Vidal-Gonzalez et al., 2006) and primates (Taub et al., 2018). Functional magnetic resonance imaging (fMRI) studies have provided evidence for the presence of this circuit in humans (Robinson et al., 2012; Vytal et al., 2014). Moreover, the degree of engagement appears to interact with the affective content of stimuli (e.g., facial expressions) and individual differences in trait anxiety (Robinson et al., 2012; Vytal et al., 2014). As such, recruitment of this circuit, above and beyond regional activation (Nord et al., 2017, 2019; Sambuco et al., 2020), is thought to drive attentional amplification of threat-relevant features in the environment, a core component of anxiety (Robinson et al., 2014). However, studies to date have primarily investigated this using static stimuli (i.e., faces) presented without context. Consequently, the relationship between this circuit and anxiety in more dynamic, naturalistic contexts remains poorly understood. Extending study of this circuitry to more naturalistic stimuli offers the opportunity to validate these findings in more ecologically rich settings and observe how this circuit may be modulated as a function of dynamic contextual features.

A small number of studies have demonstrated anxiety-relevant within-subject amygdala-prefrontal coupling during movie-watching. Specifically, these have demonstrated increased functional connectivity between the amygdala and dorsomedial prefrontal cortex (dmPFC) during fear-inducing/anxiogenic scenes within movies (Hudson et al., 2020; Kinreich et al., 2011). However, we know little about how individual differences in anxiety interact with this connectivity during movie-watching. Therefore, in chapter 2, I explored how between-subject differences in anxiety modulated this circuitry. I did not find convincing interactions between circuitry and individual differences in anxiety using traditional, static (time-invariant) feature- and seed-based approaches. Put more simply, when looking for connectivity patterns that were stable (irrespective of specific scenes within movies), I did not see differences as a function of anxiety. Given the emotional complexity of movies, an approach that is sensitive to ongoing dynamics (e.g., how anxiety-inducing a scene is) within the stimuli may be more suitable.

Studies have now started to implement intersubject representational similarity analysis for movie stimuli (for an introduction to representational similarity analysis, see (Kriegeskorte et al., 2008). Broadly speaking, this treats other subjects as a control measure for each subject at every point in the movie. By comparing two subjects' brain activity across a movie, I can generate a measure of how neurally similar the two subjects were. If differences in neural similarity correlate with differences in self-reported similarity (i.e., trait anxiety), I can infer that brain activity in region X during movie-watching varies as a function of individual difference Y. This technique is unconstrained in that it does not rely on traditional onset-convolved regressors, which require the researcher to specify exact events. However, unlike stimulus-independent analyses, such as seed-based functional connectivity analysis, it remains stimulus driven and time locked: the movie elicits a generally shared experience, but similarity measures will capture, in this instance, anxiety-relevant deviations from this. This approach has demonstrated sensitivity to detecting shared and idiosyncratic representations relevant to affective systems (G. Chen et al., 2020; P.-H. A. Chen et al., 2020; Finn et al., 2020; Finn & Bandettini, 2021). With a goal of predicting individual differences (i.e., self-report scores), it has been argued that this approach allows for greater sensitivity for predicting brain-behavior relationships versus traditional resting-state paradigms (G. Chen et al., 2020). However, despite being sensitive to the content within a movie, results do not allow for temporal specificity (which time points in the movie are driving effects) because similarity measures are based on comparisons across the entirety of movie-viewing. Thus, this approach may offer sensitivity for detecting phenotypic variation and clustering, but its ability to inform biopsychological theories of affective systems is inherently limited.

A complementary method to deriving similarity measures across an entire movie is through dynamic (time-varying) analyses. This provides information regarding neural connectivity at each time point, allowing brain measures to be mapped back onto stimulus information (e.g., anxiogenic features). This works in a similar manner to the aforementioned analyses (looking at similarity across the entire movie), except neural similarity is based on specific time points throughout the movie. Similar to traditional techniques (feature-based regression), this allows for inferences concerning which time points are driving effects. However, unlike traditional modeling approaches, this also makes fewer assumptions regarding properties of the fMRI

signal, such as shape of the hemodynamic response across anatomy and time. Consequently, this has the potential to increase sensitivity while retaining stimulus-relevant specificity. Little work has been done in this domain; yet, this approach has demonstrated that the relationship between depressive symptoms and brain activity (i.e., medial PFC and posterior cingulate) tracks ongoing emotional intensity/valence of movie stimuli (Gruskin et al., 2020). It is thus plausible that the impact of trait anxiety on connectivity may vary as a function of ongoing anxiogenic features within a movie.

By one view, impaired amygdala-prefrontal functioning in anxious populations might emerge through stable deficits in brain function (as tested within the resting-state framework). An alternate possibility is that idiosyncrasies in activity/functional connections change alongside the emotional content of movies (as evidenced in Gruskin et al., 2020). Analogously, it has been demonstrated that increasing cognitive demands (via cognitive tasks) boosts brain-based predictions of cognitive variation compared with rest (J. Chen et al., 2022; Finn et al., 2017). Likewise, it may therefore be that individual differences in trait anxiety only surface within specific emotional contexts, most prominently state anxiety (as theorized by the diathesis-stress model, Brozina & Abela, 2006, and as implicated by threat of shock studies, Robinson et al., 2012). In this project, I aimed to extend my previous work (chapter 2) on the engagement of threat circuitry during naturalistic viewing. Specifically, I aimed to explore the extent to which intersubject similarity in amygdala connectivity during movie-watching was modulated as a function of trait anxiety. Moreover, I sought to test how anxiety-relevant differences in connectivity may vary as a function of the anxiogenic content within the movie (i.e., suspense).

Hypotheses

I made the following preregistered (https://osf.io/hfc9n/) predictions in regard to a movie-watching fMRI dataset. Each were tested on left and right amygdala connectivity separately:

1. Pairwise similarity in self-reported anxiety will positively correlate with similarity in amygdala-dorsomedial prefrontal connectivity during movie-watching. In other words, I will observe anxiety-relevant idiosyncrasies when comparing

subjects' amygdala-prefrontal connectivity time courses across an entire movie clip.

2. Pairwise similarity in self-reported anxiety will show greater correlations with amygdala-dorsomedial prefrontal similarity during highly suspenseful scenes. In other words, I will observe a greater impact of trait anxiety on amygdala-prefrontal connectivity during high (vs low) suspenseful scenes.

3.3 Methods

Cam-CAN Dataset

fMRI Data

I conducted analyses on the Cambridge Centre for Ageing Neuroscience database (Cam-CAN; n = 652, Mean Age = 54.81, Age SD = 18.54, 329 Female, 50/589 left/right handed, 11 ambidextrous, 2 missing hand data; (Shafto et al., 2014; Taylor et al., 2017). Participants were required to be cognitively healthy and free of neurological or serious psychiatric conditions. Experimental procedures relevant to the present study included viewing a clip from Alfred Hitchcock's '*Bang! You're Dead*'. BOLD signal was acquired on a 3T Siemens TIM Trio System using multi-echo T2* EPI (32 axial slices 3.7mm thick, 0.74mm gap, TR = 2470ms; TE = [9.4, 21.2, 33, 45, 57] ms, FA = 78deg; FOV = 192x192mm; voxel size = 3x3x4.44mm, TA = 8mins 13s). Functional data were preprocessed using: realignment and unwarping with fieldmaps, slice-time correction, transformation to MNI space, and despiking using outlying wavelet coefficients (no smoothing). For a full overview of database details, see (Taylor et al., 2017).

Self-Report and Behavioral Data

Prior to scanning, participants completed the Hospital Anxiety and Depression Scale (HADS; Stern, 2014). The anxiety section of this scale constituted my self-report metric for hypothesis-testing (figure 3.1). Subjects with no available HADS data (n = 3) were omitted from the relevant analyses. In addition to self-report measures, I made use of canonical suspense ratings previously collected as 21 subjects viewed the same '*Bang! You're Dead*' clip (Schmälzle & Grall, 2020). To account for hemodynamic lag, I shifted the ratings backward by 2TRs (~5s; consistent with prior work in this domain,

(Finn & Bandettini, 2021), removing the last two data points and imputing the first two with the mean of the first 5 TRs of the original ratings. This regressor therefore acted as a continuous, block-wide parametric modulator and did not require convolution with the hemodynamic response function.

In a set of exploratory analyses, I derived an anxiety-relevant cognitive bias measure from behavioral data. I was interested in whether individual differences in affective bias (Aylward et al., 2020), namely greater vigilance toward threat-relevant stimuli in the environment (Mogg & Bradley, 2006), also demonstrated effects of movie-dependent connectivity (as observed in threat of shock studies, Robinson et al., 2012). For this, I calculated threat vigilance measures from the face perception task participants completed prior to scanning ('emotion expression recognition'). This included labeling faces morphed between emotional expressions (happiness-surprise, surprise-fear, fear-sadness, sadness-disgust, disgust-anger, anger-happiness; stimuli derived from (Ekman, 1976). My threat vigilance measure was calculated through a simplified drift-diffusion model. I extracted summary statistics pertaining to accuracy and mean/variance of RT for correctly-labeled trials where morphs contained 70/90% fear (summary statistics used as trial-by-trial data is not provided within Cam-CAN). Summary statistics were then inputted into E-Z drift-diffusion modeling (Wagenmakers et al., 2007). The drift parameter constituted my threat vigilance metric. RT variance values of 0 (one correct trial) and accuracy values of 0, 0.5, and 1 were increased (or decreased for the latter) by .000001 to avoid division errors. Subjects with no available face data (n = 15) were omitted from relevant analyses. Spearman correlation suggested a small, positive relationship between self-reported anxiety and threat vigilance ($\rho = .13$, $p = .0008$).

My choice for the use of fearful (vs angry) faces was based on a theoretical distinction between immediate, direct threat (an angry individual) vs uncertain threat (fearful expressions, which may signal the presence of nearby danger). The former could be conceptualized as evoking panic/fearful responses, whereas the latter may be more related to states of anxiety (though both likely fall on a continuum, see Mobbs et al., 2020).

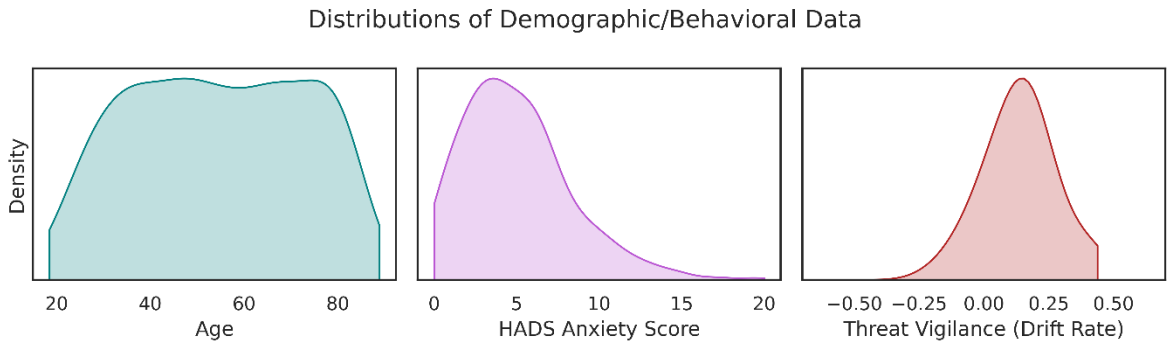

Figure 3.1. *Kernel density plots for age, self-reported anxiety, and threat vigilance (cut at minimum/maximum). Due to a very low accuracy (5%, z = −10) in the face perception task, 5 subjects had a drift rate parameter of −3.1, which is not visualized here (but was retained in analyses). HADS, Hospital Anxiety and Depression Scale.*

Analysis

fMRI timeseries extraction and modeling were conducted using AFNI (Cox, 1996) and Python. Relevant functions are denoted in parentheses. Analyses were preregistered (https://osf.io/hfc9n/) and used two-sided tests thresholded at α=.05. Post-hoc analyses are reported separately in the results section. Visualizations were generated with Seaborn (Waskom, 2021) and Matplotlib (Hunter, 2007). Data can be accessed via a request to Cam-CAN (Cam-CAN-archive.mrc-cbu.cam.ac.uk). I have made my scripts openly available (https://osf.io/5xsp6/).

Regions of Interest Masks

My amygdala ROIs were selected through individual anatomical parcellations of T1 images in Freesurfer (Fischl, 2012) constrained with an inflated ('3dROIMaker', -inflate 3; figure 3.2) MNI amygdala mask (AAL atlas; Rolls et al., 2020). dmPFC was defined via a functional mask from a previous meta-analysis of anxiety relevant task-based activations; specifically, I used a conjunction map of adaptive/maladaptive anxiety ('Induced vs. Transdiagnostic 20mm', cluster at ~[0, 23, 45], Chavanne & Robinson, 2021; https://neurovault.org/images/384691/; figure 3.2). For whole-brain analyses, functional volumes were segmented via a canonical parcellation (Schaefer et al., 2018; 400 parcels) constrained within subject-specific, inflated gray matter masks.

Participants with failed Freesurfer segmentations (n = 10) or no overlap between auto-masked EPIs and at least one canonical parcel (n = 9) were excluded from analyses. Combined with missing self-report/behavioral data, this left 630 participants for my analyses on self-report measures (3.37% dropout) and 618 participants for analyses on threat vigilance measures (5.21% dropout).

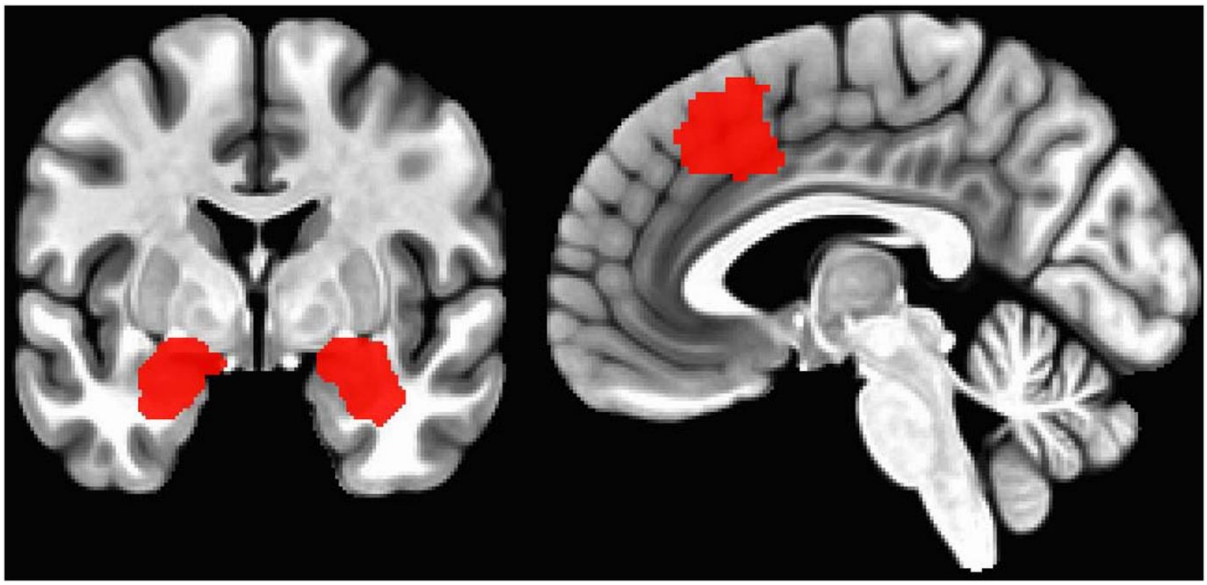

Figure 3.2. *Region of interest definitions for hypothesis testing. Left: Subject-specific regions of interest were defined using FreeSurfer before being constrained within an inflated Montreal Neurological Institute amygdala mask (coronal slice at y = −2). Right: the dorsomedial prefrontal cortex mask was generated from a meta-analysis looking at the conjunction between adaptive and maladaptive anxiety (sagittal slice at x = −3; Induced (+) vs. Transdiagnostic (+) 20 mm, Chavanne & Robinson, 2021).*

For exploratory analyses, I was also interested in the hypothalamus, bed nucleus of the stria terminalis (BNST), periaqueductal gray (PAG), medial orbitofrontal cortex/anterior ventromedial prefrontal cortex (vmPFC), subgenual anterior cingulate cortex (ACC), and anterior insula. Subcortical structures were defined anatomically: BNST and periaqueductal gray masks were based on previous manual tracings (in MNI space) of 10 and 53 subjects respectively (Theiss et al., 2017; Weis et al., 2022); and hypothalamus was defined through Freesurfer parcellations (Billot et al., 2020; all subunits combined due to the EPI voxel resolution). Cortical structures were defined

functionally from meta-analytic clusters (Chavanne & Robinson, 2021; Anterior Insula = 'Induced (+) vs. Pathological anxiety (+) 20mm', anterior vmPFC = 'Induced anxiety (threat > safe) 20mm'; subgenual ACC = 'Transdiagnostic anxiety (patients > controls)'). Medial structures where lateralizations were neighboring (BNST, PAG, hypothalamus, dmPFC, anterior vmPFC, subgenual ACC) were collapsed bilaterally. Given the spatial resolution of scans and likelihood of minor misalignments, the BNST and periaqueductal gray masks were dilated (3 times) and eroded (2 times) in order to create masks which would overlap with more participants.

Within-Subject and Pairwise Modeling

fMRI Data. I removed effects of no interest from raw timeseries ('3dDeconvolve') by regressing out baseline signals with drift (-polort A; detrending) and 24 motion parameters (raw + derivatives + squares) to produce a cleaned timeseries for each voxel. I then extracted ROI seeds ('3dmaskave') from the cleaned volumes. These were taken forward to produce TR-wise functional connectivity measures based on sliding window analyses ('timecorr', Owen et al., 2021; width = 8TRs/~20s, gaussian kernel weighting) between the amygdala and cortical ROIs (which were subsequently Fisher transformed and Z-scored).

Movie-wide intersubject representational similarity matrices were then constructed as a function of between-subject Pearson correlations in amygdala (left and right separately) -cortical connectivity (bilateral cortex) measures. TR-wise intersubject representational similarity matrices were constructed as a function of differences in between-subject amygdala-dmPFC connectivity measures.

Self Report and Behavioral Data. Self-report similarity measures were first calculated as the difference in self-reported anxiety. This allowed me to test a 'one-to-one' relationship between anxiety and connectivity; namely, whether high-high or low-low anxiety pairwise comparisons showed greater similarity than high-low anxiety comparisons. In other words, participants who differ on the low end of the HADS scale (e.g., 1 vs 2) will show the same differences in connectivity than those who differ on

the higher-end (e.g., 19 vs 20). In instances of undirected brain measures (i.e., movie-wide correlations) I used absolute differences in self-reported anxiety.

I also generated an exploratory matrix using the 'AnnaK' approach (Finn et al., 2020). Each cell in this matrix was calculated as the pairwise means of self-reported anxiety scores. Unlike the previous matrix, this allowed me to test a nonlinear relationship, namely that high-high anxiety pairwise comparisons would show greater similarity than low-low comparisons (or vice-versa). For instance, a negative correlation would suggest that participants who differ on the higher-end of the HADS scale (e.g., scores of 19 vs 20) will show similar connectivity profiles, whereas participants who differ on the low-end of the scale (e.g scores of. 1 vs 2) show greater variability in connectivity profiles. Finally, I created a matrix for my threat vigilance measures, using both differences and 'AnnaK' mean scores.

Group Modeling

I compared neural and behavioral similarity matrices using Partial Spearman Rank correlations using age, sex and motion (mean framewise displacement) as covariates. TR-wise analyses against suspense ratings used Pearson correlations. Significance was based on null distributions derived from 10,000 permutations of cells in the neural similarity matrices. For an overview of my analysis pipeline, see figure 3.3.

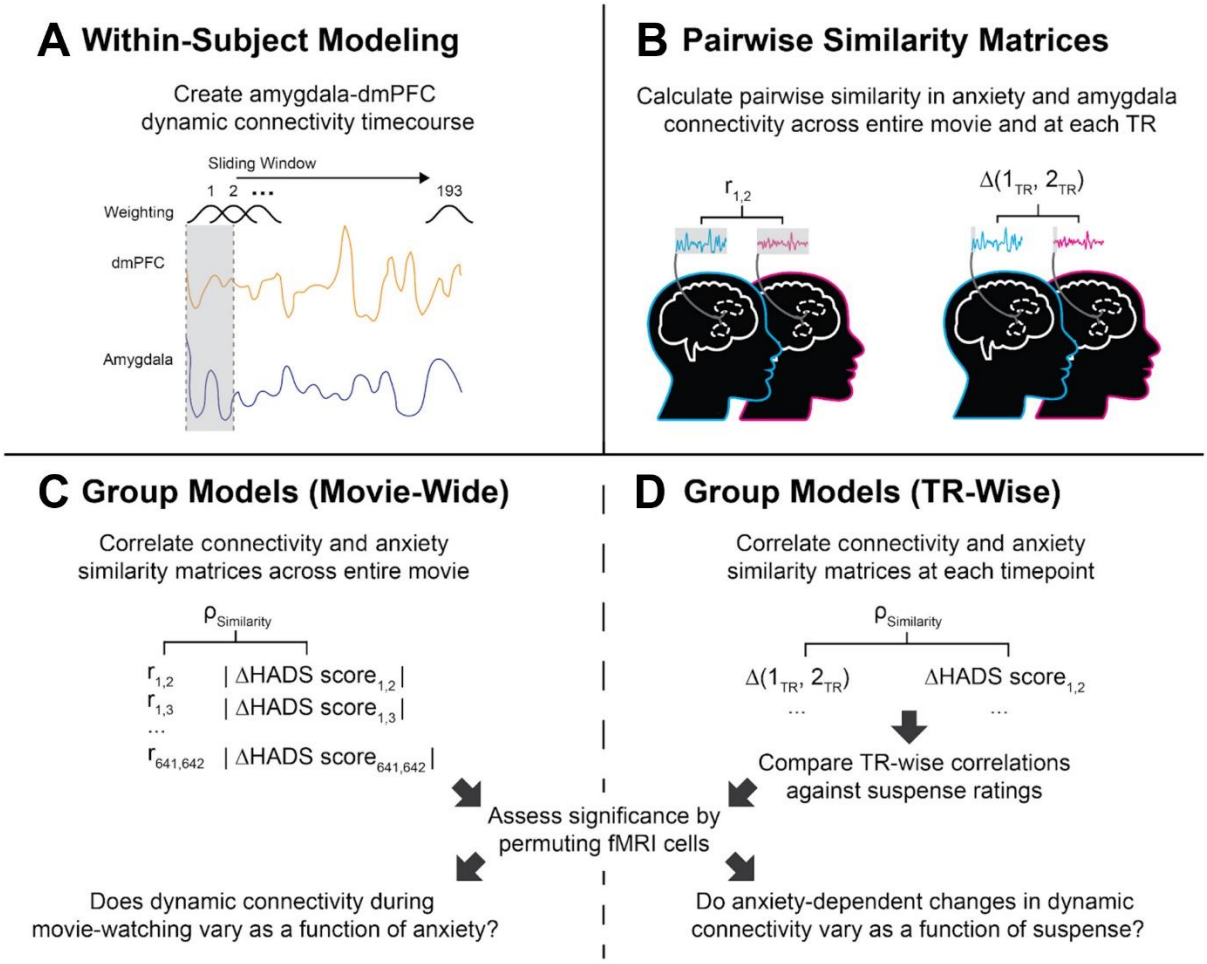

Figure 2.2.3. *Illustration of analysis pipeline. (A) I first derived time series of dynamic connectivity using a sliding window approach, with data points in the window weighted using a Gaussian function. (B) Pairwise similarity matrices were produced by correlating dynamic connectivity time series, calculating differences in connectivity at each repetition time (TR), and calculating differences in anxiety measures. (C) To test my first hypothesis, I correlated pairwise similarities in anxiety and amygdala–dorsomedial prefrontal cortex (dmPFC) connectivity across the entire movie. (D) Testing my second hypothesis, I repeated this procedure but at every TR in the movie and compared anxiety-related differences in connectivity against canonical suspense ratings. Significance for all group-level models were based on permutation testing. fMRI, functional magnetic resonance imaging; HADS, Hospital Anxiety and Depression Scale.*

3.4 Results

Movie-Wide Connectivity Tests

Firstly, I conducted Spearman correlations between self-report similarity (absolute difference) and functional connectivity similarity across the entire movie clip (Hypothesis 1). I did not observe effects in either left (ρ = .002, *p* = .581) or right (ρ = .001, *p* = .819) amygdala-dmPFC connectivity. Planned exploratory analyses also failed to show this for my threat vigilance (left: ρ = -.003, *p* = .517; right: ρ = -.003, *p* = .508) or 'AnnaK' models (left: ρ = .002, *p* = .620; right: ρ = -.0002, *p* = .968). In further planned exploratory analyses, I re-conducted movie-wide tests (self-report) using time series from 400 cortical parcels (Schaefer et al., 2018) for both absolute difference and 'AnnaK' models. Although some parcels surpassed Bonferroni correction (400 parcels, $p < .000125$), effect sizes were marginal (max |ρ| = .03). In other words, when comparing connectivity across the entirety of the movie clip, no single amygdala-parcel timeseries explained greater than 0.09% of the variance associated with anxiety.

Anxiety x Connectivity x Suspense Tests

I next produced Spearman correlations between self-reported anxiety (constant) and neural similarity matrices for each TR (dynamic). TR-wise coefficients were then taken forward to Pearson correlations against the canonical suspense ratings timeseries. This allowed me to test whether mapping between amygdala connectivity and self-report similarity was most prominent during high suspense scenes (Hypothesis 2). Unlike my movie-wide analyses, the TR-wise representational similarity matrices were directional in nature, meaning relative connectivity strength could be compared across subjects. I observed an inverse relationship to that which I predicted: there was a significant negative correlation between canonical suspense ratings and anxiety-dependent increases in right amygdala-dmPFC connectivity ($r = -.16$, $p = .02$; though not apparent for left amygdala: $r = -.05$, $p = .53$). Moreover, planned exploratory analyses demonstrated a stronger relationship between suspense and the impact of threat vigilance on right amygdala-dmPFC connectivity ($r = -.19$, $p = .006$; left amygdala-dmPFC: $r = -.05$, $p = .51$; figure 3.4).

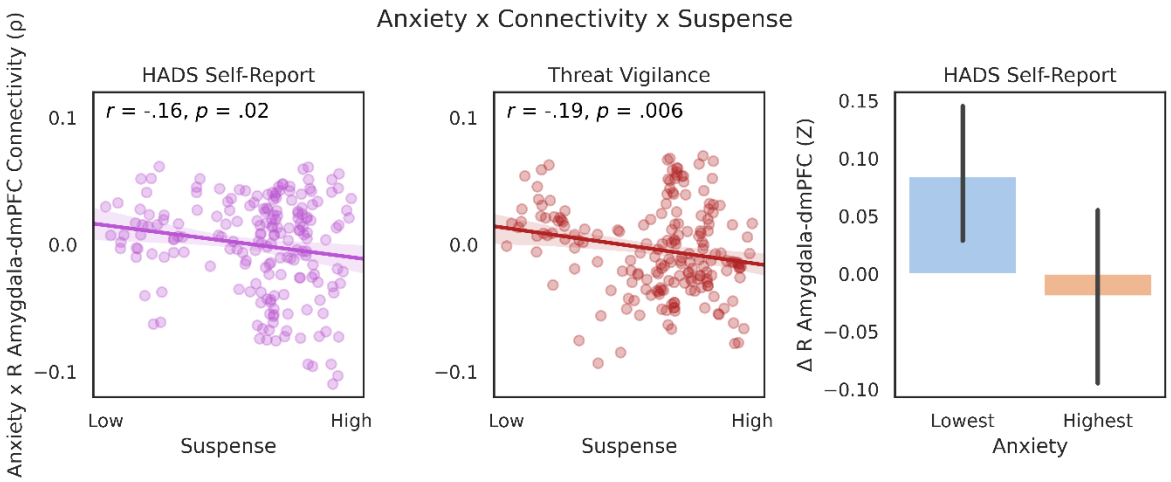

Figure 3.4. *Left/Middle: Scatterplots demonstrating a negative correlation (with 95% confidence intervals) between repetition timewise suspense ratings and anxiety-relevant increases in right (R) amygdala–dorsomedial prefrontal cortex (dmPFC) connectivity. Left: Hospital Anxiety and Depression Scale (HADS) anxiety scores. Middle: threat vigilance (drift rate from drift-diffusion models of fearful face responding). Right: bar plot demonstrating change in average connectivity (z scores) from low to high suspense (highest − lowest quartiles of suspense) across the lowest and highest quartiles of self-reported anxiety (with 95% confidence intervals).*

These results suggested that amygdala-dmPFC circuitry was modulated by suspense in low anxiety individuals, but this circuitry was less sensitive to suspense in high anxiety individuals. However, there are several interpretations for this result (see *Discussion*). To aid in interpretation, I ran post-hoc amplitude-based peak-detection (SciPy's find_peaks; Virtanen et al., 2020) across the suspense ratings timeseries (smoothed) to mark events of relative increases in suspense; this allowed me to visualize how anxiety-relevant alterations in amygdala-dorsomedial prefrontal cortex connectivity altered alongside anxiogenic scenes (figure 3.5). One event (#8) was manually adjusted to better reflect the plateau of suspense.

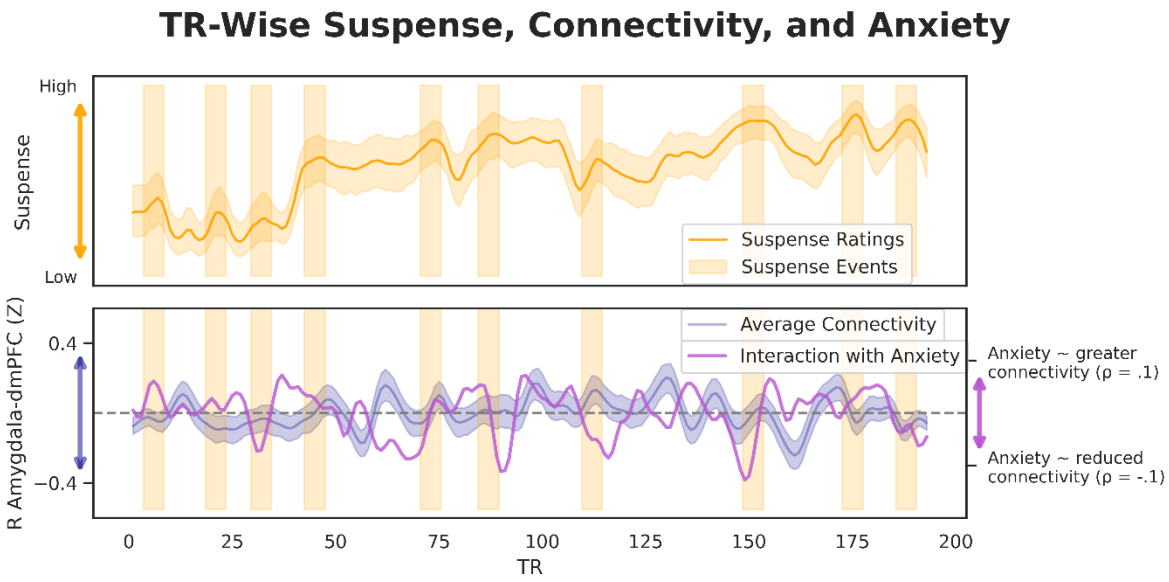

Figure 3.5. *Time series of canonical suspense ratings (orange line), suspenseful events (orange rectangles, marked using amplitude-based peak detection), average right (R) amygdala–dorsomedial prefrontal cortex (dmPFC) dynamic connectivity, and the correlation between anxiety and dynamic connectivity at each repetition time (TR) (smoothed). Shading denotes 95% confidence intervals.*

Post-Hoc Tests

As the relationship between dynamic connectivity and anxiety appeared dependent on the presence of suspense, I reconducted TR-wise tests across a wider 'defensive response network' (Abend et al., 2022) consisting of amygdala, bed nucleus of the stria terminalis, hypothalamus, periaqueductal gray, subgenual anterior cingulate cortex, an anterior section of the ventromedial prefrontal cortex, dorsomedial prefrontal cortex, and anterior insula. Most prominently, suspense showed the strongest relationship with anxiety-relevant differences in amygdala-periaqueductal gray connectivity (left amygdala: r = -.41, p < .0001; right amygdala: r = -.35, p < .0001; figure 3.6).

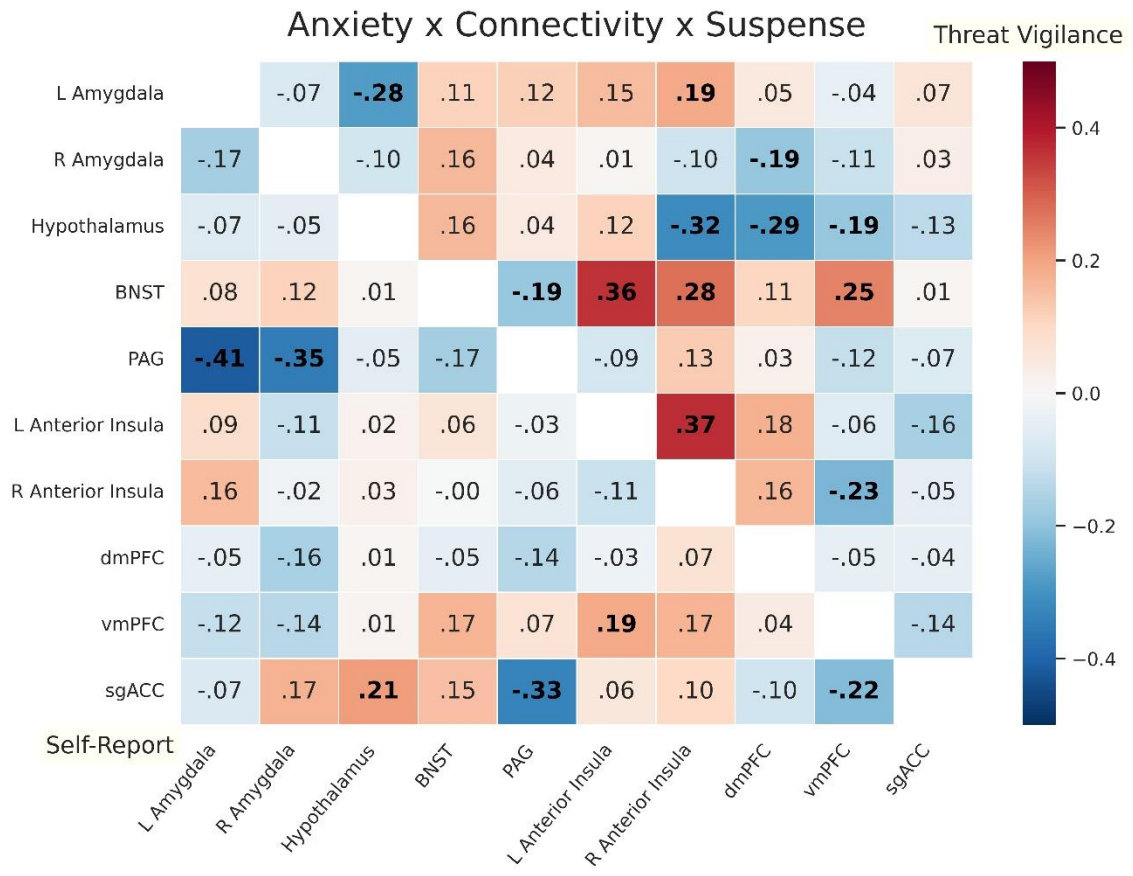

Figure 3.6. *Pearson correlations between TR-wise suspense ratings and trait anxiety-relevant increases in dynamic connectivity (lower triangle = self-report; upper triangle = threat vigilance). Bolded cells refer to correlations surviving Bonferroni correction across 45 comparisons across connectivity measures, p < .0011.*

Working with unconstrained naturalistic data (i.e., movies), it is difficult to orthogonalize features across the stimulus. As such, results should be interpreted with an understanding that a degree of collinearity likely exists between low- and high-level stimulus features. Using the pliers package (McNamara et al., 2017), I extracted features to demonstrate such collinearity. Suspense showed small-to-moderate correlations with power of the audio signal (a proxy for volume; r = .41), brightness (r = .23), and number of faces present (r = -.25). I also demonstrated correlations between age and anxiety (self-report: ρ = -.23, p < .001; threat vigilance: ρ = -.47, p < .001). Thus, there may be interactions between age and anxiety-dependent connectivity.

I used a sliding window approach due its utility demonstrated from previous work (E. A. Allen et al., 2014; Handwerker et al., 2012; Hutchison et al., 2013). However, a possible limitation of a sliding window approach to dynamic connectivity is that results may be sensitive to window length, offset, and filtering (Shakil et al., 2016). As such, I tested the robustness of these effects when using differing window lengths. Using window length of 6TRs (~15s), 8TRs (~20s), or 10TRs (~25s) did not change inference regarding suspense, right amygdala-dmPFC connectivity, and self-reported anxiety (6TRs: r = -.17, p = .02; 10TRs: r = -.17, p = .02). The same was true for threat vigilance measures (6TRs: r =-.18, p =.01; 10TRs: r = -.20, p = .006). These analyses suggest my reported findings are robust across a variety of window lengths.

3.5 Discussion

There is a wealth of task-based literature implicating an amygdala-prefrontal circuit that underlies threat-relevant biases fundamental to anxiety (Milad et al., 2007; Robinson et al., 2012, 2014; Taub et al., 2018; Vidal-Gonzalez et al., 2006; Vytal et al., 2014). Yet, little has been done to test whether individual differences in this circuit arise in more naturalistic settings. In the present study, I aimed to extend this work to a movie-watching paradigm using a dynamic, flexible analytical framework, inter-subject representational similarity analysis. Here, I tested whether anxiety would correlate with amygdala-dorsomedial prefrontal cortex dynamic connectivity throughout movie-watching. I failed to find evidence for this. I next tested the hypothesis that the relationship between anxiety and connectivity would vary depending on anxiogenic features within the movie (i.e., canonical suspense ratings). I observed effects in the inverse direction to what I predicted: the relationship between anxiety and right amygdala-prefrontal connectivity was negatively correlated with suspense (r = -.16, p = .04). Additionally, a planned exploratory analysis suggested a measure of threat vigilance (i.e., accuracy/reaction time to fearful faces) was slightly more sensitive to these effects (r = -.19, p = .001). I offer several interpretations for how this effect may have arisen.

At face value, the negative relationship suggested high (vs low) anxiety individuals had relatively increased connectivity during low suspense scenes (and/or reduced connectivity during high suspense). This would suggest that high anxiety individuals chronically engage this circuit, irrespective of anxiogenic scenes in movies, whereas low anxiety individuals selectively engage this circuit in response to anxiogenic scenes. Some resting-state studies have evidenced greater sustained engagement of this circuit irrespective of stimuli (Roy et al., 2013), though there is mixed evidence (see Mizzi et al., 2022). However, I suggest this is not the most plausible inference. In my previous study (chapter 2), I did not find evidence for differences in seed-based functional connectivity measures of amygdala-dmPFC circuitry across significantly longer movie scans. Moreover, this interpretation is in direct contrast to findings from threat of shock studies, which suggests individual differences emerge primarily when under a state of anxiety (Robinson et al., 2012).

One possibility is that anxiogenic, vicarious features of the movie evokes different affective processes to those elicited by direct, personal threat (threat of

shock). There is scant evidence explicitly investigating how the medium of anxiety-induction impacts brain response. Behavioral research has suggested differential impacts of physical vs social threats on emotional face perception amongst socially anxious individuals (Buckner et al., 2010). Additionally, given the very distinction between social and generalized anxiety disorders (Counsell et al., 2017), social vs direct threats may indeed differentially impact anxiety-relevant processes. Self-reported findings from media psychology offer additional insight. Unlike threat of shock, which is not typically thought of as a desirable experience, many people seek out anxiogenic media such as horror movies (Bantinaki, 2012). Indeed, it has been suggested that while the initial experience of anxiogenic scenes may be aversive, individuals scoring high in sensation-seeking may feel an aftermath of positive emotions and emotionally unstable individuals may show greater evoked anxiety (Clasen et al., 2018). Moreover, viewers of anxiogenic media may be worried about characters within the movie, but not themselves. Given that the anxiety measure was self-oriented, the suspense ratings may also be impacted by trait empathy. Therefore, the affective state elicited by suspenseful movies is likely multifaceted and more dynamic in nature than states evoked by threat of shock. Given the absence of these effects in seed-based functional connectivity measures (chapter 2), I conclude that these individual differences are likely arising in response to the emotional context elicited by the movie.

Visualization of the results further supports a distinct interpretation to suspense-insensitive, chronic engagement. Although temporal fluctuations in suspense resulted in initially similar right amygdala-dmPFC connectivity patterns between participants, toward the end of and/or following a lag after these events there seems a divergence in coupling as a function of individual differences in anxiety. This is in line with a body of literature demonstrating anxious individuals have reduced habituation to threat-relevant stimuli (Blackford et al., 2013; Campbell et al., 2014; Protopopescu et al., 2005). This also relates to findings demonstrating an association between personality and emotions experienced after anxiogenic scenes (Clasen et al., 2018). In other words, I suggest engagement of amygdala-prefrontal connectivity was slower to disengage following anxiogenic scenes in high anxiety individuals. Inversely, due to the short intervals between suspenseful scenes, this could be explained by earlier, amplified effects of anxious anticipation (Abend et al., 2022; McMenamin et al., 2014;

Najafi et al., 2017; engagement of amygdala-prefrontal connectivity was stronger when high anxiety individuals started to expect a forthcoming suspenseful scene).

I did not submit these timeseries to any formal analyses as this would have relied on post-hoc assumptions regarding data which I had already observed, such as the specific lag following suspense events. Moreover, the aforementioned delay appears non-constant and/or could be impacted by other features not modeled in the current study (which was limited to canonical suspense ratings). Thus, the explanations I offer are of course provisional. Nonetheless, the present results provide evidence that anxiety, dynamic connectivity, and anxiogenic features of a movie do interact in a time-varying manner. An opportunity movie-watching fMRI offers for future research is elucidating exactly how anxiety, connectivity, and nested features of the stimuli may interact. Based on the present findings, I encourage future research explicitly: test temporal lags in modeling (e.g., vector auto-regression); embed various features of the stimulus, ranging from high-level affective dynamics (e.g., suspense) to visual onsets (e.g., facial expressions), in analyses of dynamic connectivity; and compare connectivity profiles between anxiogenic scenes and threat of shock.

I also draw attention to the convergence between the self-report (Hospital Anxiety and Depression Scale; Stern, 2014) and behavioral measures (threat vigilance; derived from accuracy and reaction times to fearful facial expressions). The correlation between self-report and behavior was small ($\rho = .13$, $p = .0008$), suggesting that—although overlapping—these measures could tap into different latent constructs. Whereas the latter targeted attentional biases to threat, a key feature of anxiety (Aylward et al., 2020; Mogg & Bradley, 2006), the self-report measure summated multiple symptoms of pathological anxiety (e.g., “Worrying thoughts go through my mind”, “I get a sort of frightened feeling like 'butterflies' in the stomach”) which may measure distinct dimensions (e.g., worry, somatic symptoms, interoception; Andrews & Borkovec, 1988; Garfinkel et al., 2016). I was unable to assess item-level correlations in the present dataset. Nonetheless, both self-report and threat vigilance measures demonstrated correlations with dynamic right amygdala-dmPFC connectivity and suspense. Yet, the relationship between threat vigilance, connectivity, and anxiogenic features of the movie ($r = -.19$, $p = .006$) was slightly stronger than self-reported anxiety ($r = -.16$, $p = .02$). These results lend support for previous theorizations that this circuit is involved in attentional amplification of threat-

relevant stimuli, such as emotional facial expressions (Robinson et al., 2014; this may also be related to how the threat vigilance measure was based on a perception of others and the stimulus presented posed danger to other characters).

I note several facets of the present findings which may warrant further investigation. Firstly, I highlight the relationship between anxiety and right (but not left) amygdala-prefrontal connectivity. This lateralization is congruent with previous threat of shock studies (Gold et al., 2015; Robinson et al., 2012; Vytal et al., 2014), yet little is known about this dominance. Given that lateralization is apparent both within and outside of traditional paradigms, this warrants further investigation (e.g., whether this is related to handedness). Second, I noted potential interactions between age, anxiety, connectivity and suspense. Future work should seek to detail the exact nature of this relationship. Third, I was unable to test whether the observed associations are apparent in those with a clinical diagnosis. There is evidence to suggest the impact of induced anxiety may vary as a function of clinical diagnosis (Makovac, Meeten, Watson, Herman, et al., 2016). Therefore, it is possible these effects may not manifest in the same manner for those with clinically-significant levels of anxiety and results need to be interpreted in the context of subclinical variation. To my knowledge, there are currently no available movie-watching datasets which have explicitly sought to test clinically-diagnosed individuals. This may prove fruitful for further exploration of the impact of anxiety on brain responses to movie-watching.

Finally, I highlight the unconstrained nature of the present paradigm. Given the naturalistic basis of the stimulus (a movie), it is unsurprising there are confounded features within the stimuli. I noted small-to-moderate correlations between suspense ratings, power of the audio signal (~loudness), brightness, and faces present. However, these aesthetics likely culminate to give rise to overall suspense (Lehne & Koelsch, 2015). It is therefore difficult to elucidate sensory processing from affective phenomena. Given that the relationship between anxiety and connectivity manifested in a slightly different manner to that seen in threat of shock studies, it will be important to: demonstrate generalizability of this effect across different movie stimuli, preferably with less collinearity between features; and ensure future task-based and movie fMRI studies are conducted in compliment to each other.

Conclusion

In the present study, I investigated whether dynamic connectivity during movie-watching related to individual differences in anxiety. Across the entirety of the movie clip, comparisons of dynamic amygdala-prefrontal connectivity did not relate to individual differences in anxiety. However, anxiety appeared to have a variable impact on dynamic connectivity dependent on the presence of anxiogenic features in the movie (i.e., suspense). I suggest anxiety could be associated with: suspense-insensitive, chronic engagement of threat circuitry in high anxiety individuals; slowed disengagement of threat circuitry following anxiogenic scenes; or greater apprehension of anxiogenic scenes. Elucidating exactly how and when these individual differences appear offers opportunity for future study.

4. Anxiety and 'Intrinsic' Amygdala Connectivity

4.0 Preface

In chapter 3, I demonstrated that associations between trait anxiety and amygdala-dmPFC responses are modulated as a function of suspense during movie-watching. However, effects were in the inverse direction to what I predicted. The correlation between trait anxiety and amygdala-dmPFC responding was greatest during low suspense scenes. On the one hand, this could reflect individual differences in chronic 'intrinsic' connectivity that are apparent in the absence of states of anxiety. On the other hand, these effects could be movie driven, but arose due to nuances temporal dynamics that may reflect processes such as earlier anticipation or slowed disengagement to suspense. In chapter 4, I sought to contextualize these prior results by testing the association between trait anxiety and resting-state derived connectivity using the same participants, brain regions, and anxiety measures as chapter 3. The key hypothesis here was that trait anxiety measures would demonstrate a positive correlation with amygdala-dmPFC functional connectivity.

For the final published versions of this study, please refer to the below reference:

Kirk, P. A., Holmes, A. J., & Robinson, O. J. (2022). Threat vigilance and intrinsic amygdala connectivity. *Human Brain Mapping*, *43*(10), 3283–3292. https://doi.org/10.1002/hbm.25851

4.1 Abstract

A well-documented amygdala-dorsomedial prefrontal circuit is theorized to promote attention to threat (“threat vigilance”). Prior research has implicated a relationship between individual differences in trait anxiety/vigilance, engagement of this circuitry, and anxiogenic features of the environment (e.g., through threat of shock and movie-watching). In the present study, I predicted that—for those scoring high in self-reported anxiety and a behavioral measure of threat vigilance—this circuitry is chronically engaged, even in the absence of anxiogenic stimuli. My analyses of resting-state fMRI data (n = 639) did not, however, provide evidence for such a relationship. Nevertheless, in my planned exploratory analyses, I saw a relationship between threat vigilance behavior (but not self-reported anxiety) and intrinsic amygdala-periaqueductal gray connectivity. Here, I suggest this subcortical circuitry may be chronically engaged in hypervigilant individuals, but that amygdala-prefrontal circuitry may only be engaged in response to anxiogenic stimuli.

4.2 Introduction

A growing body of literature has outlined a cortico-subcortical network which is engaged during states of anxiety. A core feature of this network appears to be amygdala-dorsomedial prefrontal circuitry (Milad et al., 2007; Roy et al., 2013; Vidal-Gonzalez et al., 2006), which has been theorized to drive attentional amplification of threat-relevant stimuli in the environment (Robinson et al., 2012, 2013). Individual differences in attention to threat ("threat vigilance") are thought to be a key feature driving variation in trait anxiety (Grupe & Nitschke, 2013; MacLeod & Mathews, 1988). Indeed, hyper-engagement of this amygdala-prefrontal circuit while under induced anxiety has been observed in clinically anxious populations (Robinson et al., 2014). As such, the study of amygdala-prefrontal circuitry has been a primary line-of-inquiry in the anxiety literature. However, there is likely a significantly wider network beyond this core circuit.

The bed nucleus of the stria terminalis (BNST), a region dorsal to- and highly connected with the amygdala is also thought to play a core role in coordinating adaptive responses to potential dangers (Hur et al., 2020). Early work suggested that—while the amygdala was thought to react to immediate threats (fear responses)—the BNST was associated with processing chronic, uncertain threats (anxious responding; Davis, 2006). However, this framework remains disputed (Fox & Shackman, 2019). Nonetheless, the BNST, also known as the "extended amygdala", has an established role in processing ambiguous threats and, consequently, is heavily implicated in anxiety (Hur et al., 2020). Additionally, other subcortical regions such as the hypothalamus and periaqueductal gray may form a key junction between threat-relevant perceptual/cognitive processes and the embodied responses associated with anxiety: engagement of fight-flight-freeze behaviors and alterations in autonomic functioning (Deng et al., 2016).

A breadth of research has demonstrated top-down, cortico-subcortical projections. In addition to the dorsomedial prefrontal cortex, other regions in the cortex have been implicated, such as: the anterior insula, associated with a range of functioning including anxiety-relevant interoceptive sensitivity, anticipation of future events, and controllability of stressors (Grupe & Nitschke, 2013; Limbachia et al., 2021; Terasawa et al., 2013); subgenual anterior cingulate, related to threat-relevant memory processes (Hakamata et al., 2020); and an anterior section of the

ventromedial prefrontal cortex/medial orbitofrontal cortex (Pujara et al., 2019), which may relate to positive affect and/or safety signal integration (Myers-Schulz & Koenigs, 2012; Tashjian et al., 2021), though relatively less attention has been paid to its functional distinction from subgenual ACC. Broadly speaking, projections from the cortex have typically been thought of as providing regulatory, evaluative, and contextual inputs to fundamental threat processes in subcortical regions (Tillman et al., 2018). While the aforementioned regions have been selectively associated with specific perceptual/cognitive processes, these likely operate as a broad, interactive network to orchestrate defensive behaviors (Chavanne & Robinson, 2021; Gorka et al., 2018).

We have now seen a plethora of anxiety research implicating this “defensive response network” spanning cortical and subcortical regions (Abend et al., 2022). However, despite a wide range of research establishing an association between anxiety and this network, our understanding of individual differences in this circuitry nonetheless remains limited. There is substantial literature establishing the presence of amygdala-dorsomedial prefrontal connectivity while under induced anxiety, but designs are not typically powered for large-scale individual differences research. On the other hand, large-scale resting-state studies sometimes include such circuitry within multivariate models, which have started to emerge as useful in the prediction of self-reported anxiety (Li et al., 2019). However, there can be difficulties interpreting the contributions of feature weights in these models (e.g., nonlinear support vector machines; Misaki et al., 2010) and how they relate to true brain activity/connectivity. Thus, model parameters often do not directly grant access to physiological information and necessitate transformations before attempting to estimate this information (Haufe et al., 2014). With the frequent goal of behavioral prediction, these studies often focus on models' decoding accuracy of psychiatric symptoms; consequently, there is often less of a focus on elucidating low-level mechanisms associated with this circuitry.

Among resting-state research that has included amygdala-prefrontal circuitry as a focal point, there is little consensus. For individuals with clinical anxiety, multiple studies have demonstrated both increased and aberrant amygdala-prefrontal connectivity at rest (see Mizzi et al., 2022). Despite accelerated developments in analytical tools, much of this research has remained dependent on diagnostic criteria and/or self-report measures which may have high underlying heterogeneity (Cuthbert

& Insel, 2013; Insel et al., 2010). Secondly, as these measures depend on introspection, they may not be comparable across individuals nor tap into precise internal processes (Baumeister et al., 2007; Watson et al., 2017). Moreover, individual scales are often associated with differing and/or multiple latent factors (Rose & Devine, 2014). While these measures may be a useful tool to implicate whether regions and connections are generally implicated in anxiety, they provide little theoretical precision as to the processes underlying brain circuitry.

In chapter 3, I demonstrated that individual differences in anxiety were associated with amygdala-dorsomedial prefrontal dynamics during movie-watching; however, effects were notably stronger for a targeted behavioral measure of threat vigilance than self-reported anxiety. I noted that subjects' reaction time/accuracy to fearful faces correlated slightly higher with suspense and amygdala-prefrontal dynamics (r = −.19) than did self-reported scores from the Hospital Anxiety and Depression Scale (r = −.16). This supports the notion that perceptual/attentional processes related to threat are a key function of this circuitry. Counter to my predictions, I saw effects primarily during low suspense scenes. As visualizations demonstrated the possibility of a more nuanced, dynamic relationship between anxiety, connectivity, and suspense than that indicated by linear correlation, I offered three interpretations for these effects: (1) high trait anxiety individuals chronically engage amygdala-prefrontal threat circuitry irrespective of the anxiogenic features of the environment; (2) high trait anxiety individuals show greater apprehension of anxiogenic scenes; or (3) anxiety slows disengagement of threat circuitry following anxiogenic scenes. In order to explore this “chronic engagement” hypothesis of amygdala-prefrontal connectivity, I sought to test whether anxiety was associated with functional connectivity in the same individuals, but during resting-state scanning. Specifically, I investigated whether this relationship was apparent for “intrinsic functional connectivity” of the amygdala and dorsomedial prefrontal connectivity as derived from eyes-closed resting-state scans in a separate imaging run, but in the same subjects using the same anxiety measures. In planned exploratory analyses, I also sought to test whether intrinsic connectivity of a wider defensive response network was associated with anxiety measures.

Hypotheses

I made the following key predictions regarding a resting-state fMRI dataset. Each tested left and right amygdala connectivity separately and were preregistered on the Open Science Framework (https://osf.io/cfdq7/):

1. Self-reported anxiety will positively correlate with 'intrinsic' amygdala-dorsomedial prefrontal connectivity (seed-based functional connectivity).
2. A behavioral measure of threat vigilance will positively correlate with 'intrinsic' amygdala-dorsomedial prefrontal connectivity (seed-based functional connectivity).

4.3 Methods

The present resting-state project was conducted following a related analysis on movie-watching data. Experiment code and data derivatives are available at the Open Science Foundation (https://osf.io/cfdq7/). Raw data are available on request from the Cam-CAN website (https://Cam-CAN-archive.mrc-cbu.cam.ac.uk/dataaccess/).

Cam-CAN dataset

fMRI Data

I conducted analyses on the Cambridge Centre for Ageing Neuroscience database (Cam-CAN; n = 652, Age Mean = 54.81, Age SD = 8.54, Age Range = 18.5 - 88.9, 330 Female, 320 Male, 50/589 left/right handed, 11 ambidextrous, 2 missing hand data; Shafto et al., 2014; Taylor et al., 2017). The present study made use of volumes acquired during eyes-closed resting-state scanning. BOLD signal was acquired with a T2* GE EPI (32 axial slices 3.7mm thick, 0.74mm gap, TR = 1970ms; TE=30ms, FA=78 deg; FOV=192 mm x 192 mm; 3 x 3 x 4.44mm, TA=8mins 40s). The functional data were already preprocessed using the following steps: realignment and unwarping with fieldmaps, slice-time correction, transformation to MNI space, and despiking using outlying wavelet coefficients (no smoothing). For a full overview of database details, see (Taylor et al., 2017).

Self-Report and Behavioral Data

Prior to scanning, participants completed the Hospital Anxiety and Depression Scale (HADS; Zigmond & Snaith, 1983). The anxiety section of this scale (7 items; Cronbach's α = ~.83; Bjelland et al., 2002) constituted my self-report metric for my first hypothesis. Subjects with no available HADS data (n = 3) were omitted from the relevant analyses, leaving 649 participants (mean/SD of anxiety scores = 4.96/3.30).

I also previously conducted analyses on whether a behavioral measure of attentional bias to threat ('threat vigilance') also correlated with connectivity. For this, I calculated threat vigilance measures from a face perception task participants completed prior to scanning ('emotion expression recognition'). This included labeling faces morphed between emotional expressions (happiness-surprise, surprise-fear, fear-sadness, sadness-disgust, disgust-anger, anger-happiness; stimuli derived from Ekman, 1976). My threat vigilance measure was calculated through drift diffusion modeling of fearful facial expressions. My choice for the use of fearful faces was based on the notion that these signal uncertain threats, a feature of the environment typically associated with anxiety/vigilance (Mobbs et al., 2020).

I first extracted accuracy and mean/variance of RT for correctly-labeled trails where morphs contained 70/90% fear (summary statistics used as trial-by-trial data for each morph step are not provided within the Cam-CAN dataset). These were then inputted into E-Z drift-diffusion modeling (Wagenmakers et al., 2007). The drift parameter constituted my threat vigilance metric. RT variance values of 0 (one correct trial) and accuracy values of 0, 0.5, and 1 were increased (or decreased in the last case) by .000001 to avoid division errors. Subjects with no available face data (n = 15) were omitted from the relevant analyses. In chapter 3, I reported a small but significant positive relationship between self-reported anxiety and threat vigilance measures (ρ = .13, p = .0008; distributions plotted in figure 4.1).

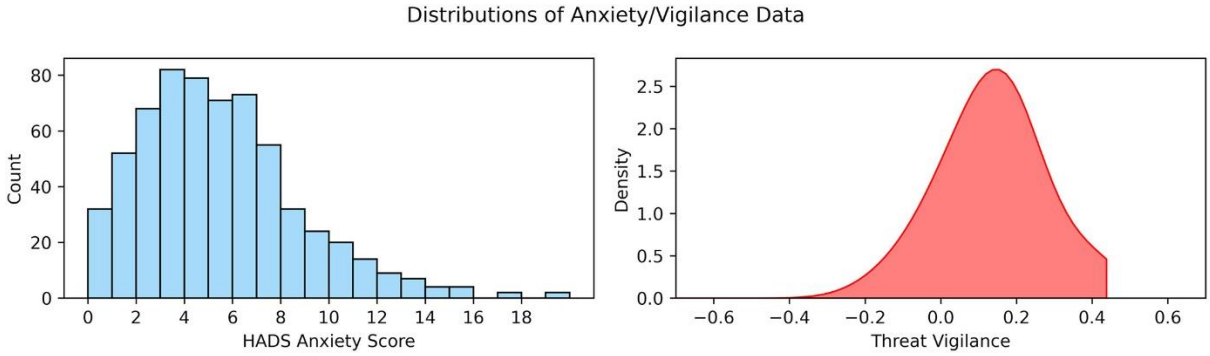

Figure 4.1. *Kernel density plots of self–reported HADS anxiety and threat vigilance measures. Due to low accuracy in the facial emotion task, 5 subjects had extremely low drift rate parameters of −3.1 (not visualized, but retained in analyses).*

Analyses

fMRI time series extraction and modeling was conducted using AFNI (Cox, 1996) and Python. Relevant functions are denoted in parentheses. All analyses used two-sided tests thresholded at α=.05 unless otherwise stated. All tests were preregistered (https://osf.io/cfdq7/). The only deviation to preregistration was repeating analyses on data following global signal regression as a robustness check (see *within subject modeling*; I report below which tests were conducted post-hoc).

Regions of Interest Masks

All masks were the same as used in chapter 3 (as such, language will largely overlap). Specifically, for hypothesis-testing, my key regions of interest were the amygdala and dorsomedial prefrontal cortex: my amygdala ROIs were selected from previously-generated anatomical parcellations of T1 images in Freesurfer (Fischl, 2012) constrained within a dilated MNI amygdala mask; my dmPFC mask was selected from a meta-analysis demonstrating the conjunction of adaptive/maladaptive anxiety ('Induced (+) vs. Transdiagnostic (+) 20mm', Chavanne & Robinson, 2021; https://neurovault.org/images/384691/; figure 4.2). The latter mask was generated based on an overlap between activation-based results contrasting unpredictable-threat vs safe conditions and clinical vs healthy subjects (pooled across two or more anxiety disorders). This was chosen so as to be atheoretical regarding the distinction between the neural manifestation of adaptive and maladaptive anxiety.

For exploratory analyses, I was also interested in the hypothalamus, bed nucleus of the stria terminalis (BNST), periaqueductal gray (PAG), medial orbitofrontal cortex/anterior ventromedial prefrontal cortex (vmPFC), subgenual anterior cingulate cortex (ACC), and anterior insula. Subcortical structures were defined anatomically: BNST and periaqueductal gray masks were based on previous manual tracings (in MNI space) of 10 and 53 subjects respectively (Theiss et al., 2017; Weis et al., 2022); and hypothalamus was defined through Freesurfer parcellations (Billot et al., 2020; all subunits combined due to the EPI voxel resolution). Cortical structures were defined functionally from meta-analytic clusters (Chavanne & Robinson, 2021; Anterior Insula = 'Induced (+) vs. Pathological anxiety (+) 20mm', anterior vmPFC = 'Induced anxiety (threat > safe) 20mm'; subgenual ACC = 'Transdiagnostic anxiety (patients > controls)'). Medial structures where lateralizations were neighboring (BNST, PAG, hypothalamus, dmPFC, anterior vmPFC, subgenual ACC) were collapsed bilaterally. Given the spatial resolution of scans and likelihood of minor misalignments, the BNST and periaqueductal gray masks were dilated (3 times) and eroded (2 times) in order to create masks which would overlap with more participants (though at the cost of potential unrelated noise/signal; see chapter 4.5 *discussion*).

Participants with failed Freesurfer segmentations (n = 10) were excluded from analyses. Combined with missing self-report/behavioral data, this left 642 participants for main-effects tests (1.53% dropout), 639 participants for tests on self-report measures (1.99% dropout), and 627 participants for tests on threat vigilance measures (3.83% dropout). I note here that 9 participants who were not included in my previous movie-watching analysis (due to issues of timeseries extraction from a canonical 400 parcel solution) were included in the present analysis.

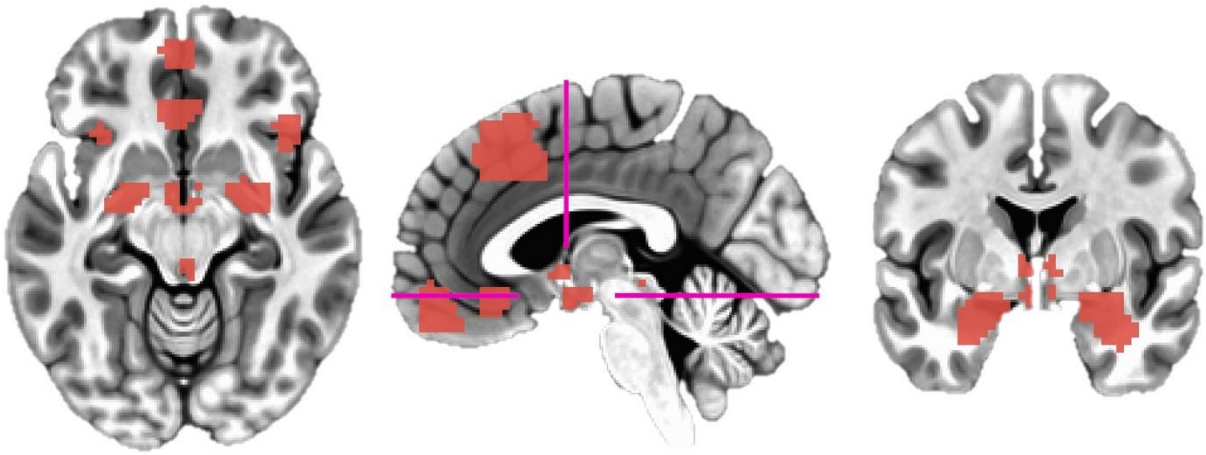

Figure 4.2. *ROI definitions. Left: axial view showing anterior vmPFC, sgACC, anterior insula, hypothalamus (80% Freesurfer overlap), amygdala (dilated MNI mask used for constraining segmentations), and periaqueductal gray masks. Middle: sagittal view showing the dmPFC mask; pink lines refer to axial and coronal slices. Right: coronal view showing BNST, hypothalamus, and dilated amygdala masks.*

Within-Subject Modeling

fMRI data. I first removed effects of no interest from my raw time series ('3dDeconvolve') by regressing out baseline signals with drift (-polort A; demeaning and detrending) and 24 motion parameters (raw + squares + temporal derivatives + derivatives squared) to produce a cleaned time series (I also highlight here that data the was previously despiked and I included motion parameters in my between-subjects modeling, see *Group-Level Results*). I then extracted ROI seeds ('3dmaskave') from the cleaned time series. For each subject I then calculated (Fisher-transformed) Pearson correlations between all ROIs to generate functional connectivity measures.

As my original analysis didn't remove global BOLD signals, this had the potential to mask anti-correlated regions (Murphy & Fox, 2017). Following planned analyses, I generated a second preprocessed dataset (post-hoc) by including a 25th, global signal regressor which was generated by taking the mean timeseries of all voxels within auto-masked volumes. For visualization purposes, I also calculated whole-brain, voxel-wise amygdala correlations on this data ('3dTcorr1D'), which I projected onto a surface using NIlearn (Abraham et al., 2014).

Group-Level Modeling

For group-level tests I first looked at average within-subject connectivity via t-tests for all pairs of ROIs. For between-subjects effects, I conducted partial Spearman correlations between anxiety measures (self-report/threat vigilance) and amygdala-dmPFC connectivity, including age, sex, and motion (mean framewise displacement) as covariates of no interest. All tests were reconducted post-hoc on data which had been preprocessed with global signal regression. I report uncorrected results and those which surpass Bonferroni correction across 45 edges ($p < .0011$).

4.4 Results

Average Connectivity

In my original, planned analysis, all regions demonstrated positive functional connections and surpassed Bonferroni correction for all 45 edges (t (641) range = 11.3:110.8, $p < .00001$)). Following global signal regression, the polarity of some of these connections was altered (figure 4.3). Applying global signal regression had no impact on the direction or significance of inference for my subsequent, statistically-corrected results.

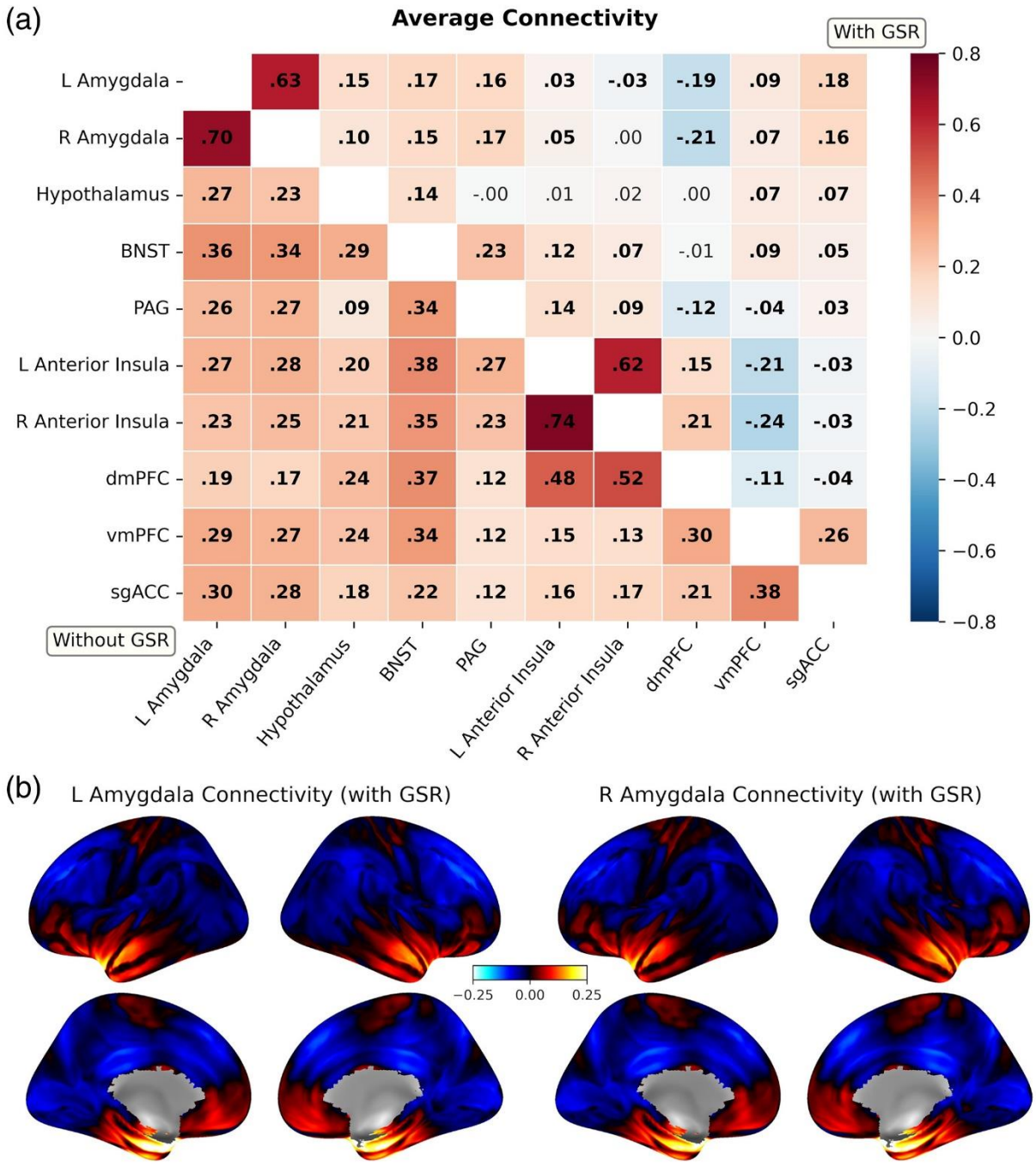

Figure 4.3. *Average correlations of timeseries between all ROIs ("functional connectivity"). (a) Heatmap of average functional connections between regions before (lower triangle) and after (upper triangle) global signal regression (GSR). Displayed are non-Fisher transformed r for visualization purposes. Bolded cells refer to connections significant at Bonferroni-corrected p < .05. (b) Average amygdala-whole brain functional connectivity (r) following global signal regression (no thresholding). BNST, Bed nucleus of the stria terminalis; PAG, periaqueductal gray; dmPFC, dorsomedial prefrontal cortex; vmPFC, (anterior) ventromedial prefrontal cortex; sgACC, subgenual anterior cingulate cortex.*

Hypothesis-Testing

To test my key hypotheses, I conducted partial Spearman correlations between anxiety measures and amygdala-dmPFC connectivity, including age, sex, and motion (mean framewise displacement) as covariates of no interest. Self-reported anxiety did not demonstrate a significant relationship with amygdala-dmPFC connectivity (left amygdala: $\rho = .004$, $p = .90$; right amygdala: $\rho = .03$, $p = .51$). Threat vigilance also did not demonstrate a significant relationship (left amygdala: $\rho = .04$, $p = .37$; right amygdala: $\rho = .06$, $p = .13$; figure 4.4).

Exploratory Tests

For planned exploratory analyses, I repeated partial Spearman correlations between anxiety measures and all pairs of ROIs, applying Bonferroni correction for all 45 edges ($p < .0011$). Self-reported anxiety was not significantly related to any of my connectivity measures. However, threat vigilance was significantly associated with increased left amygdala-periaqueductal gray functional connectivity ($\rho = .15$, $p = .0001$). I subsequently repeated my analyses post-hoc on data which had been preprocesed with global signal regression. This did not alter my inference: threat vigilance again demonstrated a significant relationship with increased left amygdala-periaqueductal gray functional connectivity ($\rho = .14$, $p = .0005$; figure 4.4).

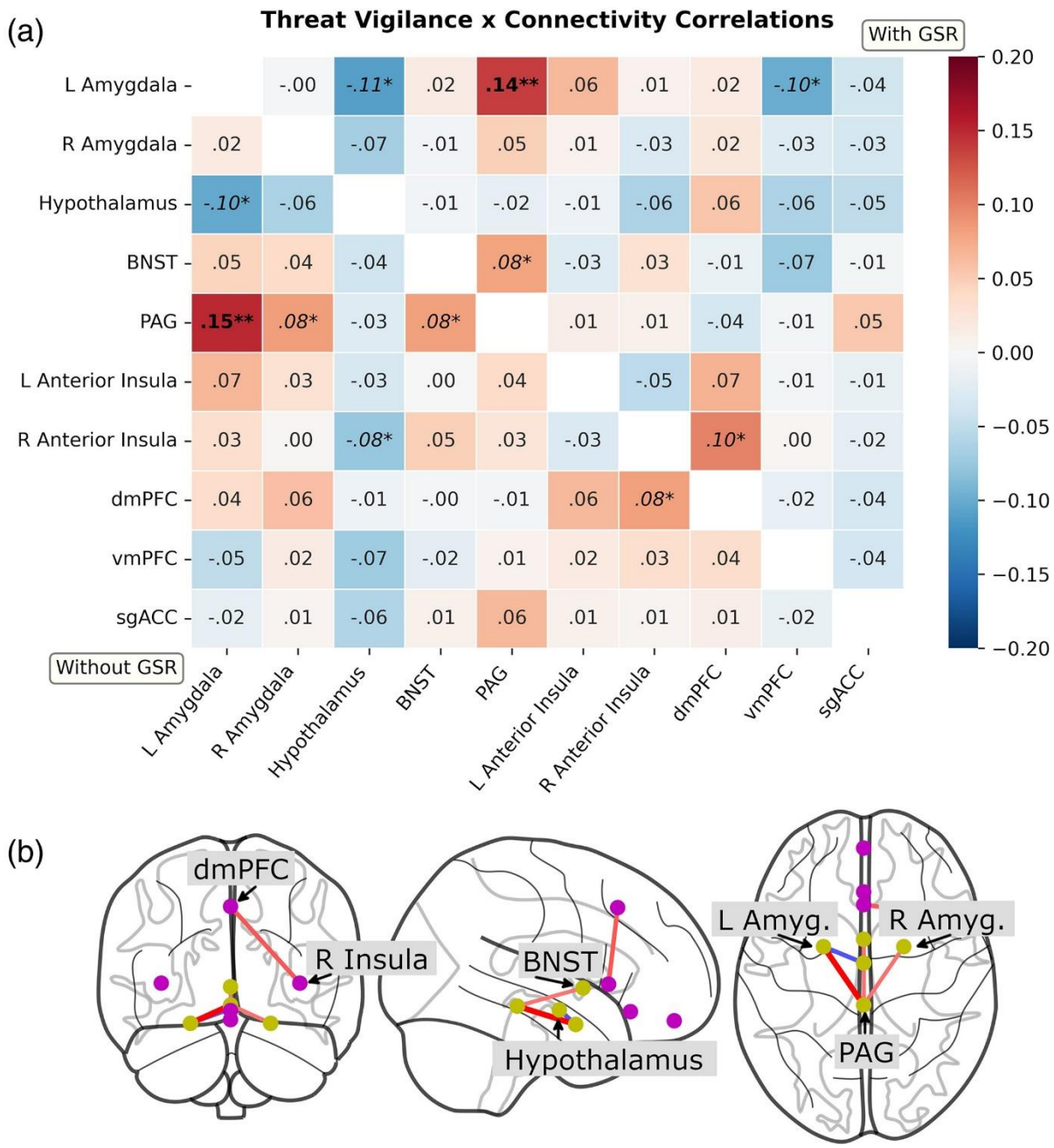

Figure 4.4. *Partial Spearman correlations between threat vigilance and functional connectivity (ρ; adjusting for age, sex, and mean motion). (a) Heatmap of correlations before (lower triangle) and after (upper triangle) global signal regression (GSR). **p <.05, Bonferroni-corrected across 45 edges; *p <.05, uncorrected. Only the left amygdala-periaqueductal gray connection survives correction regardless of GSR. (b) Glass brain plot of uncorrected correlations (p <.05 uncorrected with GSR). BNST, bed nucleus of the stria terminalis; PAG, periaqueductal gray; dmPFC, dorsomedial prefrontal cortex; vmPFC, (anterior) ventromedial prefrontal cortex; sgACC, subgenual anterior cingulate cortex.*

4.5 Discussion

In the present study I sought to test whether 'intrinsic' (seed-based, resting-state) functional connectivity of an amygdala-dorsomedial prefrontal circuit, thought to underlie attention to threat, correlated with individual differences in anxiety. I looked at both self-reported anxiety and a behavioral measure of attention to threat ('threat vigilance'), derived from the accuracy and reaction times of face perception data. Testing my key hypotheses, I did not observe a relationship between the anxiety measures and amygdala-dorsomedial prefrontal connectivity. In my planned exploratory analyses, I did however observe a correlation between threat vigilance and heighted functional connectivity between the amygdala and periaqueductal gray.

My hypothesis—that trait anxiety measures would positively correlate with intrinsic amygdala-prefrontal connectivity—was motivated by threat of shock studies (Robinson et al., 2012), the resting-state literature (which has shown mixed results, see Mizzi et al., 2022), and my previously observed results associating individual differences in anxiety and this circuitry to suspenseful dynamics during movie-watching (chapter 3). In the latter, I observed a negative correlation between anxiety-relevant alterations in amygdala-dmPFC connectivity and suspense: one interpretation of my findings was that—while low anxiety individuals selective engage this circuit in response to threat—highly anxious individuals chronically engage this circuit, irrespective of anxiogenic cues; another interpretation was that highly anxious individuals have slowed disengagement and/or greater apprehension to anxiogenic scenes. If highly anxious individuals chronically engage this circuit even in the absence of salient stimuli, I would expect differences to also be apparent during resting-state scanning.

Here, I report no significant relationship between self-report/vigilance and amygdala-dmPFC connectivity. This provides insight into how this circuitry is recruited across individuals. In line with the threat of shock literature, engagement of this circuitry may only arise in response to anxiogenic stimuli; consequently, individual differences are not observed in the absence of such perturbations (i.e., eyes-closed resting-state scanning). Even if a true effect were present in the dataset, I highlight here: a) the relatively large sample size (n = 639); b) lack of detecting these effects across two differing preprocessing pipelines c) inconsistent findings from prior resting-state studies (Mizzi et al., 2022); and d) and previously observed effects in the same

subjects with the same mask during movie-watching (chapter 3). Consequently, I argue that any potential effect—if true—is of a theoretically negligible magnitude compared to effects following induced anxiety.

After hypothesis-testing I conducted a series of planned exploratory tests on a wider 'defensive response' network which included other subcortical and cortical regions implicated in anxiety and threat vigilance (Abend et al., 2022; Grupe & Nitschke, 2013). Here, I detected a relationship between amygdala-periaqueductal gray connectivity and threat vigilance (but not self-report). Main effects tests suggest these were functionally positive connections that were heightened among those scoring high in threat vigilance. In other words, more behaviorally vigilant individuals demonstrated heightened positive amygdala-PAG connectivity These regions—and their connection—have been repeatedly implicated in top-down, anxiety-relevant regulation of autonomic functioning and fight-flight-freeze behaviors (for reviews, see Faull et al., 2019; Lefler et al., 2020).

Stimulation-based work in rodents has demonstrated populations of neurons within the periaqueductal gray which respond to threat detection and threat responsive behaviors (Deng et al., 2016; Y. Wang et al., 2021). In addition to typical evoked activation, primate research has demonstrated associations between per individual differences in anxious temperament and functional connectivity between the amygdala and periaqueductal gray (Fox et al., 2018). Human fMRI work has demonstrated an association between evoked anxiety and periaqueductal gray activation (Hur et al., 2020; Mobbs et al., 2007). One study in humans using an anxiety induction did not find interactions between functional connectivity of the periaqueductal gray and the degree of evoked anxiety or clinical diagnosis (Abend et al., 2022). Here, I provide evidence that, unlike amygdala-prefrontal connections, individual differences in amygdala-periaqueductal circuitry may be apparent at rest; specifically, this circuitry may be chronically engaged in hypervigilant individuals, even in the absence of threatening cues/anxiogenic stimuli.

Prior resting-state research contrasting clinical groups has failed to observe such associations with amygdala-periaqueductal gray connectivity (Arnold Anteraper et al., 2014). I believe this is likely related to the different latent factors measured by self-report scales and the functional processes underlying this subcortical circuitry. I

previously noted only a small association between self-report and threat vigilance measures (ρ = .13; chapter 3). The former measure (Hospital Anxiety and Depression Scale; Zigmond & Snaith, 1983) summates multiple dimensions of the symptomatology underlying anxiety disorders (e.g., worry/rumination, somatic sensations, panic attacks) which may each engage different internal processes (Baumeister et al., 2007; Watson et al., 2017). By having a self-report measure which averages across multiple latent dimensions, there is likely reduced sensitivity to detecting specific internal processes.

'Higher-order' symptoms (e.g., worry) vs more 'fundamental' processes (e.g., threat vigilance) have been traditionally discussed in the context of relying more so on cortical vs subcortical structures respectively (Paulesu et al., 2010; Somerville et al., 2010). This is one interpretation of why effects on subcortical connectivity were not apparent for the self-report measure. However, given recent evolutionary change of the human subcortex, this distinction may not be as clear as previously thought (Chin et al., 2023). Instead, these dimensions likely differentially engage cortico-subcortical circuitry (Grupe & Nitschke, 2013; Kolobaric et al., 2022). Consistent with the small correlation between self-report and behavior, it may also be that the self-report measure is not a strong indicator of behavioral responding to threat. Therefore, this connection may also be specific for behavioral responses to threat and unrelated to a person's consciously-aware feelings of anxiety (LeDoux & Pine, 2016). In line with recent calls (Moriarity et al., 2022), these findings further highlight the need for greater emphasis in anxiety research to investigate when/how measures of symptom subtypes, behavior, and brain function converge and diverge. I recommend future work to expand the present analytical framework to better identify specific phenotypic variation by assessing item-level self-report scores (which were unavailable in the present study) as well as additional self-report scales assessing different aspects of anxiety.

I have provided evidence that a behavioral index of threat vigilance may be a key process underlying chronic engagement of amygdala-periaqueductal gray circuitry. However, the use of human fMRI provides us with only one perspective for clarifying the function of this circuitry in humans. Due to the inherent associations between the BOLD signal and anxiety with autonomic functioning (Hu et al., 2016; Iacovella & Hasson, 2011), fMRI constrains the ability to draw causal conclusions as to

mechanisms underlying this circuitry. In order to further delineate the psychophysiological processes driving this circuitry in humans and why it is associated with threat vigilance (e.g., are these signals directly or indirectly related to vigilance and/or autonomic regulation?), I encourage the use of modalities that enable stronger causal inferences (albeit, see chapter 6 for my application with fMRI). For instance, regional recordings and stimulation via intracranial electroencephalography may help further tease apart how these regions contribute to the perceptual, cognitive, and autonomic processes associated with anxiety (Parvizi & Kastner, 2018).

In the present study, I took a theory-driven approach for pre-selecting regions of interest. This was done to study a variety of anxiety-relevant connections (i.e., 45 edges) while minimizing the degree of statistical correction. However, this comes with inherent inferential constraints. First, as my approach did not use a whole-brain parcellation, there may be other connections relevant to self-reported anxiety and threat vigilance which were missed by taking this approach. Therefore, I cannot infer that regions with no significant connections in the present results would be the same when using a whole-brain parcellation. Second, several of the regions were not defined on a subject-specific basis, which may reduce sensitivity to related signals. Arguably, the lack of detected effects (particularly in cortical regions) could thus have arisen due to the use of standard-space masks. In the context of my hypothesis-testing however, I do note that the dmPFC mask was sufficient for capturing anxiety-relevant processes in the same subjects during movie-watching (chapter 3). I also noted effects in subcortical regions such as the BNST and periaqueductal gray. These masks were based on previous manual tracings in MNI space (on n = 10 and n = 53 subjects respectively). As these regions are notably small, this risked missing relevant signals due to minor misalignments between subjects. I therefore dilated and eroded these masks to create better overlap between subjects. However, as these regions neighbor other small, subcortical structures, white matter, and ventricles, this procedure risked bringing in noise and unrelated signals.

Moreover, the dataset was collected using a 3 Tesla magnet with relatively large voxels (3 x 3 x 4.44mm) which—compared to 7 Tesla scanning—may not be as spatially precise for the small, subcortical regions defined in the present study (Huggins et al., 2021). Lastly, although I describe an absence of stimuli during scanning, I highlight that resting-state scanning may not be a passive state. Rest can

be considered a task in and of itself, with different effects across populations, and thus may not offer a completely neutral backdrop for studying intrinsic connections (Finn, 2021). Understanding this, my inferences regarding these structures are of course tentative and research using more refined spatial resolutions, other neuroimaging modalities, and subject-specific definitions is needed.

Conclusion

The present study aimed to investigate whether an association was present between individual differences in anxiety and amygdala-prefrontal connectivity whilst at rest; I did not observe such a relationship. I suggest this circuitry may only be engaged in response to anxiogenic stimuli; and thus individual differences only emerge under such conditions. On the other hand, I noted a relationship between a behavioral measure of attentional bias to threat ('threat vigilance') and amygdala-periaqueductal connectivity. Much of the prior literature has discussed the role of this subcortical circuitry in responding to threatening cues. Here, I provide evidence that this may be chronically engaged, irrespective of anxiogenic features of the environment. Moreover, why this was observed for the threat vigilance measure, but not self-report, I argue is due to the function role of this circuitry in more fundamental processes related to threat vigilance. Future research using higher magnet strengths and other imaging modalities will likely prove fruitful for elucidating precise contributions of this subcortical circuitry to anxiety-relevant processes.

5. RapidHRV: An Open-Source Toolbox for Extracting Heart Rate and Heart Rate Variability

5.0 Preface

Anxiety is a multi-faceted construct engaging numerous perceptual, cognitive, and physiological processes ranging from threat vigilance to worry to autonomic responding. In chapters 2-4, I explored the extent to which trait anxiety was associated with amygdala-dmPFC responding during movie-watching. Here, we found evidence that there are associations, but this likely fluctuates as a function of suspense. Whether these dynamics reflect altered subjective experiences and engagement of peripheral physiological systems remains unanswered. As such, I sought to test the extent to which trait anxiety also shapes peripheral physiological responding, as well as subjective experiences of state anxiety, during movie-watching. Before conducting such a study, in this chapter I developed an analytical pipeline to be used for deriving subsequent measures of autonomic balance (i.e., heart rate).

For the published version of this software, please refer to the below references. Note: for brevity, I have included a significantly compressed version of this report so as to be adapted toward the primary aims of the thesis.

Kirk, P. A., Bryan, A. D., Garfinkel, S. N., & Robinson, O. J. (2022). RapidHRV: An open-source toolbox for extracting heart rate and heart rate variability. *PeerJ*, *10*, e13147. https://doi.org/10.7717/peerj.13147

5.1 Abstract

Heart rate and heart rate variability have enabled insight into a myriad of psychophysiological phenomena. There is now an influx of research attempting using these metrics within both laboratory settings (typically derived through electrocardiography or pulse oximetry) and ecologically-rich contexts (via wearable photoplethysmography, i.e., smartwatches). However, these signals can be prone to artifacts and a low signal to noise ratio, which traditionally are detected and removed through visual inspection. Here, I developed an open-source Python package, RapidHRV, dedicated to the preprocessing, analysis, and visualization of heart rate and heart rate variability. Each of these modules can be executed with one line of code and includes automated cleaning. In simulated data, RapidHRV demonstrated excellent recovery of heart rate across most levels of noise (>=10 dB) and moderate-to-excellent recovery of heart rate variability even at relatively low signal to noise ratios (>=20 dB) and sampling rates (>=20 Hz). Validation in real datasets shows good-to-excellent recovery of heart rate and heart rate variability in a range of recording modalities, dependent on the degree of artifacts such as motion.

5.2 Introduction

Evidence has outlined a link between heart rate, heart rate variability, and health-related risks, including mental illnesses (Hillebrand et al., 2013; Jandackova et al., 2016; Makovac, Meeten, Watson, Garfinkel, et al., 2016; Pham et al., 2021)(Hillebrand et al., 2013; Jandackova et al., 2016; Makovac et al., 2016a; Pham et al., 2021). Consequently, there is now an influx of research looking into whether these measures can be derived in naturalistic settings to track clinically-relevant outcomes, namely through wearable devices (Georgiou et al., 2018; Mulcahy et al., 2019). However, a key issue when opting to use the measures in naturalistic settings are the low signal to noise ratios (e.g., photoplethysmography (PPG), a typical measure for cardiac monitoring in wrist wearables, Caizzone et al., 2017). Moreover, heart rate variability measures generally require relatively longer windows for extraction compared to heart rate (Baek et al., 2015). Thus, significant noise poses a problem for out-of-laboratory applications, as point estimates can contain large errors from technological limitations and motion artifacts within windows of extraction. In experimental settings, noise has often been dealt with through visual inspection of data (Makovac, Watson, Meeten, Garfinkel, et al., 2016; Rae et al., 2020); but when approaching time courses in relatively larger-scale samples, manual outlier detection is not a pragmatic solution.

Whilst some open-source packages are already available for the analysis of heart rate and heart rate variability, these are typically modality-specific, and not targeted at wrist-worn measures (e.g., pyVHR for video-based estimation, Boccignone et al., 2020). Some modality-general packages do exist, but these often still require manual visual inspection and/or can require custom scripting on the users end for tailoring to e.g., noisy PPG measures (‘Analysing_Smartwatch_Data’ in HeartPy, van Gent et al., 2019; NeuroKit2, Makowski et al., 2021). As such, these are often less suited for dealing with datasets collected across large time frames. Consequently, I set out to develop a simple yet flexible toolbox for the extraction of time-domain heart rate and heart rate variability measures with automated artifact rejection applicable across recording modalities, including wrist-worn PPG. Here, I present the development and validation of an open-source Python package, ‘RapidHRV’.

5.3 Pipeline

RapidHRV was developed in Python (V 3.7.9). RapidHRV source code and tutorials are available to download through PyPi (https://pypi.org/project/rapidhrv/) and GitHub (https://github.com/peterakirk/RapidHRV). Below I provide an overview of RapidHRVs preprocessing, analysis (figure 5.1), and visualization. Each of the three modules only requires one function (one line of code) to run, for which I have embedded examples at the end of the relevant sections below.

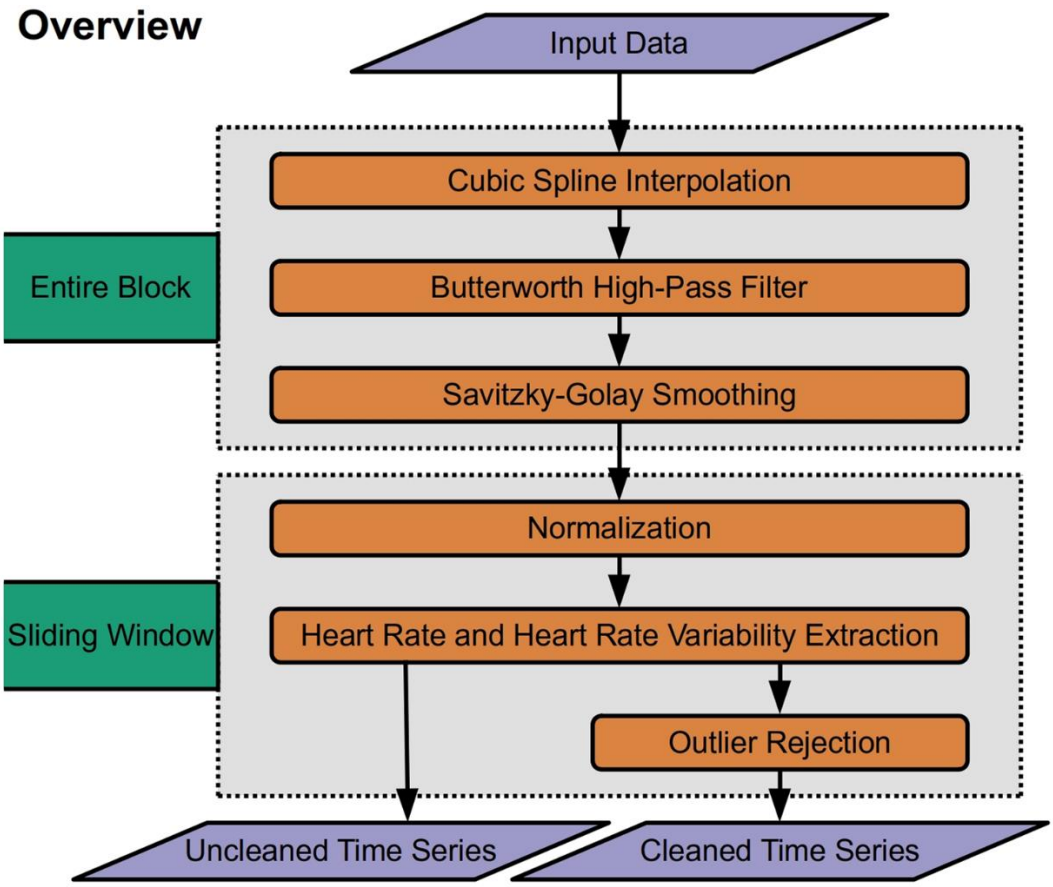

Figure 5.1. *Overview of RapidHRV pipeline. Across an entire block, the pipeline initially processes data with high-pass filtering, upsampling, and smoothing. RapidHRV then applies a sliding window across the entire block. Within each window, the data are scaled. Heart rate (beats per minute) and heart rate variability (root mean squared of successive differences + standard deviation of intervals) are calculated for each window, and data is submitted to outlier rejection. RapidHRV produces both a cleaned and uncleaned time series of heart rate and heart rate variability.*

Preprocessing

First, data is upsampled with cubic spline interpolation (3rd order polynomial; default = 1kHz) to increase temporal accuracy of peak detection. To mitigate potential long-term drifts in the signal, the pipeline then applies a high pass butterworth filter (0.5Hz) across the input data. Finally smoothing with a Savitzky-Golay filter (3rd order polynomial; default = 100ms) is applied to reduce spiking (sharp increases in the signal caused by artifacts such as motion) whilst retaining temporal precision.

Extracting Heart Rate and Heart Rate Variability

Following preprocessing, the pipeline scales the data (between 0 and 100) and runs peak detection on every window (default width = 10s; for a methodological discussion and prior validation of using ultra-short, 10s windows in heart rate variability estimation, see Munoz et al., 2015). This outputs peaks and their properties (e.g., heights, amplitudes, width; SciPy 'find_peaks', Virtanen et al., 2020). As RapidHRV uses fixed movements for the sliding window, a window can start or end at any point during the cardiac signal. This can occasionally result in underestimation of the first/last peak's amplitude as the baseline value may—for example—be set during the P wave. Therefore, RapidHRV recalculates amplitudes of the first/last peaks using baseline imputation from the neighboring peaks.

For extracting beats per minute (BPM) the number of peaks, k - 1, is multiplied by 60 (seconds), and divided by the difference in time between the first and last peaks, i and j:

$$\mathrm{BPM} = ((k-1) * 60)/(j-i)$$

The root mean square of successive differences is calculated by obtaining: (1) the interbeat interval, IBI_i, between neighboring peaks; (2) the successive differences in interbeat intervals, IBI_i - IBI_{i+1}; (3) the square of differences; (4) the mean of squared differences (dividing by the number peaks, N, - 1); and (5) the root of the mean square of successive differences (RMSSD):

$$\mathrm{RMSSD} = \sqrt[2]{\frac{\Sigma_{i=1}^{N-1}(\mathrm{IBI}_i - \mathrm{IBI}_{i+1})^2}{N-1}}$$

BPM and RMSSD were selected as the primary measures as they appear to be the most stable metrics when derived from ulta-short recordings (Baek et al., 2015). RapidHRV also supplements these measures with the standard deviation of N-N intervals (SDNN), standard deviation of successive differences (SDSD), proportion of successive differences greater than 20ms (PNN20), proportion of successive differences greater than 50ms (PNN50), and high-frequency power (HF; note: as this requires more data points than time-domain analyses NaN is returned if there is insufficient data).

Outlier Detection

The last phase of the pipeline is to pass measures derived from peak extraction to outlier rejection (figure 5.2). This is applied at the level of the sliding window. If a window is declared an outlier, heart rate and heart rate variability measures are removed from the cleaned time series. By default, RapidHRV returns both the cleaned and the uncleaned time series. In addition to default parameters listed below, the package has optional arguments embedded to allow users to override these presets. Given that not all users may be entirely comfortable manually adjusting these, RapidHRV additionally contains semantically-labeled arguments as inputs for outlier constraints (‘liberal’, ‘moderate’ [default], and ‘conservative’; corresponding parameters are parenthesized under Biological Constraints and Statistical Constraints).

Biological Constraints

RapidHRV first applies restrictions to exclude data that are highly unlikely given known physiology:

1. Screening for sufficient peaks in a window (default: number of peaks > (window width / 5) + 2), floored at 3; default at 10s = 3 peaks). This is primarily for computational applicability and efficacy, screening data prior to further processing. The minimum number of peaks required to enable calculation of RMSSD is 3. As such, this is also applied to the uncleaned time series.

2. Minimal and maximal heart rate (‘moderate’ [default]: 30 > BPM > 190; ‘liberal’: 20 > BPM > 200; ‘conservative’: 40 BPM > 180). These boundaries were based on typical heart rate at rest and during exercise in the healthy population (Pierpont & Voth, 2004; Sandvik et al., 1995; Savonen et al., 2006).
3. Minimal and maximal heart rate variability (‘moderate’ [default]: 5 > RMSSD > 262; ‘liberal’: 0 > RMSSD > 300; ‘conservative’: 10 > RMSSD > 200). Default arguments correspond to the minimum/maximum 2nd/98th percentiles of resting RMSSD across ages 16-89 years (van den Berg et al., 2018).

Statistical Constraints

RapidHRV next applies statistical constraints to account for noisy data that may otherwise appear to provide measures within the range of known physiology:

1. Median absolute deviation (MAD) of peak heights (distance from minimum value of signal in window; i.e., 0) and prominence (amplitude from baseline height; ‘moderate’ [default] = 5 MAD units; ‘liberal’ = 7; ’conservative’ = 4). Unlike Z-scoring, this quantifies each peak’s height and prominence in a given window in terms of its deviation from the median value in the same window (for a discussion of median absolute deviation see Leys et al., 2013). Applying these constraints to height and prominence helps exclude windows with noise-driven inaccuracies in peak detection.
2. Median absolute deviation of interbeat intervals (‘moderate’ [default] = 5 MAD units; ‘liberal’ = 7; ’conservative’ = 4). This was also implemented to account for inaccuracies in peak detection, either where spiking may cause detection of an irrelevant peak shortly after e.g., an R wave, or low signal to noise ratio may result in missing relevant peaks.
3. Time from the first peak to the last peak does not recede 50% of the fixed window width. This is to ensure the user that the actual length of time for extracting HR/HRV is not less than half of that which is specified in the window width argument. Given debates surrounding adequacy of different window lengths for HRV extraction (Baek et al., 2015; Munoz et al., 2015), this was implemented primarily as a theoretical constraint (rather than for just cleaning

per se) to ensure the user is not provided data that deviated significantly from their specified window.

4. Analysis can be executed with one line of code, which returns a pandas DataFrame (McKinney, 2012; The pandas development team, 2023) containing the analyzed data.

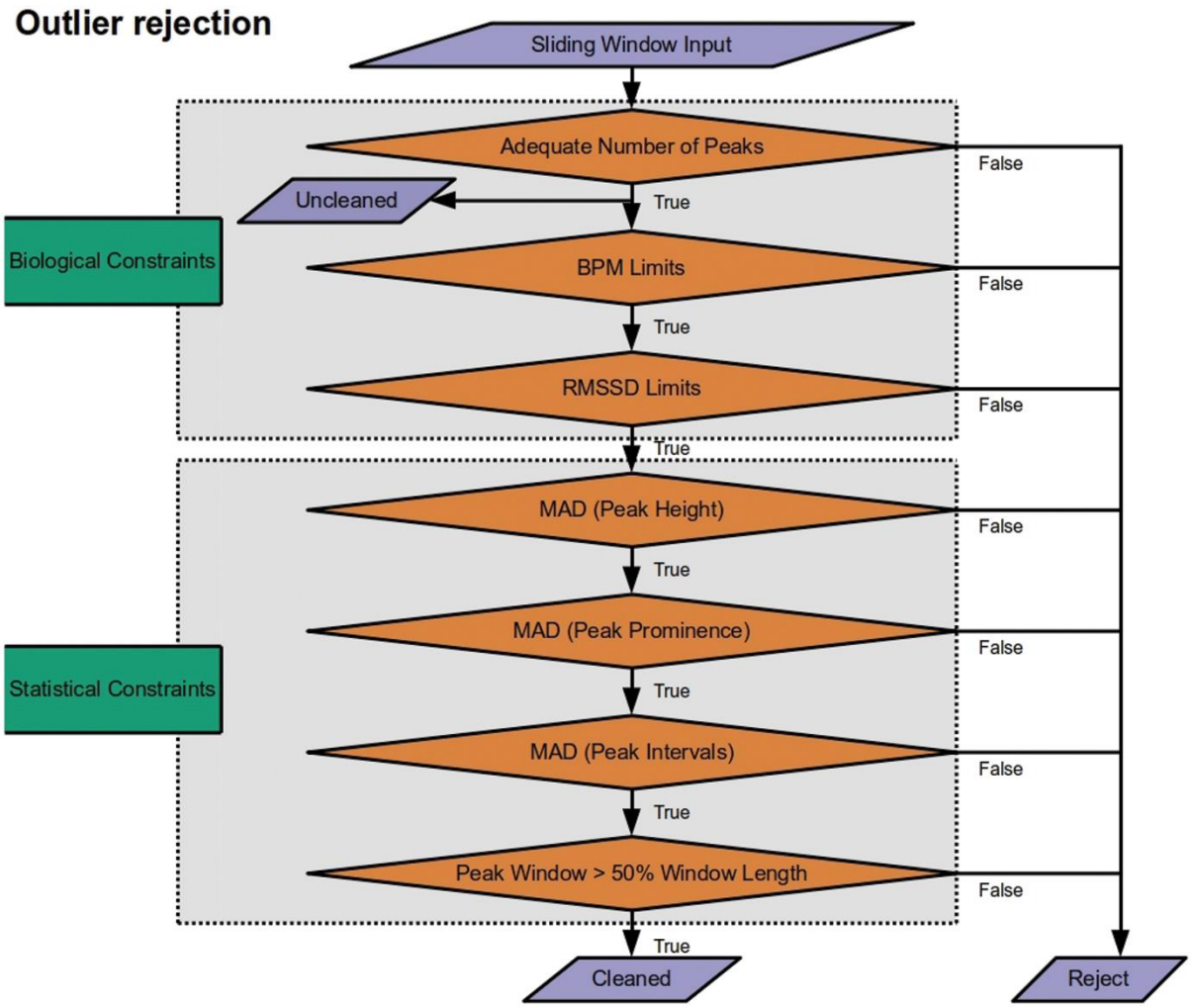

Figure 5.2. *The only outlier rejection method applied to the uncleaned time series is screening for a sufficient number of peaks to derive metrics. The cleaned time series then goes through a battery of biological constraints (thresholding minimum/maximum beats per minute (BPM) and root mean square of successive differences (RMSSD)) and statistical constraints (median absolute deviation (MAD) of peak heights, prominences, and intervals; ensuring adequate duration from first to last peaks).*

Visualization

To allow for selected manual inspection, I have also implemented optional interactive visualizations via matplotlib (Hunter, 2007) which allow the user to plot the time course of heart rate and heart rate variability. The user can then select and view specific data points to see the window of extraction.

5.4 Validation Methods

Datasets

To validate the above pipeline I subjected it to a series of tests across both simulated and real data (table 5.1). I first started by testing RapidHRV's estimations in two sets of simulated data (PPGSynth; Tang et al., 2020). Next, I ran validation in real data across successively noisier modalities: electrocardiography (ECG), finger photoplethysmography (PPG), and wrist PPG data. Database information and code generated in validation tests are available through the open science framework (https://osf.io/7zvn9/). For full details of simulation parameters and datasets, please refer to the published report (Kirk et al., 2022). For purposes of the present report, I only report validation in two of the datasets (1. Simulations across sampling rates and noise; and 4. Data collected from finger PPG recordings; Table 5.1).

Table 5.1. *Simulated and real datasets used for validation of RapidHRV.*

Dataset	Modality	N	Conditions	Duration	Hz
1. Simulation	PPGSynth	273	None (noise)	5 min	20–250 Hz
2. Simulation	PPGSynth	172 (10 repeats)	'Anxiety'	5 min	20–250 Hz
3. (Iyengar et al., 1996)	ECG	40	Movie	~2 h	250 Hz
4. (de Groot et al., 2020)	Finger IR PPG	39	Anxiety (sitting)	2 × 28 min	1,000 Hz
5. (Reiss et al., 2019)	Wrist PPG	15	Varying activities	~2.5 h	64 Hz

Analyses

Unless otherwise stated, all analyses were conducted using RapidHRV's default arguments: window width = 10s; window movement = 10s; outlier method = 'moderate' (peak/height median absolute deviation = 5, interbeat interval median absolute deviation = 5, BPM range = 30-190, RMSSD range = 5-262); minimum window successful extraction = 5s, minimum amplitude for peak detection = 50, minimum distance between peaks = 250ms; for ECG data, ecg_prt_clustering = True). To assess performance across datasets, I used: visualizations; intraclass correlation coefficients (ICC; two-way mixed effects, absolute agreement, single measure); root-mean-square-error (RMSE); and sensitivity to experimental effects (Cohen's *d*). For ICC values, I used the following semantic labels for interpretation: ICC < .5 as 'poor', .5 < ICC < .75 as 'moderate'; .75 < ICC < .90 as 'good', and .90 < ICC as 'excellent' (Koo & Li, 2016).

5.5 Validation Results

Parameter Recovery in Simulated PPG

I first took the pipeline forward to validation using simulated photoplethysmography (PPG) data from PPGSynth (Tang et al., 2020) in MATLAB. This allowed me to test how well RapidHRV recovered known parameters under specified conditions, such as sampling rate and noise. RapidHRV was able to accurately recover heart rate across most sampling frequencies and noise in my initial simulations. Accurate detection of BPM primarily started to degrade when signal to noise ratios were less than 10dB (table 5.2). RapidHRV cleaning provided improvements in simulations with a signal to noise ratio of 10dB.

Table 5.2. *Intraclass correlations between simulated BPM and RapidHRV for uncleaned (and cleaned) data as a function of sampling rate and noise.*

Sampling rate	0.01 dB	10 dB	20 dB
20 Hz	0.06 (*0.18*)	0.37 (*0.80*)	0.98 (*1.0*)
50 Hz	0.18 (*0.13*)	0.80 (*0.96*)	1.0 (*1.0*)
100 Hz	0.33 (*0.26*)	0.96 (*1.0*)	1.0 (*1.0*)
250 Hz	0.62 (*0.7*)	1.0 (*1.0*)	1.0 (*1.0*)

Performance in recovery of heart rate variability was again primarily based on signal to noise ratio. At 20dB RapidHRV recovery of RMSSD was good-to-excellent for higher sampling rates (>=100Hz), whereas lower sampling rates (<100Hz) required slightly lower levels of noise (>30dB) for excellent recovery. RapidHRV cleaning provided clear improvements when signal to noise ratio was below 30dB (figure 5.3).

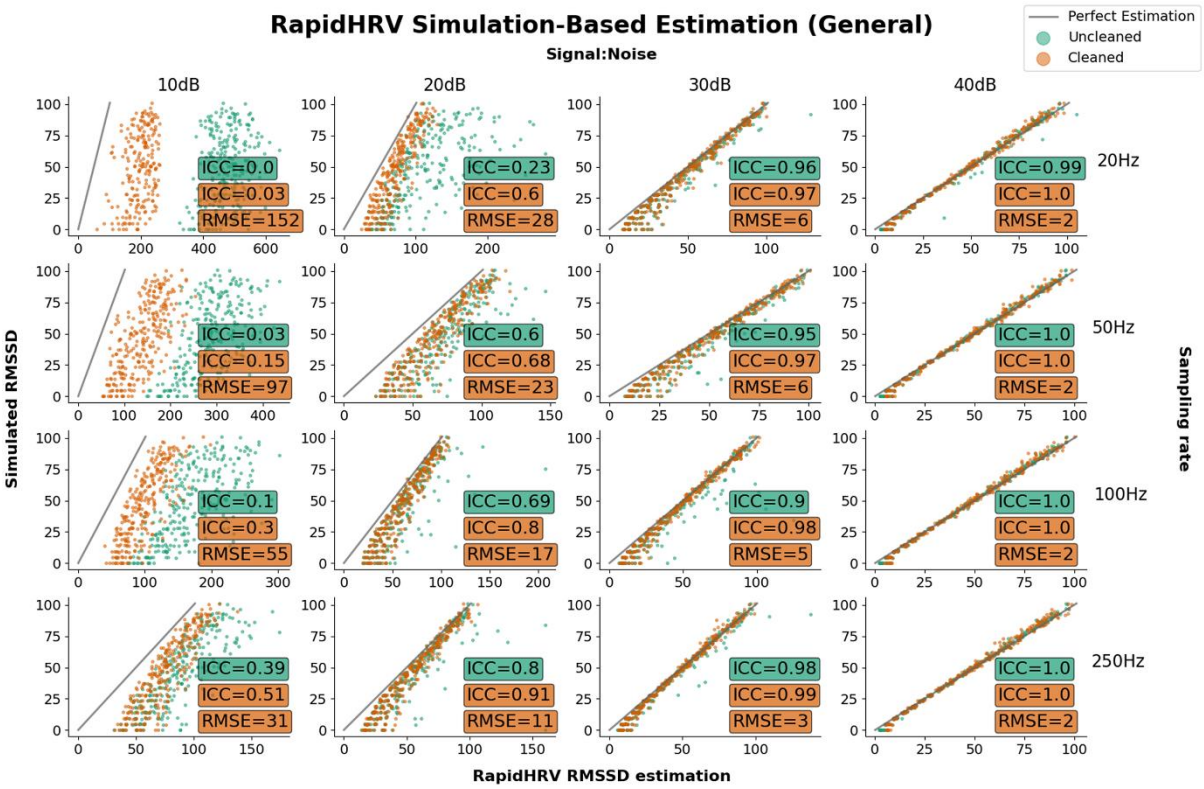

Figure 5.3. *Parameter recovery of simulated PPG data as a function of heart rate variability (Intraclass Correlations (ICC) and Root Mean Square Error (RMSE) against ground truth). Y axes reflect the true RMSSD in the data, whilst the X axes reflect RapidHRV's estimation. For readability, data is only plotted in a key range of performance (subplots: 10–40 dB noise left-to-right; 20–250 Hz top-to-bottom).*

Estimation via Finger PPG

I next took the pipeline forward to validation in finger PPG data. Here, I used a dataset of 39 subjects (Age: Mean = 22.67; Range = 18–38 years; demographics reported prior to n = 1 exclusion) watching 2 × 28 min blocks of documentary and horror video clips undergoing finger PPG recording (1,000 Hz, de Groot et al., 2020). This allowed

me to contrast psychological conditions of the experiment, testing whether RapidHRV was able to detect effects of anxiety. Moreover, in the original study, data had been preprocessed and analyzed using a commercially available software (Labchart; ADInstruments, Sydney, Australia; analyzed using built-in 'Peak Analysis' module). This allowed me to benchmark RapidHRV against another software. The full dataset and description is available via the Open Science Framework (https://osf.io/y76p2/).

Sensitivity to Anxiety

In the finger PPG data, RapidHRV was able to capture previously reported (de Groot et al., 2020) effects of anxiety on BPM (table 5.3). RapidHRV additionally demonstrated an influence of anxiety on RMSSD. Effects on BPM were greater following cleaning, whereas detection of effects on RMSSD was entirely dependent on cleaning.

Table 5.3. *Effect size (Cohen's d) of heart rate and variability between conditions as a function of software and cleaning method.*

Software	BPM effect (*d*)	RMSSD effect (*d*)
Labchart	0.52	0.19
RapidHRV (Uncleaned)	0.45	−0.04
RapidHRV (Cleaned)	0.54	0.35

Benchmarking

Overall, there was excellent agreement between RapidHRV and previous estimates (de Groot et al., 2020; implemented using LabChart, ADInstruments, Sydney, Australia) of BPM (ICC > .99; figure 5.4). For heart rate variability, there was good agreement between the two when using the cleaned time series (ICC = .89), but poor agreement when using the uncleaned time series (ICC = .32).

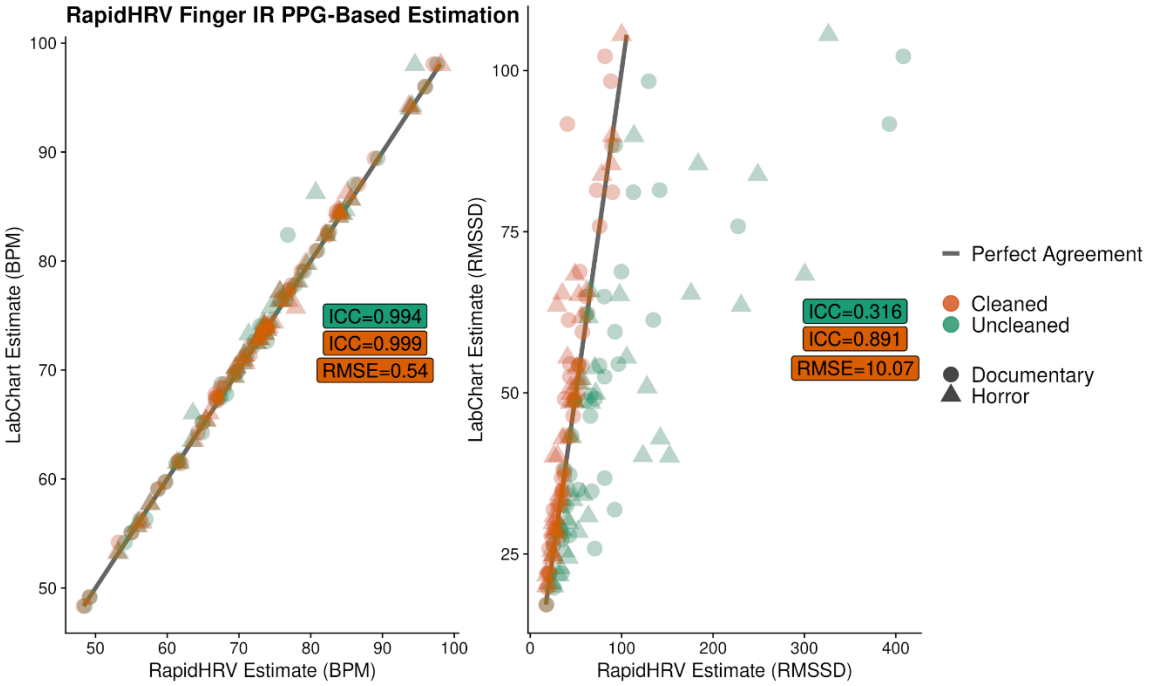

Figure 5.4. *Agreement between RapidHRV and a previous Labchart analysis (de Groot, Kirk & Gottfried, 2020) of heart rate and variability in a finger PPG dataset (n = 39).*

5.6 Discussion

RapidHRV is an open-source toolbox for extracting heart rate and heart rate variability measures. RapidHRV was developed in response to the need for software dedicated to dealing with extensive cardiac data collected across large time frames, such as out-of-laboratory PPG recordings, which may require point estimates from very short time windows (~10 seconds). Python packages currently exist which can analyze cardiac data (e.g., Systole, Legrand & Allen, 2022; NeuroKit2, Makowski et al., 2021; pyHRV, Gomes et al., 2019). However, outlier rejection algorithms often require visual inspection and/or extensive scripting on the user's end. While suitable for the cardiac data collected during laboratory experiments, this may not be feasible when dealing with data collected across large time-scales, such as weeks or months. Here, I have attempted to fill this gap by developing a programmatically easy-to-use toolbox which extracts HRV measures from ultra-short windows and automates artifact detection and rejection. In general, this is applied via a series of biological and statistical constraints. Moreover, for ECG data, I have also implemented a k-means clustering algorithm for delineating P, R, and T waves. Across simulated and real datasets, I scrutinized RapidHRV, testing scenarios where it was and was not able to extract meaningful metrics. I show that signal to noise ratio, sampling rate, and recording modality had a clear impact on sensitivity of estimation. Here, I summarize these validation tests and make modality-specific recommendations for users.

Simulations

Within simulated data, RapidHRV was able to recover heart rate across most levels of noise (white gaussian noise filter >=10dB), even at relatively low sampling rates (>=20Hz). RapidHRV's recovery of heart rate variability was excellent at relatively low levels of signal to noise ratio (>=20dB), though there was degradation of performance as sampling rate decreased. Additional simulations of cardiac responses to an anxiety induction demonstrated RapidHRV estimations fully captured effects at moderate levels of noise (>=30dB) even at relatively low sampling rates (i.e., 20Hz). RapidHRV was able to partially capture effects (~50% reduction in effect size) at very high levels of noise (>=10dB when Hz >50). Simulations revealed RapidHRV cleaning was particularly beneficial at lower sampling rates and higher levels of noise, but was not

necessary (or could be relaxed) when signal and sampling rates were high. Moreover, these simulations were able to clarify the validity of RapidHRV's default window (10s) for estimation of heart rate variability across a longer time period (i.e., 5 minutes).

Finger PPG

Using RapidHRV-estimates, I noted effects of anxiety on heart rate and heart rate variability in a database of participants watching horror and documentary videos while undergoing finger infrared PPG recordings. Notably, the estimated effect size was analogous to that noted in threat of shock studies (Gold et al., 2015). Moreover, when contrasting subject-specific estimates, I found good-to-excellent agreement between RapidHRV and a previous analysis using a commercially available software. Effect sizes between conditions and convergence of estimates between softwares was significantly improved following RapidHRV cleaning.

Conclusion

In the present paper, I have outlined RapidHRV: an open-source Python pipeline for the estimation of heart rate and heart rate variability. Across simulated datasets, RapidHRV showed good-to-excellent recovery of heart rate and heart rate variability at relatively high levels of noise in simulation data. Estimates in finger PPG demonstrated RapidHRV was able to recapitulate known effects anxiety, and showed excellent agreement with visually-inspected analyses and commercial software. Given the increased interest in the use of wearable measures of heart rate metrics and how they relate to other domains such as mental health, I hope that this toolbox will be of wide use to the community, and that the simulation and benchmarking tests provided may help inform the design and analysis of such studies.

6. Anxiety and Subjective-Autonomic Responding to a Suspenseful Movie Clip

6.0 Preface

In chapter 2-4, I demonstrated associations between trait anxiety and amaygdala-dmPFC responding during movie-watching. In this chapter, I sought to test whether these responses may be associated with activation within the autonomic nervous system. Before doing so, I developed an analytical pipeline (RapidHRV) in chapter 5 to extract heart rate measures as a proxy for autonomic balance. In chapter 6, I use RapidHRV to test the extent to which trait anxiety also shapes heart rate, as well as subjective experiences of state anxiety, during the same suspenseful video clip used in chapter 3.

For a pre-printed version of this study, please refer to the below reference:

Kirk, P. A., Foret-Bruno, P., Lowther, M., Garfinkel, S., Skipper, J. I., & Robinson, O. J. (2023). *Anxiety symptomatology and subjective-cardiac responding to a suspenseful movie clip*. PsyArXiv. https://doi.org/10.31234/osf.io/dqhnx

6.1 Abstract

There are known associations between the presence of experimentally-presented threats and peripheral physiological responses, such as a heart rate, which holds implications for understanding pathological levels of anxiety. Moving away from traditional task-based paradigms, I investigated whether subjective experience (i.e., continuous state anxiety ratings) and heart rate were associated with individual variations (n = 133) in trait anxiety symptomatology (items on the hospital anxiety and depression scale) during an anxiogenic movie clip. In other words, do different trait anxiety-related processes manifest in altered anxiety states vs heart rate during movies? Using intersubject representational similarity analysis, I report a significant association between pairwise similarities in trait anxiety symptom profiles and continuous state anxiety ratings during suspenseful movie-watching. On the other hand, I failed to detect associations between trait anxiety symptoms and heart rate. This suggested that trait anxiety symptoms are associated with differences in subjective experiences of anxiety during suspenseful movies, but are potentially not associated with autonomic responses.

6.2 Introduction

It has long been theorized that—when faced with potential dangers—peripheral physiology is engaged to facilitate defensive behaviors in response to threat, namely fighting or fleeing (Cannon, 1929). One significant mechanism which gives rise to these responses is the autonomic nervous system. This includes two branches, sympathetic and parasympathetic, which are differentially engaged based on homeostatic demand (including, but not limited to, defensive responding; (Morrison, 2001). When posed with a direct threat, humans typically alter activity within and across sympathetic/parasympathetic branches Friedman & Thayer, 1998). Effects of this include increased respiration, perspiration, pupil dilation, and altered cardiac activity (e.g., increased heart rate; McCorry, 2007). Many of these responses may confer benefits to an individual when encountering threat; for example, increased heart rate provides oxygen-rich blood to support musculature when fighting and fleeing (Critchley, 2009; McCorry, 2007).

A large body of literature has detailed which physiological responses may relate to state anxiety, conceptualized as an emotion evoked by uncertain threat, and trait anxiety, one's predisposition to anxious states. One of the most studied aspects of physiology in this context is cardiac reactivity. When inducing state anxiety through personally-direct threat, such as exposing participants to unpredictable shocks, increases in heart rate are often observed (Petry & Desiderato, 1978). Moreover, cross-sectional comparisons reveal trait anxiety to be associated with greater increases in heart rate to personally-directed threat (Somerville et al., 2010; albeit, effects are not always consistent, see Qiao et al., 2022). Consequently, how physiological measures, such as heart rate, may correlate with pathological levels of trait anxiety have come under investigation. Following induced state anxiety, pathologically high trait anxiety appears to be associated with relatively greater increases in heart rate (Pittig et al., 2013). However, such effects may not be apparent for all types of anxiety, such as social anxiety disorders (Pittig et al., 2013). This implicates a key role of symptomatology (the types of symptoms experienced; e.g., vigilance vs worry vs somatic sensations) in the relationship between trait anxiety and autonomic activity. This also speaks to a larger issue of overlooking subsets of anxiety-related mechanisms and differences in the types of conscious experience of anxiety.

Despite physiological responses being associated with the presence of threats or clinical diagnosis, the relationship to subjective experience in this relationship is often neglected. The mapping of biological measures onto self-reported experiences of anxiety can be, and is often, weak (Boeke et al., 2020; Marek et al., 2022; Rosenberg & Finn, 2022). If the eventual goal of this research is to aid individuals experiencing pathological levels of anxiety, there are clear needs to distinguish threat responding (which includes autonomic responses) from consciously experienced anxiety (LeDoux & Pine, 2016; Taschereau-Dumouchel et al., 2022). This discrepancy is often not directly addressed in studies. Moreover, even if such measures are included, our knowledge remains constrained to the experimental environment of these paradigms. There is clear evidence that, when an explicit, direct threat is presented in a laboratory, there are increases in heart rate, and this might vary between individuals based on their symptomatology. However, the extent to which anxiety symptomatology may be associated with psychological/physiological responding outside of constrained experimental contexts remains unknown.

One approach to move anxiety research toward more ecological paradigms is through the use of movies, which may allow us to probe the biology of anxiety in a manner more naturalistically representative than that of traditional task-based paradigms (Eickhoff et al., 2020; albeit, the extent of this ecological validity is debatable, see Grall & Finn, 2022). Additionally, it offers several analytical benefits. By having stimulus presentation time-locked between participants (participants are viewing the same videos, so time series are comparable), we can apply techniques such as intersubject representational similarity analysis (P.-H. A. Chen et al., 2020; Finn et al., 2020; Kriegeskorte et al., 2008). Like intersubject correlation, this computes pairwise similarities in time courses between participants. However, it also allows us to study individual differences by comparing how intersubject similarities in responding to movies maps onto similarities in trait anxiety. This technique has often been applied to neuroimaging data. To my knowledge, no work has used this technique in the study of individual differences in anxiety and autonomic/subjective responses (one recent study has investigated cardiac responses in the context of mentalizing, see R. Wang et al., 2022).

Using intersubject representational similarity analysis, I have previously demonstrated movie-watching's utility for understanding the neurobiology of anxiety,

reporting individual differences in trait anxiety to be associated with threat circuitry dynamics during suspenseful movie-watching (chapter 3). However—while I was able to demonstrate that suspenseful movie-watching elicits anxiety-relevant idiosyncrasies in neural circuitry—the extent to which trait anxiety is manifested in altered: (1) self-reported state anxiety; and (2) autonomic responding remains unclear. In the present study, I collected data on participants watching the same suspenseful movie clip that has been used in multiple prior neuroimaging datasets. However, less work has linked this data to behavioral/physiological responses, especially in regard to individual differences. As participants watched this same suspenseful stimulus, I took an intersubject representational similarity approach to test the extent to which anxiety symptomatology was associated with both ongoing state anxiety and cardiac responses during suspenseful movie-watching.

Hypotheses

In the present preregistered study (https://osf.io/w2duj/), I compared between-subjects, pairwise similarities in anxiety symptom profiles (comparisons of item-wise scores on an anxiety questionnaire) with pairwise similarities in psychophysiological responding. I predicted that pairwise similarities in anxiety symptom profiles would positively correlate with pairwise similarities in:

1. Continuous reporting of state anxiety during suspenseful movie-watching.
2. Heart rate during suspenseful movie-watching.

6.3 Methods

Participants

Experiments using cognitive tasks have found associations between trait anxiety and cardiac responding in the range of r = ~.25-.40 (Shinba et al., 2008; Williams et al., 2017). At the floor end of such study effects, I calculated approximate sample size using G*Power 3.1 (Faul et al., 2007). Given my strong priors and that the tests for my hypothesis are only interpretable in one direction (i.e., intersubject representational similarity analysis, see analysis), I calculated power for a one-tailed effect, specifying

r = .25, α = .05, 1-β = .9 ('Correlation: bivariate normal model'). This suggested an approximate sample size of n = 134. To account for ~10% data rejection, this was raised to n = 150. As preregistered, a stopping rule was implemented wherein signal quality and confirmatory tests were checked at n = 50 (Bonferroni correction on confirmatory tests was adjusted accordingly). I continued to collect the full sample but discontinued the use of certain measures, as described below. Following exclusion criteria (see Design and Analysis), 133 subjects were retained for hypothesis-testing and 115 for exploratory analyses. Participants were required to be: aged between 18 and 64; have normal or corrected to normal vision; right handed; have no current or past diagnosis of psychiatric disorder; no current prescription or non-prescribed use of any psychiatric drugs; and not be pregnant.

Design

Self-Report and Behavioral Data

Participants completed: a demographic questionnaire (age [M = 25.53, SD = 8.26, range 18-58], gender [118 female, 29 male, 2 gender variant/non-binary, and 1 transgender female], and ethnicity [94 Asian or Asian British, 41 White, 6 Mixed or multiple ethnic groups, 6 Black/Black British/Caribbean/African, 3 Other ethnic groups]); the Hospital Anxiety and Depression Scale (HADS; (Zigmond & Snaith, 1983; the trait section only of the Spielberger State-Trait Anxiety Inventory (STAI; Spielberger, 1983); Generalised Anxiety Disorder Assessment Questionnaire (GAD-7; Spitzer et al., 2006); and the short form of the Perth Emotional Reactivity Scale (PERS-S; Preece et al., 2019). The anxiety section of the HADS scale constituted my self-report metric for hypothesis-testing (see figure 6.1 for distribution), so as to be able to draw direct comparisons to my prior work using the same scale (chapters 3 and 2.3). Each questionnaire included a catch item for inattention ('Please select [OPTION X]'). Participants who failed the catch item in the HADS were excluded from all analyses as this was my key hypothesis-testing measure (n = 3). Participants who failed a catch item on the other questionnaires were only excluded from exploratory analyses (n = 24; full exclusions in table 6.1). I also included a slider (ranging from "Not Anxious" to "Very Anxious"; figure 6.2) at the bottom of the screen for participants to continually rate state anxiety.

Table 6.1. *Participants fulfilling exclusion criteria. Criteria below are not mutually exclusive within-subjects. These included, failing catch items on the questionnaires (HADS, GAD, STAI, or PER-S); issues with stimulus presentation/recording (audio/logging error), and subjects who provided unresponsive ratings.*

Hypothesis-testing exclusions (n = 17)			*Exploratory exclusions* (n = 35)
Failed HADS check	Audio/logging error	Unresponsive	Failed GAD, STAI, or PERS-S check
3	6	8	24

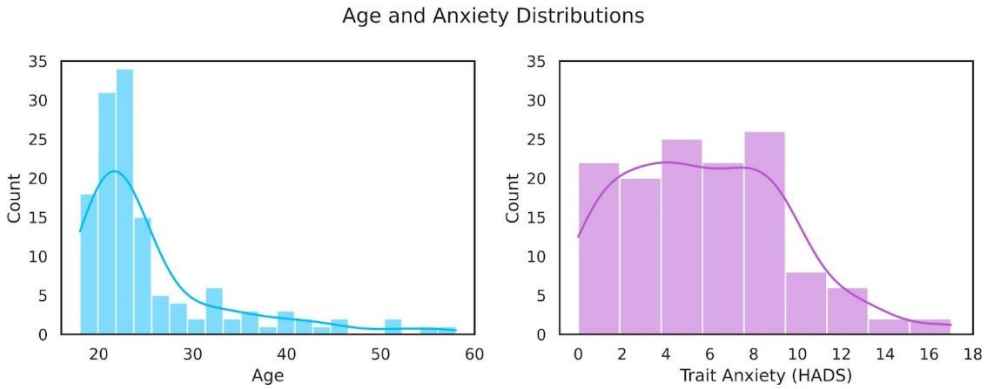

Figure 6.1. *Histograms plots (with kernel density estimation) of age and scores on the Hospital Anxiety and Depression Scale (HADS; anxiety section).*

Figure 6.2. *During the movies, participants provided continuous ratings of subjective state anxiety using a slider at the bottom of the screen.*

Video Clips

Participants watched two clips, both ~8 minutes in duration. My suspense clip was from Alfred Hitchcock's 'Bang! You're Dead', which has been used extensively in neuroimaging studies (Kliemann et al., 2022; Kolisnyk et al., 2023; Shafto et al., 2014). The edited, black and white clip consists of a child who finds what he believes to be a toy gun, not realizing it is a loaded gun. The child continues to play with the weapon until the clip ends with him triggering the gun and almost shooting a maid. The non-suspense clip was from the comedy show 'Friends' (Season 10 episode 6; ~8 minutes following the opening titles). This was selected so as to match the social/sensory complexity of my suspenseful clip and to allow for comparisons with databases which use the same video (Boyle et al., 2020). The clip was edited to grayscale so as to match 'Bang! You're Dead'.

The video clips were counterbalanced between subjects. To establish a baseline and mitigate the influence of emotional states between conditions, participants also viewed a picture of a relaxing beach scene (as used in de Groot et al., 2020) for 2 minutes before, between, and after video clips (see figure 6.3 for full schematic). 6 subjects were excluded due to audio/logging errors during the experiment.

Physiological Recordings

Cardiac signals were recorded using a Nonin 8000S finger pulse oximeter (https://www.nonin.com/products/8000s/). Before my stopping rule, supplementary physiology measures were recorded using an EmotiBit (https://www.emotibit.com/), but this was dropped due to issues of signal quality.

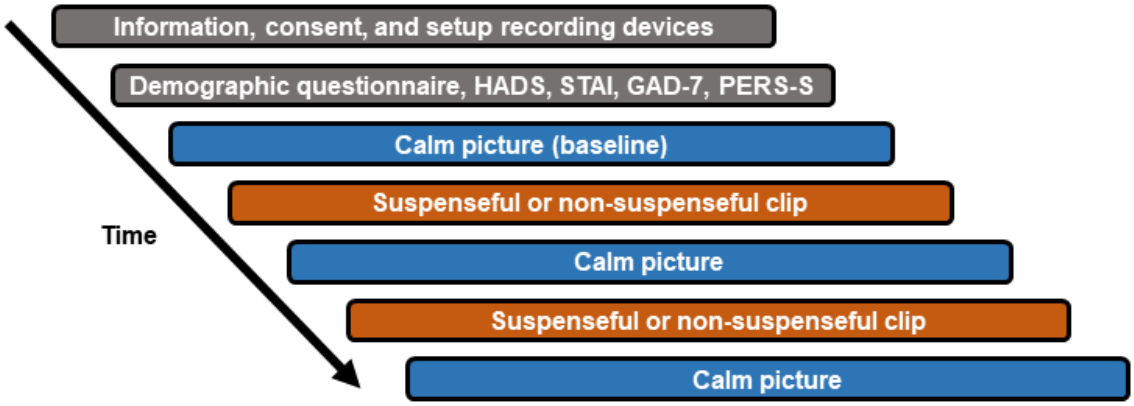

Figure 6.3. *Experimental Protocol. Participants consented, had the recording devices attached, and completed questionnaires before watching a series of video clips. HADS = Hospital Anxiety and Depression Scale, STAI = State-Trait Anxiety Inventory, GAD-7 = Generalised Anxiety Disorder Assessment, PERS-S = Perth Emotional Reactivity Scale (short form).*

Analyses

Analyses were conducted in Python, including the use of the RapidHRV (chapter 5), SciPy (Virtanen et al., 2020), and Pingouin (Vallat, 2018) packages. As preregistered (https://osf.io/w2duj/), all analyses used one-sided tests thresholded at α=.05 unless otherwise stated.

Within-Subject Processing

Continuous anxiety ratings. Continuous state anxiety ratings underwent minimal processing. Subjects whose responses never changed (n = 8) were excluded from all analyses. Data was resampled down to 1000ms windows so as to be able to match the resolution of the cardiac data. Time series from each clip were baseline subtracted (average response from 2 minute calm picture scene prior to videos), then smoothed using a Savitzky-Golay filter ('scipy.signal.savgol_filter', 3rd order polynomial, window length 10s), before finally being Z-scored. For 49 subjects, an

error resulted in the first 30 seconds of slider data not logging properly. This was interpolated using backwards fill based on the first valid rating.

Cardiac Data. Cardiac data from the pulse oximeter were processed using RapidHRV. Heart rate (beats per minute, BPM) time series were extracted using a window length of 10,000ms, window offset of 1000ms, and outlier rejection handled using RapidHRV defaults (outlier_detection_settings = 'liberal'). Rejected/missing heart rate and heart rate variability data points were interpolated using a cubic spline and smoothed with a Savitzky-Golay filter (3rd order polynomial, window length 10 samples).

Typicality Measures. For each subject, I produced typicality measures. I define this as every subject's similarity to ‘canonical’ measures of affect (as measured in separate datasets); in other words, how ‘typical’ their responses are to group averages. This was done for two reasons: (1) my continuous state anxiety ratings scale (figure 6.2) was generated for the purposes of this study and I wanted to benchmark the collected ratings as a confirmatory check; and (2) for use in exploratory tests of individual differences in trait anxiety. Typicality measures were calculated using Fisher-transformed bivariate correlations between primary dependent measures collected in my study (continuous state anxiety ratings and BPM) and independent, external ratings of suspense (Schmälzle & Grall, 2020), tension (Sun et al., 2022), arousal, valence, and uncertainty (Majumdar et al., 2022). All external ratings were resampled to the same resolution as the features generated from the present study.

Subjective-Autonomic Coherence. For each subject, I produced a similarity measure between continuous state anxiety ratings and BPM time series. This was achieved through the use of dynamic time warping—in ‘dtaidistance’ (Meert et al., 2020)—constrained using a Sakoe-Chiba band size of 10 seconds (in other words, allowing for a maximal lag of 10 seconds between the two signals). I also re-calculated measures post-hoc without this constraint.

Intersubject Similarity Measures. My main question, whether individual differences in trait anxiety were associated with altered behavioral and physiological responses within the clip, was assessed using intersubject representational similarity analysis (Finn et al., 2020; Kriegeskorte et al., 2008; Figure 6.4). I first created a trait anxiety symptom profile similarity matrix. Each cell in the matrix was a comparison between two subjects; specifically, Euclidean distance for pairwise comparisons of mean-centered item-wise scores on the anxiety section of the Hospital Anxiety and Depression Scale. Next, I generated behavioral/physiological similarity matrices. I calculated Fisher-transformed bivariate correlations on continuous anxiety rating/BPM time series for each pairwise comparison of subjects. For exploratory analyses, I also looked at anxiety sum scores on the HADS. Unlike trait anxiety symptom profiles, which refer to differences between subjects for *every item* on the questionnaire, the trait anxiety sum score refers to differences in *total scores* on the questionnaire.

Group-Level Modelling

All group-level analyses used one-sided tests thresholded at α=.05 unless otherwise stated. This was chosen a priori as many of my analyses (i.e., intersubject representational similarity analysis) are not theoretically interpretable in negative directions or because I had strong priors (e.g., increased state anxiety during the suspenseful clip compared to baseline). Multiple comparisons corrections were applied using Bonferroni correction. I inverted all distance measures for interpretability so that higher values are associated with greater similarity.

For confirmatory tests, I first validated the use of my continuous anxiety ratings scale by submitting my typicality measures against one-sample t-tests against 0. I then contrasted primary dependent measures (continuous anxiety ratings and BPM) across conditions (suspenseful, non-suspenseful, baseline) as a manipulation check.

Hypothesis-testing was conducted on intersubject similarity matrices (figure 6.4). The two similarity matrices (1: trait anxiety symptom profiles vs. 2: continuous state anxiety ratings or BPM) were compared using partial Spearman correlations with age and gender as covariates. Significance was assessed using permutation-testing; the trait anxiety symptom profile similarity matrix was randomly permuted 10,000 times and correlated against the continuous anxiety ratings/BPM similarity matrices to create

a null distribution for which the original coefficients were compared against. This was run separately for each hypothesis.

For exploratory analyses, I submitted typicality measures and subjective-autonomic coherence measures to two-tailed partial Spearman rank correlations with gender and age as covariates and HADS anxiety sum scores as the predictor.

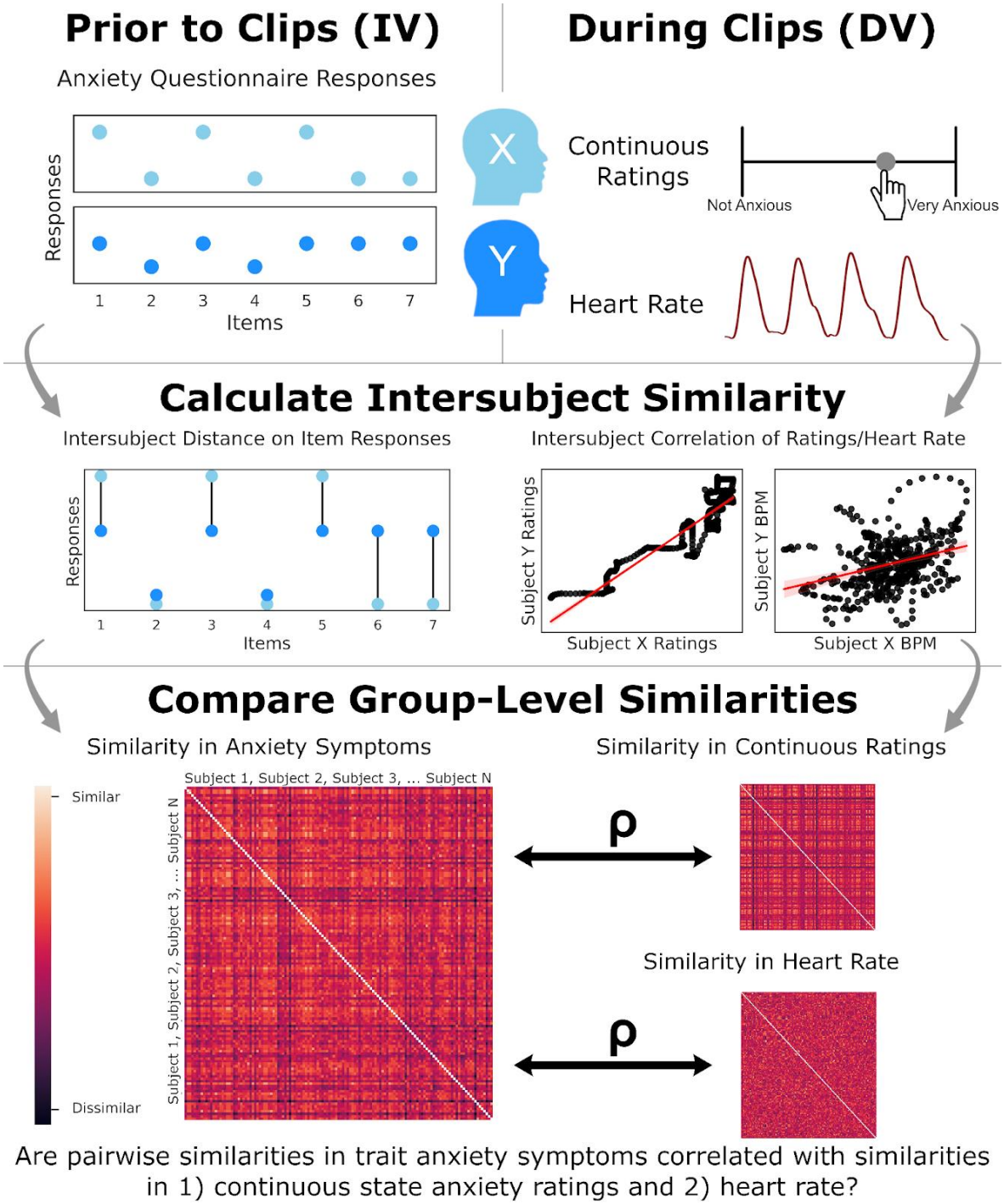

Figure 6.4. *Overview of analysis pipeline. Prior to watching clips, participants filled out the hospital anxiety and depression scale (independent variable). Continuous state anxiety ratings and heart rate (HR) were then recorded as they watched a suspenseful movie clip (dependent variables). Intersubject similarity in trait anxiety symptom profiles was calculated as pairwise Euclidean distance across anxiety items. Similarity for: 1) continuous anxiety ratings; and 2) heart rate were calculated as fisher transformed correlations. At the group level, I compared intersubject similarities in trait anxiety symptom profiles against: 1) continuous state anxiety ratings; and 2) HR.*

Deviations from Preregistration

I note the following deviations from preregistration.

- For 49 participants, a logging error resulted in no slider ratings for the first 30 seconds. This was interpolated using backward filling. I also preregistered to exclude participants whose variability exceeded 3 median absolute deviations of the sample. This was implemented primarily to exclude participants who did not respond at all or moved the mouse a minimal amount. No subjects exceed 3 deviations above the median. However, invariant responses—i.e., people who didn't *ever* move the slider—did not exceed 3 deviations below the median. As such, participants who also provided invariant responses on the slider (n = 8) were excluded from all analyses.
- I originally preregistered to include heart rate variability as a primary outcome. However, a recording error from the pulse oximeter device resulted in leakage from other channels into the pulse oximeter channel from the first 59 subjects. Using an altered preprocessing pipeline (i.e., clipping and additional smoothing; see available code), I was able to recover heart rate data, but was unable to recover heart rate variability due to lack of temporal precision in pulse timings following said processing. As such, I only focus on heart rate for my analyses. For transparency, I have still retained the associated original Bonferroni-corrected thresholds, but note that adjusting this either way does not alter inference.
- I originally preregistered to compare item-wise scores on questionnaires between participants using Spearman rank correlations. However, I instead used Euclidean distance as correlations of -1, 0, and 1 could not be Fisher transformed, correlation coefficients could not be calculated where there was no variance in scores (which changes dependent on implementation of reverse coding), and correlations are insensitive to the magnitude of discrepancies in scores (e.g., participants who deviate on an item by 1 are treated the same as those who deviate by 4).
- Before the implementation of my stopping rule, I included an Emotibit device to record skin conductance. However, due to issues of signal quality, I stopped use of this device for the remainder of the experiment, which meant dropping

skin conductance as a measure in my analyses. Alteration of Bonferroni correction did not alter inference.

- I have added additional tests between conditions to help contextualize my findings and as robustness checks. Other post-hoc tests are listed in the results as such.

6.4 Results

Confirmatory Tests

As my continuous anxiety ratings scale (figure 6.5) were generated for the purposes of the planned study, I benchmarked the collected continuous state anxiety ratings to those from independent samples. Specifically, I correlated my continuous ratings against previously collected ratings of suspense (Schmälzle & Grall, 2020), tension (Sun et al., 2022), arousal, valence, and uncertainty (Majumdar et al., 2022) to produce 'typicality' measures for each subject. Fisher transformed correlation coefficients were submitted to one-sample t-tests against 0. Anxiety ratings were significantly associated with independent ratings of suspense (average r = .62, $t(132) = 31.66$, $p < .0001$), tension (average r = .64, $t(132) = 31.46$, $p < .0001$), arousal (average r = .60, $t(132) = 29.41$, $p < .0001$), valence (average r = -.59, $t(132) = -28.16$, $p < .0001$), and uncertainty (average r = .44, $t(132) = 21.49$, $p < .0001$).

t-tests also confirmed increases in self-reported anxiety in the suspenseful condition compared to baseline (2 minute rest prior to video clips; $t(132) = 20.24$, $p < .0001$) and to the non-suspenseful condition ($t(132) = 18.64$, $p < .0001$). On the other hand, BPM was not significantly increased in the suspenseful compared to baseline ($t(132) = -2.798$, $p = .98$) nor the non-suspenseful condition ($t(132) = .37$, $p = .713$).

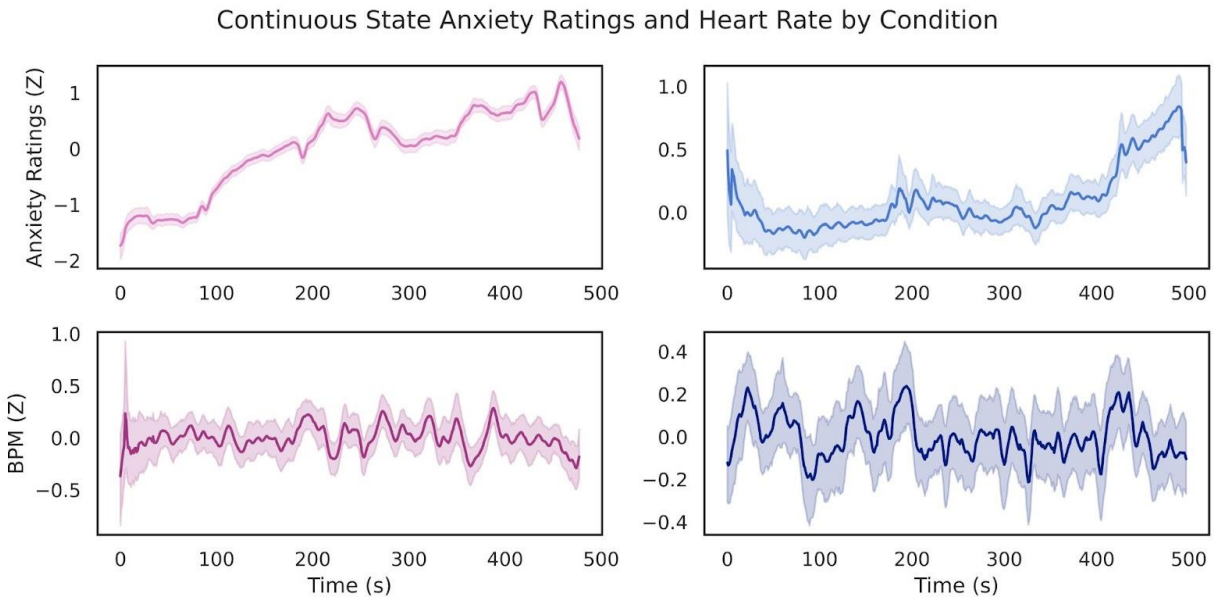

Figure 6.5. *Z-normalized time series (with 95% confidence intervals) for continuous state anxiety ratings (first row) and BPM (second row) for the suspense (first column) and non-suspense (second column) clips.*

Hypothesis-Testing

I predicted that pairwise similarities in trait anxiety symptoms (item-wise differences) would be associated with pairwise similarities in continuous state anxiety responses and BPM during the suspenseful condition. Partial Spearman rank tests of intersubject similarity matrices revealed that trait anxiety symptom profiles were correlated with continuous state anxiety ratings ($\rho = .1$, $p < .0001$; figure 6.6) but not BPM ($\rho = -.01$, $p = .845$). I conducted a post-hoc test which demonstrated that the association between trait anxiety symptoms and continuous ratings dissipated when using just trait anxiety sum scores as a predictor ($\rho = .01$, $p = .171$). There was a small correlation between pairwise differences in sum scores and symptom similarity ($\rho = -.02$). As such, I re-conducted my analysis (between trait anxiety symptoms and continuous ratings) post-hoc with pairwise similarities in sum scores as a covariate which did not change my inference ($\rho = .1$, $p < .0001$).

To inspect the specificity of my effects post-hoc, I tested whether associations were the same for my non-suspenseful condition and when accounting for change in similarity between conditions. I did not detect an association between trait anxiety

symptom profiles and continuous state anxiety ratings during the non-suspenseful condition (ρ = -.037, *p* = .999). Effects for the suspenseful clip also remained after contrasting measures against the non-suspenseful condition (ρ = .097, *p* < .0001). For BPM, I again did not detect an association during the non-suspenseful condition (ρ = .001, *p* = .457) nor when contrasting conditions (ρ = -.006, *p* = .722).

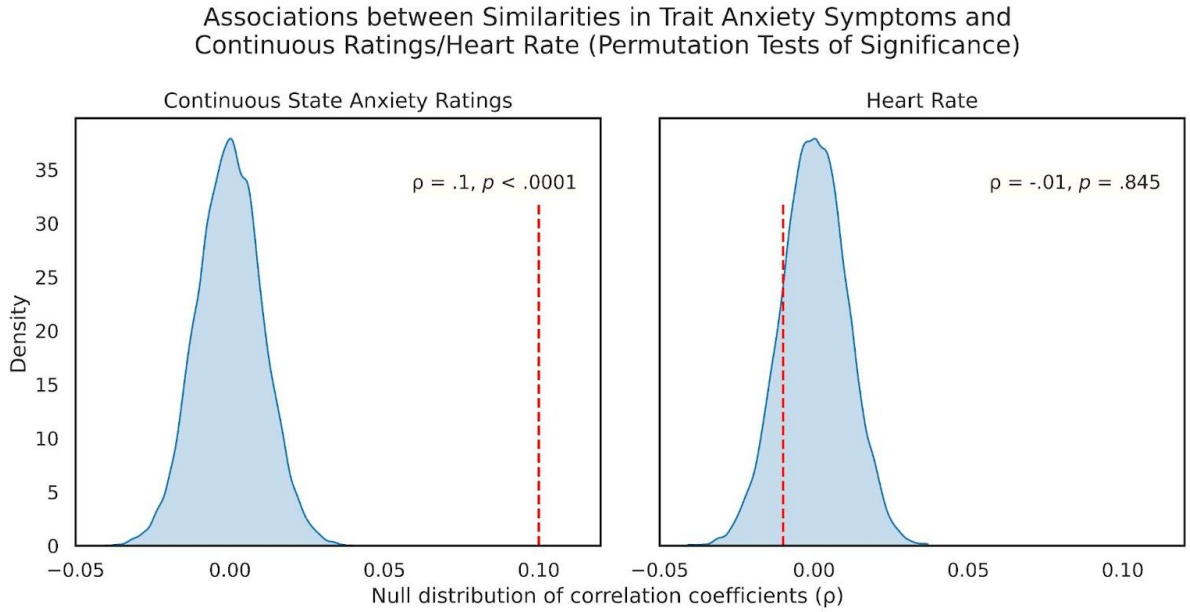

Figure 6.6. *Intersubject similarity matrices (left) were calculated via pairwise comparison between subjects: 1) trait anxiety symptoms from self-report questionnaires prior to watching movie clips (lower triangle); and 2) continuous state anxiety ratings during anxiogenic movie-watching (upper triangle; see figure 6.4 for more details). Partial Spearman correlations were run between intersubject similarity measures to test for the association between trait anxiety symptoms and continuous ratings during the movie clip. The correlation was re-run with 10,000 random permutations of the anxiety symptoms matrix to produce a null distribution; the original coefficient was compared against to derive significance (right).*

Exploratory Tests

For exploratory tests, I conducted typicality analyses. This refers to how ‘typical’ (i.e., Fisher transformed correlation coefficient) each subject's time series is when compared to ‘canonical’ ratings of suspense and uncertainty (group averages from independent data). Using this approach, I failed to find an association between trait anxiety sum scores and typicality scores for continuous anxiety ratings (suspense: ρ = .04, p = .646; uncertainty: ρ = .17, p = .074) nor BPM (suspense: ρ = .01, p = 894; uncertainty: ρ = .03, p = .793). I also investigated whether trait anxiety was associated with measures of subjective-autonomic coherence (similarity between ratings and BPM). I failed to find evidence for an association between subjective-autonomic coherence and HADS anxiety sum scores (ρ = .08, p = .379).

6.5 Discussion

Prior work has established a link between induced anxiety (for example, by threat of shock) and increased autonomic responding, including cardiac activity (Petry & Desiderato, 1978). As such, how peripheral physiological responding, such as heart rate, may be linked to pathological levels of trait anxiety has come under study (Pittig et al., 2013). However, many of the observed effects have been established using tightly-controlled experimental paradigms, such as threat of shock. The extent to which these effects generalize to more naturalistic settings has not received sufficient attention. Moreover, how these responses relate to ongoing subjective states of anxiety is often overlooked. By presenting the exact same anxiogenic stimuli as my previous work (chapter 3), the present study set out to investigate to what extent trait anxiety symptoms were associated with continuous state anxiety ratings and heart rate responses during an anxiogenic movie clip. Using an inter-subject correlation approach, I found a significant association between pairwise similarities in trait anxiety symptoms and continuous state anxiety responses, but failed to find an association with heart rate.

As the independent variable, I first derived pairwise similarities in trait anxiety symptom profiles. This was operationalized as intersubject item-wise similarities on the anxiety section of the hospital anxiety and depression scale. Put simply, I produced measures of how similar pairs of individuals were in regard to their symptoms of trait anxiety, rather than just overall scores summed across items. Testing my first hypothesis, I found trait anxiety symptoms to be significantly associated with continuously reported state anxiety ratings during a suspenseful clip. When using summary scores from questionnaires, which average responses across symptoms, this effect was not present as a separate test or when used as a covariate. As measured through self-reported questionnaires, this suggests differing types of trait anxiety symptoms—more so than overall intensity—may be associated with ongoing states of anxiety during anxiogenic movie-watching.

I demonstrated an association between trait anxiety symptoms and continuous state anxiety ratings using intersubject representational similarity analysis (Finn et al., 2020; Kriegeskorte et al., 2008). To my knowledge, no work has used this technique in the study of individual differences in anxiety and subjective/autonomic responses. While I was able to demonstrate that associations between trait anxiety symptoms and

continuous state anxiety ratings do arise within stimulus presentation, I cannot pinpoint what specific components of the movie may be driving these effects. By contrasting the effects with a non-suspenseful clip, there is evidence to suggest, overall, anxiogenic movie stimuli elicit anxiety-relevant idiosyncratic responses. However, whether these associations occur at specific spectral densities (e.g., low-frequency drifts vs spikes in anxiety) or are related to specific content in the movies (e.g., the presence of a gun vs arguments) was not-tested within this approach. It would be especially fruitful to further investigate and replicate effects using both intersubject representational analysis and other analyses which do not rely on pairwise comparisons, such as using factor analysis to produce within-subject measures of symptom types. I particularly encourage future work to investigate whether specific dimensions of anxiety (e.g., threat vigilance vs rumination) are associated with specific responses (e.g., amplitude of acute responses to weapons vs slow returns to baseline following peaks of suspense). Moreover, the data was collected from participants watching two ~8 minute clips. In addition to alternative analytical approaches, it will be important to also test the specificity of these effects across differing movies.

For my second hypothesis, I failed to find any associations between trait anxiety symptoms and heart rate responses. One interpretation is that there was no such effect or that it was of a weaker magnitude than the sample permitted me to detect. Given that I did not detect overall increases in heart rate as a function of the suspenseful video clip, it may be that the degree of anxiety induction by this clip was also insufficient to probe the autonomic nervous system in a relevant manner. Alternatively, given that such anxiogenic stimuli does not impose direct threat on an individual (unlike threat of shock), there may be no need to prepare the body for ‘fight-fight’-like responses (all the while still evoking a state of anxiety). Future work should investigate whether more anxiogenic clips could drive individual differences in autonomic responding, as is seen in studies using personally-directed threats (Abend et al., 2022).

It is also important to contextualize these null findings in light of my analytical approach. Here, I compared differences in trait anxiety symptoms (multivariate) with heart rate (univariate). It could be that the association between trait anxiety and peripheral physiology does not manifest in such a univariate manner. There is evidence to suggest that different emotional processes impact different organs

dependent on current goals (Critchley, 2005, 2009). It may be that trait anxiety symptoms are not associated with just increases in heart rate during anxiogenic movies. Rather, trait anxiety symptoms are associated with differing responses across autonomic projections (e.g., some symptoms could be more strongly associated with heart rate, while others are more so with respiration). Whilst I was able to recover heart rate measures, heart rate variability was excluded as a measure due to noise, which could have provided greater sensitivity to detecting effects (Pittig et al., 2013). Likewise, the measure of skin conductance was excluded due to poor signal quality, which could have also provided sensitivity (Abend et al., 2022). I encourage future work to look at autonomic responses to movies across physiology measures (heart rate, heart rate variability, tonic/phasic skin conductance, respiration etc.) and across differing frequency domains (drifts vs spikes in heart rate).

I lastly acknowledge there are methodological constraints present in the study. Cardiac measures in just under half of the subjects contained moderate levels of noise (due to a logging error). As such, I cannot conclude that no anxiety-relevant idiosyncrasies in autonomic responding truly exist during suspenseful movie-watching; rather, I have failed to provide evidence that anxiety symptomatology may be associated with altered heart rate during a moderately suspenseful video clip.

Conclusion

In the present study, I sought to test to what extent trait anxiety symptoms may be associated with ongoing states of anxiety and heart rate during suspenseful movie-watching. Using intersubject representational similarity analysis, I demonstrated that pairwise similarities in trait anxiety symptoms, but not overall scores on questionnaires, were associated with pairwise similarities in continuous state anxiety ratings during a suspenseful movie clip (n = 133). However, I failed to find an association between trait anxiety symptoms and heart rate responses. I recommend future studies test whether a relationship between trait anxiety and cardiac responses may be revealed when using more anxiogenic movie stimuli and by incorporating additional physiological recordings.

7. Neural-Autonomic Responses to Suspense

7.0 Preface

I previously demonstrated that *trait* anxiety was associated with amygdala-prefrontal dynamics during movie-watching. In chapter 6, my analyses indicated that individual differences in anxiety were associated with ongoing *states* of anxiety during movie-watching, but I failed to detect any associations with autonomic responding (i.e., heart rate). This initially suggests that movies do not necessarily drive anxiety-relevant increases/decreases in cardiac responding during movie-watching. However, even in the absence of observable alterations in cardiac responding, it is nonetheless plausible that communication between the brain and the autonomic nervous system could be impacted (e.g., altered interoceptive awareness). As such, in the present chapter, I sought to tie together all the previous chapters and investigate the extent to which *states* of anxiety during movies might be associated with alterations in communication between the brain and the autonomic nervous system.

For the pre-printed version of this studies, please refer to the below references:

Kirk, P. A., & Robinson, O. J. (2023). *Preliminary evidence for altered neural-autonomic coherence during anxiogenic movies*. PsyArXiv. https://doi.org/10.31234/osf.io/ce8mh

7.1 Abstract

Neuroscience research into anxiety has predominantly focused on its impact on central or peripheral nervous systems in isolation. However, there is evidence to suggest anxiety impacts the degree of communication between the brain and the autonomic nervous system. Existing literature suggests fundamental threat circuitry, typically engaged in anxiety-relevant processes such as attentional vigilance, also serves to communicate with peripheral physiology, namely the autonomic nervous system. However, whether such neural-autonomic communication occurs outside of tightly-controlled experimental settings, and in relatively more naturalistic contexts, is less clear. Here, using a suspenseful movie-watching paradigm (n = 29; Caltech Conte dataset), I hypothesized that activity in three key structures (amygdala, dorsomedial prefrontal cortex, and insula) would show increased associations with autonomic responding (i.e., heart rate) as a function of anxiety. I failed to find associations with my primary activity measures. However, in my planned exploratory analyses, suspenseful movie-watching was associated with reduced coherence between heart rate and: amygdala-dorsomedial prefrontal dynamic connectivity; amygdala-subgenual anterior cingulate dynamic connectivity; precuneus activity; vmPFC activity; and bilateral putamen activity. Taken together, I provide preliminary evidence for altered neural-autonomic coherence as a function of anxiogenic movie-watching. I encourage future work to investigate the causal directionality of these effects, the extent to which they generalize to other movie stimuli, and how they may interact with pathological levels of anxiety.

7.2 Introduction

One function of anxiety is to promote the detection, identification, and avoidance of harm (Mobbs et al., 2015). When under a state of induced anxiety, neural systems can tune perceptual/cognitive processes to promote such vigilance (attention to threat-relevant stimuli; (Eysenck et al., 2007). Underlying this is a core network of cortico-subcortical brain circuitry, which includes the amygdala; bed nucleus of the stria terminalis; hypothalamus; periaqueductal gray; insula; dorsomedial prefrontal cortex (dmPFC); subgenual anterior cingulate cortex (sgACC); and anterior ventromedial prefrontal cortex (vmPFC); all of which serve a multitude of functions (Chavanne & Robinson, 2021; Grupe & Nitschke, 2013). Here, I refer to this as the 'defensive response network' (Abend et al., 2022). Research in recent decades has focussed on how these systems link anxiety to processes such as attention, reward/punishment, and memory (Robinson et al., 2013). At the same time, anxiety also induces—and is influenced by—alterations in peripheral physiology; most notably, the autonomic nervous system. Indeed, at the outset of modern psychology, the central nervous system was not always seen as the fundamental basis of anxiety. Instead, peripheral physiology was posited to be the biological driver of affect (Dewey, 1894; James, 1894). Since then, the role of peripheral physiology in anxiety has often been studied as a separate line of inquiry to direct studies of brain activity.

Research has established a link between anxiety and activity across branches of the autonomic nervous system (typically summarized as increased sympathetic activation and/or parasympathetic withdrawal), using proxies such as heart rate, heart rate variability and skin conductance (Friedman & Thayer, 1998b; Hodges & Spielberger, 1966; Lader, 1967). Dominance of the sympathetic branch of the autonomic system can assist an individual during 'fight-flight'-like contexts; for instance, by providing increased blood flow to support musculature for physical exertion (e.g., fleeing; McCorry, 2007). Such somatic manifestations have been proposed to constitute a separable dimension of anxiety compared to 'cognitive' symptoms (Schachter, 1964). The reality is likely less clear cut; emerging evidence reveals an intrinsic link between peripheral physiology and cognitive processes in anxiety (Critchley et al., 2013; Mallorquí-Bagué et al., 2016). For instance, the cardiac cycle may modulate the perception of threat-relevant stimuli (Garfinkel et al., 2014). Likewise, there is substantial overlap in neural circuits which drive autonomic activity

and are associated with threat vigilance (albeit at the spatial resolution of fMRI), namely the amygdala, dorsomedial prefrontal cortex, and insula (Critchley, 2005; Thayer et al., 2012; Wager et al., 2009). Indeed, anxiety-induced individual differences in autonomic responding are correlated with differences in amygdala-prefrontal responding (Abend et al., 2022; Makovac, Meeten, Watson, Herman, et al., 2016). Thus, anxiety-relevant brain systems ('defensive response network'), which have largely been discussed in terms of perceptual processes such as threat vigilance, may also be responsible for communication with peripheral physiology.

Although the field has started to develop a core framework for studying the neural substrates of anxiety (both central and peripheral), one potential issue arises: most of our understanding is driven by tightly-controlled experimental paradigms. The extent to which observed effects are thus seen outside of such conditions remains relatively unknown. As such, I have since extended the research focus to movie fMRI, which consists of presenting movies to subjects while undergoing scanning. By using such unconstrained and relatively more naturalistic stimuli, we can start to develop a better understanding of whether prior results translate to more ecological settings. Studies have demonstrated within-subject effects of anxiety on amygdala-prefrontal activity during movie-watching (Hudson et al., 2020; Kinreich et al., 2011). In chapters 2 and 3, I investigated between-subject alterations in movie-evoked activity/connectivity; my results suggest individuals scoring higher in trait anxiety demonstrate some differential processing of features such as faces/words and show idiosyncrasies in threat circuitry dependent on the degree of suspense in a movie. Hence, anxiogenic movies appear to offer an effective platform for investigating anxiety in a more ecologically-rich context.

Movie fMRI studies have predominantly been constrained to studying the brain in isolation, with links primarily constrained to perceptual/cognitive processes or self-reported questionnaires. There exist few studies linking neural and autonomic systems during movie-watching. Intracranial electrophysiological recordings in humans have provided evidence for a role of insula-dorsomedial prefrontal/anterior cingulate dynamics in shaping autonomic responses during emotional face processing in movies (Sonkusare et al., 2023). One movie fMRI study suggested general arousal/valence-driven heart rate dynamics to be associated with shifts in macro-scale architecture, namely in salience, executive, and default mode networks (which includes, but is not

limited to, insula and dorsomedial prefrontal cortex/anterior cingulate; (Young et al., 2017). These studies have not assessed associations with subcortical structures. Moreover, to my knowledge, no studies have yet to directly investigate whether communication between threat circuitry and the autonomic system alters as a function of anxiety during movie-watching.

In the present study, I investigated which components of the 'defensive response network' may be associated with heart rate (as a proxy for autonomic responding) during movie-watching. While much research conceptually embeds findings in terms of top-down autonomic entrainment (e.g., brain signals to the heart), I also acknowledge that the relationship between central and peripheral nervous systems is not unidirectional. I do not aim to make causal inferences regarding directionality of communication (put simply, whether the brain is influencing the heart or the heart is influencing the brain). As such, in referring to communication between the brain and autonomic/cardiac activity, I opt for a more theoretically-neutral term, coherence.

Hypotheses

In the present study I contrasted brain activation during an anxiogenic/suspenseful clip and a non-suspenseful clip. As stated in my preregistration (https://osf.io/9vy87/), I predicted that the suspenseful movie would be associated with stronger coherence between heart rate and activity in the:

4. Left amygdala.
5. Right amygdala.
6. Left insula.
7. Right insula.
8. Dorsomedial prefrontal cortex.

Following hypothesis-testing, I also preregistered to expand my analyses to encapsulate activity within and connectivity across a 'defensive response network'. In addition to my primary regions of interest for hypothesis-testing, this included BNST, hypothalamus, PAG, sgACC, and anterior vmPFC.

7.3 Methods

Dataset

I made use of the Caltech Conte Center dataset (Kliemann et al., 2022). In brief, n = 29 (following exclusion of subjects from the full n = 55; see Cardiac Data) participants watched a grayscale, 8 minute clip from Alfred Hitchock's '*Bang! You're Dead*'. The clip consists of a child playing with a real, loaded gun, which they believe is a toy gun. The clip generally builds in suspense until the child almost shoots a maid. Its use for eliciting anxiety-relevant idiosyncrasies in neural circuitry was demonstrated in separate participants in chapter 2. Participants also watched Pixar's '*Partly Cloudy*', which served as the non-suspenseful, control condition. The 5.5 minute animated clip consists of anthropomorphized clouds which create baby humans and animals.

Whole-brain scanning was conducted on a Siemens 3T Magnetom Prisma using echo planar imaging: 2.5mm^2 voxels, TR = 700ms, TE = 30ms, FA = 53o, multiband acceleration = 6. Data had already undergone standardized preprocessing (using fMRIPrep), including distortion correction, coregistration, and motion correction (for full details, see Kliemann et al., 2022).

Analyses

Within-Subject Processing

Cardiac Data. Pulse oximeter data (200Hz) was previously processed with RapidHRV (chapter 5), making use of a 10 second moving window with a 1s offset (figure 7.1). Beats per minute (BPM) constituted my metric for heart rate. As I aimed to investigate ongoing communication between the brain and autonomic nervous system (rather than block-wide averages), heart rate variability was not used due to its instability at small time scales (Pecchia et al., 2018). Missing data points were interpolated using cubic-spline interpolation (3rd order polynomial) and backward/forward fill using the first/last valid data point (for missing data at the beginning/end of the time series). BPM time series were: smoothed using a Savitzky-Golay filter; upsampled to 700ms (to match the temporal resolution of fMRI acquisition); detrended (with the same detrending parameters applied to fMRI data, see fMRI Data); shifted by 6s (to account for hemodynamic lag, as used in chapter 3);

and Z-normalized. RapidHRV output and a priori visual inspection indicated 26 subjects had severe noise present in the signal. These subjects were excluded from the present analysis, leaving 29 subjects for subsequent analyses.

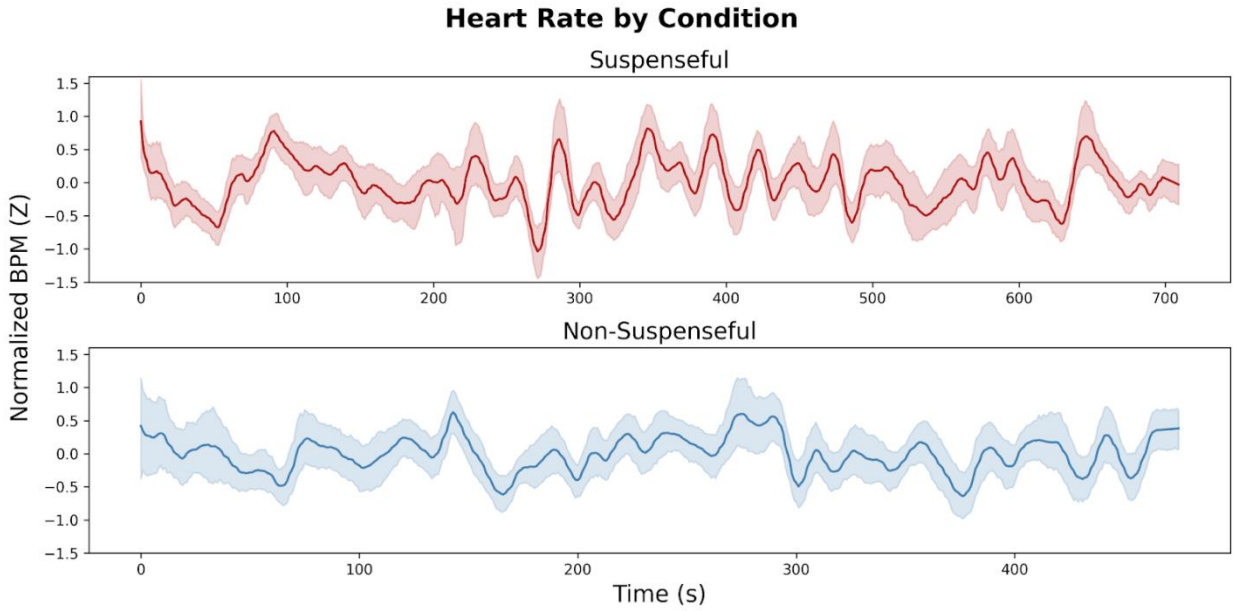

Figure 7.1. *BPM time series averaged across all participants for suspenseful movie-watching (top) and non-suspenseful movie-watching (bottom).*

fMRI Data. I denoised data by taking voxel-wise residual time series from a GLM (3dDeconvolve) which contained the following parameters: detrending (-polort 'A'); nuisance regression of CSF and WM mean signals; and 24 motion parameters (6 rotational and translation + 6 temporal derivatives + 12 squares of raw and derivatives). For voxel-wise analyses only, I applied smoothing to 6mm FWHM (3dBlurToFWHM).

For group-level analyses, time series were extracted (3dmaskave) from 10 regions (as implemented in chapters 3 and 4). For my 3 hypothesis-testing ROIs, dmPFC and insula were defined using meta-analytical clusters ("induced (+) vs. transdiagnostic (+) 20 mm" and "induced (+) vs. pathological anxiety (+) 20 mm" respectively; Chavanne & Robinson, 2021), and amygdala was defined using FreeSurfer parcellations (constrained within an inflated AAL defined amygdala mask, Rolls et al., 2020). These specific ROIs were selected: based on my hypotheses, to

allow consistency with- and comparisons to my prior studies, and because they show spatial convergence with meta-analyses indicative of neural-autonomic communication (Dugré & Potvin, 2023; Ferraro et al., 2022). The remaining 7 ROIs correspond to the rest of the 'defensive response network' (hypothalamus, bed nucleus of the stria terminalis, periaqueductal gray, anterior ventromedial prefrontal cortex, and subgenual anterior cingulate cortex; figure 7.2), following the same definition procedures as my chapters 3 and 4 (FreeSurfer segmentations for subcortex and meta-analytic clusters for cortex; Billot et al., 2020). ROIs were spatially resampled (3dresample) to match the grid spacing of the EPI data.

In addition to activation time series, I derived dynamic connectivity measures across all edges of the 'defensive response network'. Using a sliding window based analysis in the 'timecorr' package (width = 29 TRs/20.3 seconds, gaussian kernel weighting; Owen et al., 2021), I produced time series of Fisher transformed correlation coefficients. I selected my window to match that chapter 3 (note: supplementary analyses in this work suggested changing the window size did not change inference). Time series were then Z-normalized.

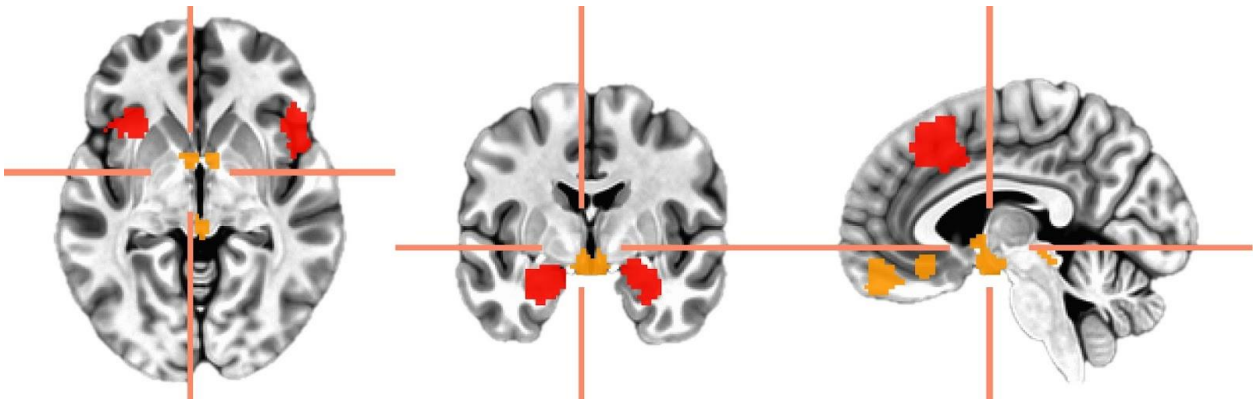

Figure 7.2. *Regions of interest for (MNI x=-4, y=-3, z=-3) primary hypothesis-testing (red) and exploratory analyses (orange). ROIs included are left slice: anterior insula (meta-analytically defined), bed nucleus of the stria terminalis (MNI anatomical mask), and periaqueductal gray (MNI anatomical mask); middle slice: amygdala and hypothalamus (Freesurfer-defined; i.e., a union of all subjects); right slice: dorsomedial prefrontal cortex, anterior ventromedial prefrontal cortex, and subgenual anterior cortex (meta-analytically defined).*

Neural-Autonomic Coherence. Traditionally, ongoing associations between HRF-convolved cardiac measures and the BOLD signal have been tested by looking at the instantaneous correspondence between the two signals (Nguyen et al., 2016; Valenza et al., 2019). I also took this approach by calculating Fisher transformed bivariate correlations between the two signals. However, I also wanted to approach the data in a way which made fewer assumptions concerning the temporal relationship between the two signals. As such, I also made use of dynamic time warping (using 'dtaidistance', Meert et al., 2020), which warps two signals non-linearly so as to best match all timepoints in regard to similarity, with the final output being a distance measure. Warping paths were constrained using a Sakoe-Chiba band corresponding to 14 TRs (~10 seconds). Due to the difference in duration between the two conditions, I matched the number of TRs between conditions (i.e., omitting the first section of the suspense condition). I lastly generated exploratory whole-brain measures. For this, I calculated voxel-wise, Fisher transformed cross-correlation maxima between HR and the BOLD signal (3ddelay; Saad et al., 2001, 2003; figure 7.3). Here, 3ddelay only tests for lags in BOLD data against the HR signal. As such, I introduced an additional 10s lag into the HR to allow for tests of neural activity both preceding and following HR.

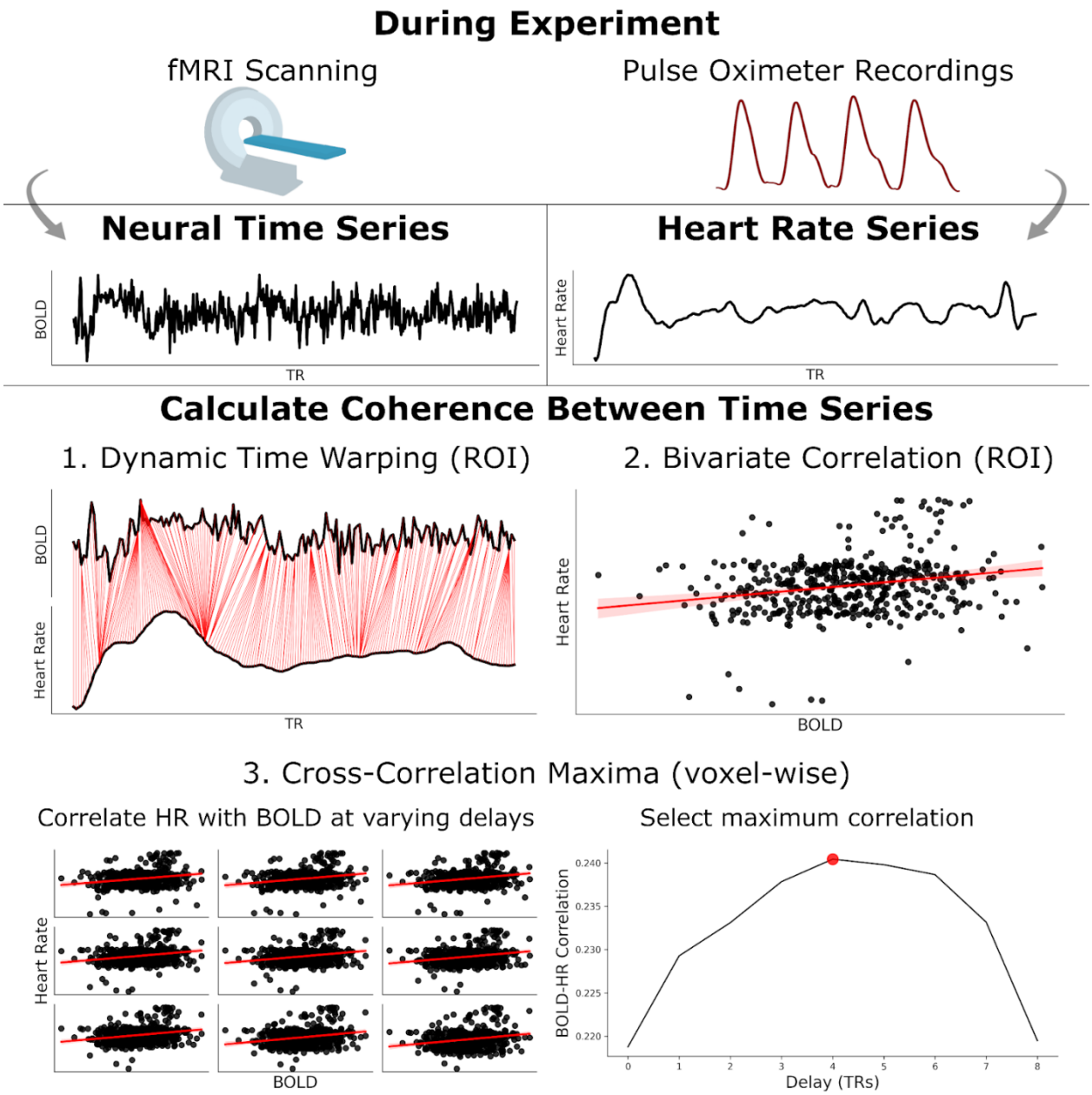

Figure 7.3. *Overview of analysis pipeline. From the fMRI and pulse oximeter signals I preprocessed/extracted BOLD and Heart Rate time series. I then calculated the similarity ('coherence') between these signals using three approaches. For my regions of interest analyses, I used dynamic time warping and bivariate correlations. For whole-brain exploratory analyses, I selected cross-correlation maxima at every voxel.*

Group-Level Modeling

All group-level models were run in Python and AFNI (Cox, 1996) and used two-tailed tests at $p < .05$. For this, ROI brain-heart similarity measures were submitted to paired-sample t-tests comparing across suspenseful and non-suspenseful conditions. Bonferroni correction was run at the level of hypothesis-testing measures (5 activation ROIs, $p <. 01$) and exploratory tests (10 activation ROIs + 45 connectivity measures, $p < .0009$). I also provided main effects, one-sample t-tests against 0 per condition to contextualize results. For my whole-brain analyses, I submitted cross correlation maxima maps to paired t-tests between conditions (3dttest++). I adjusted for a false positive rate using permutation-based cluster thresholding (-Clustsim, voxel-wise $p < .001$), resulting in cluster-corrected threshold of $k > 16$. Coordinates are reported in MNI space.

Deviations from Preregistration

I note the following deviations from my preregistration:

- I did not preregister a plan for smoothing fMRI data. This was done post-hoc, but only for exploratory voxel-wise analyses.
- To help characterize my planned tests (i.e., direction of connectivity/activation), I have provided supplemental one-sample t-tests for conditions separately.
- I originally stated 27 subjects were excluded due to excessive noise in the cardiac signal. This was an error, and the actual number of subjects excluded was 26 (leaving 29 for analyses).
- I have provided supplementary descriptive time delay statistics for cross-correlation clusters.
- In my voxel-wise analyses, I did not account for the fact that 3ddelay only delays BOLD signals and, in this instance, only tests for HR preceding BOLD activity. As such, I introduced a 10s lag to account for this.

7.4 Results

Roi Analyses

I first ran paired-sample t-tests on brain-heart coherence measures between the suspenseful and non-suspenseful conditions. For my key hypotheses, I failed to find evidence for a significant difference for either my primary (dynamic time warping) or secondary (bivariate correlation) measures of brain-heart similarity. In my planned exploratory analyses, I expanded this out to a wider 'defensive response network' which—in addition to more regions of interest—included dynamic connectivity between all regions. I failed to find evidence using my dynamic time warping measure, but found two Bonferroni-corrected results for my bivariate correlation measure (instantaneous association between BOLD and 6s-lagged HR). Within-subject changes in coherence were observed for amygdala-dmPFC dynamic connectivity ($t(28)=-3.91$, $p = .0005$) and amygdala-sgACC dynamic connectivity ($t(28)=-4.44$, $p = .0001$; figure 7.4). This was characterized by a general positive coherence between heart rate and connectivity in the non-suspenseful condition, which was then reduced during the suspense condition (figure 7.5).

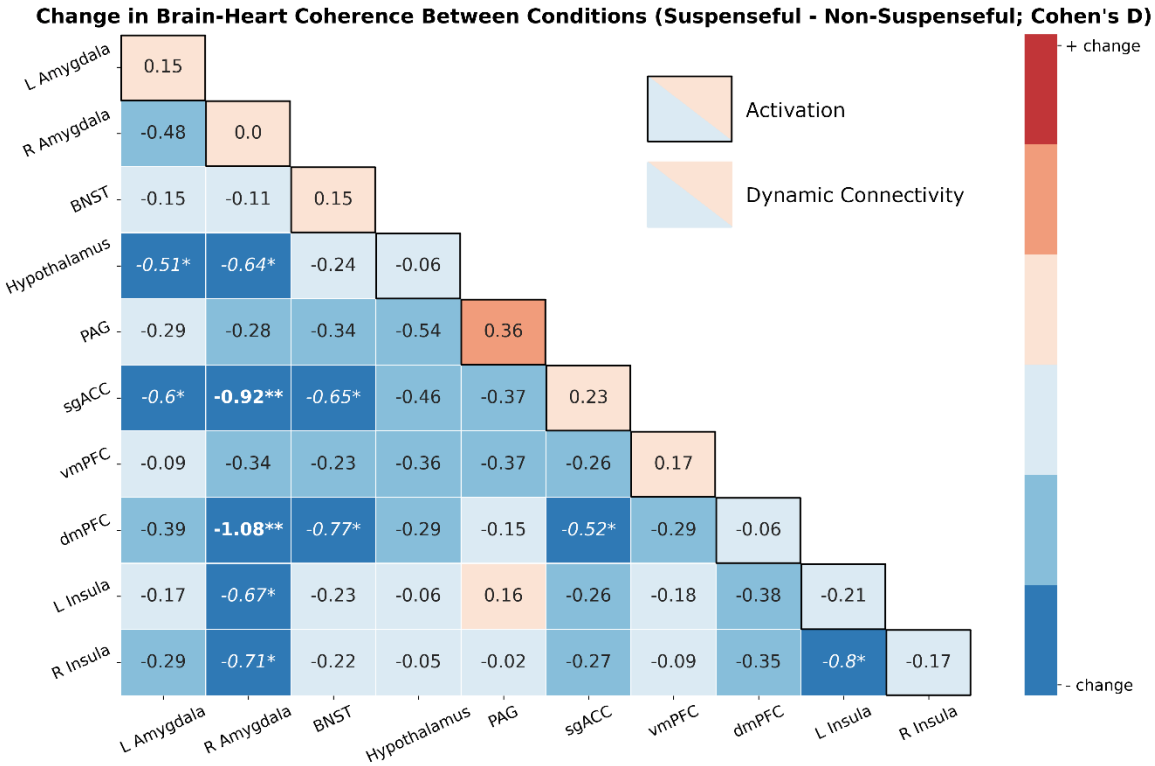

Figure 7.4. *Effect (Cohen's D) of suspenseful vs non-suspenseful movie-watching on brain-heart coherence. Coherence measures in each condition were defined as the Fisher transformed bivariate correlation between 6s-lagged HR against fMRI measures. The diagonal of the matrix represents change in activation-based coherence. Cells below the diagonal represents change in dynamic connectivity-based coherence. Blue cells refer to a reduction in coherence between heart rate and brain responses during suspenseful movie-watching relative to non-suspenseful movie-watching, while red cells refer to an increase. There were no significant changes in the coherence between activation and heart rate. Dynamic connectivity between the right amygdala and both the dmPFC and sgACC was associated with reduced coherence with heart rate during the anxiogenic movie. ** p < .0009 (Bonferroni-corrected threshold), * p < .05 (uncorrected).*

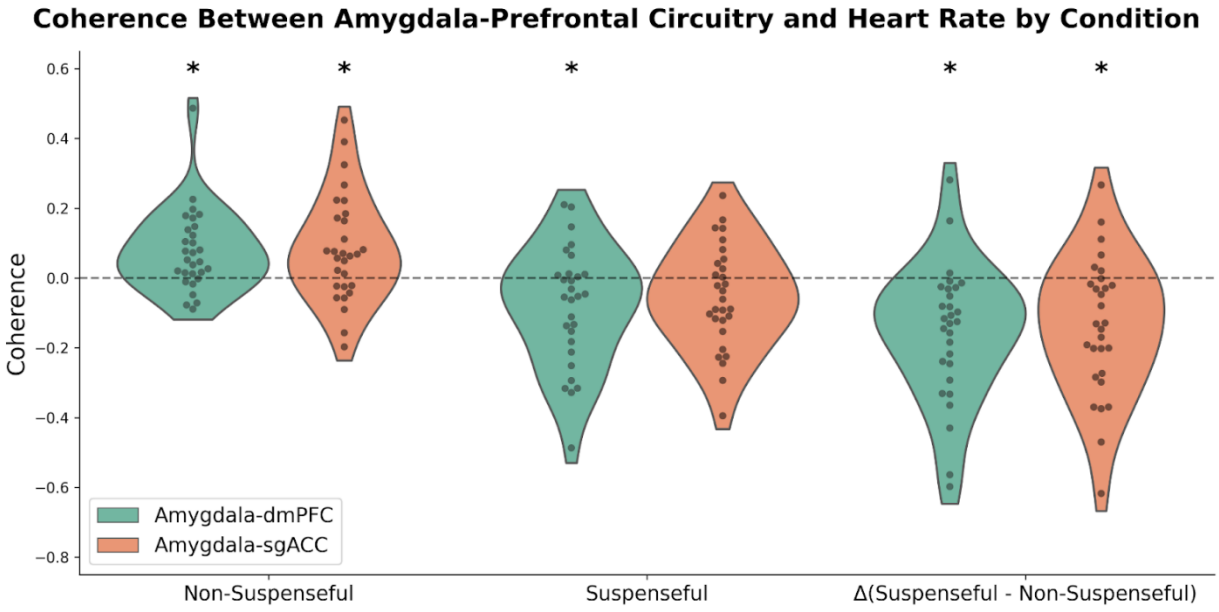

Figure 7.5. *Violin plots detailing coherence (Fisher-transformed correlation coefficients) between amygdala-prefrontal dynamic connectivity and heart rate as participants watched non-suspenseful and suspenseful movie clips. This illustrates that coherence was significantly positive during the non-suspenseful condition and was reduced during the suspenseful condition (to negative coherence for amygdala-dmPFC and to non-significant coherence for amygdala-sgACC). * p < .05.*

Whole-Brain Analysis

Next, I submitted cross-correlation maxima maps to paired-sample t-tests (3dttest++). A different technique to my ROI analyses, this method found the maximum correlation between heart rate and BOLD at varying delays (which was applied equally between conditions). While global covariation with the heart rate signal was entirely positive in both conditions, I found reduced associations between heart rate and activity in 5 regions during the suspenseful (vs non-suspenseful) movie. Of particular relevance, I observed associations in precuneus, vmPFC, and bilateral putamen (figure 7.6; results listed in table 7.1). For descriptive purposes, I also extracted mean delay across subjects and conditions in these clusters, which suggested HR was preceding activation by ~16s.

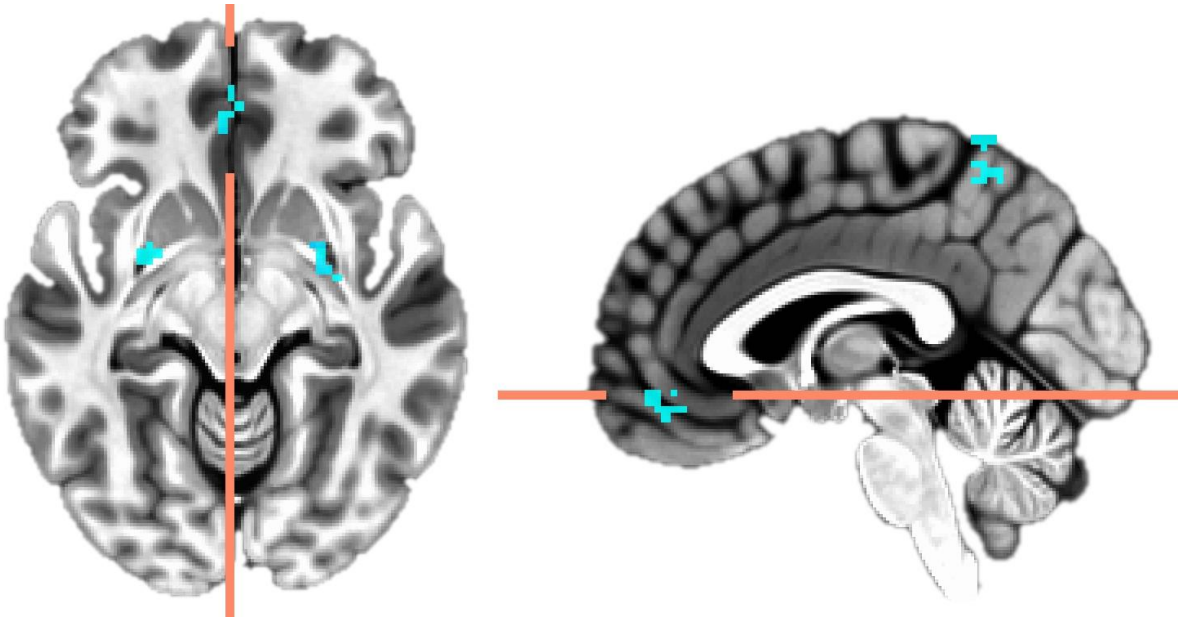

Figure 7.6. *Whole-brain differences in cross-correlation maxima (suspense - non-suspense movie) projected onto a template brain (MNI x=2, y=1, z=-8, voxel-wise p < .001, cluster-corrected k > 16). Taking a different approach to my ROI analysis, this technique selected the maximum correlation between activity and heart rate across varying lags within each voxel. When contrasting conditions, this suggested coherence between heart rate and activity in precuneus, vmPFC, and bilateral putamen was lower during the suspense condition compared to non-suspense.*

Table 7.1. *Whole-brain clusters from cross-correlation maxima maps. Delay has been calculated after accounting for a hemodynamic lag of 6s and refers to the delay from HR to BOLD.*

Region	Voxels	Peak	*p*	Mean delay (95% CI)
Precuneus	56	[-12, -58, 69]	<.01	17.2s (12.9, 21.5)
Right cuneus	33	[26, -98, 12]	<.01	14.3s (10.0, 18.6)
Left putamen	29	[-26, 2, -11]	<.02	16.1s (11.5, 20.8)
Right putamen	25	[28, -5, -11]	<.02	16.1s (11.7, 20.5)
vmPFC	25	[-2, 45, -16]	<.02	16.8s (12.4, 21.2)
Right cerebellum	17	[24, -82, -24]	<.05	21.8s (16.1, 27.5)

7.5 Discussion

Prior literature implicates a moderating role of anxiety on the communication between the brain and the autonomic nervous system (Critchley, 2005; Makovac, Meeten, Watson, Herman, et al., 2016). Whether these findings generalize to relatively more naturalistic settings has yet to be tested. Consequently, I sought to test the extent to which the association between neural responding (BOLD signal) in threat circuitry and autonomic responding (heart rate) may change as a function of an anxiety-inducing movie clip. Specifically, I tested whether the association between heart rate and activation/connectivity across the 'defensive response network' differed as participants' watched anxiogenic and control movie clips (suspenseful and non-suspenseful). I found evidence for anxiety-relevant alterations in the association between heart rate and: 1) amygdala-dorsomedial prefrontal cortex dynamic connectivity; and 2) amygdala-sgACC dynamic connectivity. Specifically, I observed positive associations between amygdala-prefrontal dynamic connectivity and heart rate in the non-suspenseful condition and this was reduced to negative (amygdala-dmPFC) or non-significant (amygdala-sgACC) associations during the suspenseful condition. Moreover, my whole-brain analyses revealed anxiety-relevant reductions in the association between heart rate and activity in precuneus, vmPFC, and bilateral putamen.

My general framework posited that states of anxiety would be associated with increased coherence between threat circuitry responses and heart rate. This was motivated by the idea that anxiety promotes both top-down signaling to increase sympathetic activity (and consequently, cardiac activity), as well as promoting increased interoceptive awareness/sensitivity to cardiac activity. However, incongruent with this framework, the results implied coherence was reduced in the suspense condition, compared to the non-suspenseful condition. I offer several interpretations.

While anxiety appears to increase sympathetic activation, this is often preceded by faster acting parasympathetic withdrawal (Critchley, 2009). Perhaps the results are thus better understood in terms of parasympathetic withdrawal. Prior studies outside the anxiety literature have also implicated associations between parasympathetic activity and suppression of sgACC/dmPFC responses (Barber et al., 2020; J. Kim et al., 2011; O'Connor et al., 2007). Especially in the context of the available scanning

durations (< 10 minutes per duration) and that the coherence measures were restricted to relatively short delays (0s to ~16s), it is plausible that the results highlight the impact of anxiety 'releasing the brake' on parasympathetic control via amygdala-prefrontal circuitry.

It is also important to consider that effects may be driven by the non-suspenseful, control condition. The non-suspenseful condition, 'Partly Cloudy', contains positively-valenced content. Anxiety is not the only emotion to impact autonomic responding. Positively valenced stimuli can still elicit responses in peripheral physiology. For instance, prior research has noted cardiac acceleration in response to happy faces (albeit of a lower magnitude than negatively-valenced faces), and this may correlate with responses in the amygdala (Critchley, 2005). It is equally plausible that the effects do not pertain to reduced communication between central and peripheral nervous systems; rather, increased communication as a function of positively-valenced content.

A final consideration for interpretation is that reduced coherence may be underpinned by bottom-up, interoceptive processes. In chapter 6, I failed to find associations between individual differences in trait anxiety and heart rate responses to the same anxiogenic stimuli used in the present study (all the while still inducing states of anxiety). Thus, the anxiogenic stimuli may not be eliciting threat-relevant top-down, autonomic responses. As such, bottom-up interoceptive influences must be considered. Prior work suggests increasing awareness of interoceptive signals, such as attention to respiration, appears to be associated with altered amygdala-prefrontal responses (Doll et al., 2016). Therefore, the measures of neural-autonomic coherence may be driven by the degree to which individuals are attending to their own cardiac signals during movie-watching. A primary function of adaptive anxiety is to attend to threats in the environment (Mobbs et al., 2015). As such, participants' engagement in the suspenseful condition could feasibly detract from interoceptive integration that was apparent during the non-suspenseful condition. Put simply, another interpretation is that participants are attending to their own heart rate more during the non-suspenseful condition compared to the suspenseful condition. However, it is outside the scope of the current study to draw conclusions regarding directional causality. It may therefore be useful for future studies to consider the implementation of active manipulations

(e.g., pharmacological or behavioral) to disentangle the contribution of interoceptive awareness to neural responses during movie-watching.

It is important to contextualize the results also with the non-significant results, as well as my methodological constraints and choices. All presented analyses were preregistered. However, I selected activation within the amygdala, dorsomedial prefrontal cortex, and insula to be the primary measures of coherence (rather than previously discussed dynamic connectivity). I failed to find evidence of altered coherence between heart rate and activation in these regions between conditions. This may represent a true null and effects primarily emerge at the level of communication between regions (connectivity) rather than activity within regions. Given the sample size (n = 29) and neuroimaging measure, it is difficult to provide evidence in favor of the null and the presented results should be considered preliminary. I therefore recommend future work to test whether the absence/appearance of anxiety-relevant alterations in neural-autonomic communication does primarily emerge at the level of circuitry, as opposed to regional activity (at least, in relation to amygdala-prefrontal circuitry).

Replication of the results is especially important given the exploratory nature of the coherence measures, which used varying methods to approximate neural-autonomic coherence (dynamic time warping, bivariate correlations, and cross-correlation maxima). The measures were either predominantly data-driven (dynamic time warping and cross-correlation maxima) or assumed instantaneous correspondence between BOLD and HRF-lagged heart rate (bivariate correlation). It will be useful to generate characterizations of the temporal relationship between ongoing neural activity (BOLD) and cardiac responding in these contexts; for instance, through the use of methods such as vector autoregression to derive exact temporal lags (and whether this varies across regions). Following this, research into neural-autonomic communication may be able to produce measures more sensitive to the effects of induced anxiety, and hold potential to disentangle associations with sympathetic vs parasympathetic activity.

Generalization of my results to other movie stimuli is also strongly encouraged. Here, I conducted the present study in order to provide generalizability from the anxiety literature to a relatively more naturalistic platform. However, the present study used a

dataset which included 29 subjects watching short movie clips (< 10 minutes per condition). It will be important to test whether these effects hold when using: different movie stimuli varying in low-level sensory features, especially as the suspense conditions was grayscale and non-suspenseful condition was not; larger/longer samples, due to the potential for instability of effects in small sample sizes (Marek et al., 2022); and other proxies for autonomic responding (e.g., skin conductance). Finally, the present findings need to be contextualized entirely in terms of induced states of adaptive anxiety. I cannot conclude whether these results translate to pathological levels of anxiety. Future work should seek to investigate between-subjects effects of maladaptive anxiety on neural-autonomic communication during movie-watching, which has been observed in task-based designs (Abend et al., 2022).

Conclusion

I aimed to investigate how communication between the brain and the autonomic nervous system may change as a function of anxiogenic movie-watching. Here, I found preliminary evidence for anxiety-relevant alterations in the coherence between heart rate and amygdala-prefrontal dynamic connectivity (amygdala-dorsomedial prefrontal cortex and amygdala-subgenual anterior cingulate). Moreover, I found evidence for alterations in coherence between heart rate and activity in precuneus, vmPFC, and bilateral putamen. However, effects were in the inverse direction to which I hypothesized. Coherence was positive during the non-anxiety condition, but reduced during the anxiogenic condition. This was demonstrated in 29 subjects undergoing < 10 minutes of scanning per condition. Future work testing the stability of these with longer (and different) movie stimuli, as well as in pathological levels of anxiety, will be important for grasping a better understanding of the contributions of this circuitry to peripheral physiology.

8. General Discussion

Traditional experimental paradigms, such as threat of shock, have build a fundamental understanding of the neurobiological basis of *state* and *trait* anxiety. Anxiety appears to be associated with a broad cortico-subcortical-autonomic network which drives processes such as threat vigilance (Chavanne & Robinson, 2021; Shackman & Fox, 2021). However, our understanding is predicated on a limited range of tightly-controlled paradigms. Whether anxiety presents itself in the same way 'in the wild', outside of such laboratory settings is unknown. One platform to bridge this gap is through movie-watching. Movies can evoke emotional states and present stimuli in a more contextually-rich, dynamic, naturalistic manner. Yet, there is scarce research investigating the neurobiological manifestiations of anxiety in response to movies. In order to test whether findings from the anxiety literature generalize to relatively more naturalistic settings, I therefore conducted a series of studies assessing the extent to which anxiety impacts neural, subjective, and autonomic responses to movies.

8.1 Summary of Findings

Chapter 2

I began by investigating the impact of anxiety on neural responses to movies. The prior literature implicates a 'defensive response network' that underpins anxiety (Abend et al., 2022). Core to this network is an amygdala-dorsomedial prefrontal circuit, posited to drive aversive amplification of threat-relevant stimuli in the environment. For instance, amygdala-prefrontal response appear to biasing perceiving faces as fearful (Robinson et al., 2012, 2014). Accordingly, chapter 2 set out to test whether individual differences in trait anxiety were associated with amygdala-prefrontal responses to faces in movies. I failed to find evidence of altered responses in this amygdala-prefrontal circuit, but did note associations between anxiety and activation to faces/speech in other regions, such as superior parietal lobe and primary auditory cortex.

Chapter 3

The anxiety literature implicates that inducing *state* anxiety through threat of shock elicits associations between *trait* anxiety and amygdala-prefrontal response, irrespective of stimulus-specific processing (Vytal et al., 2014). Therefore, I sought to assess the degree to which trait anxiety was associated with amygdala-prefrontal responses to suspenseful movies (a naturalistic approach to inducing state anxiety). I demonstrated significant associations between trait anxiety and amygdala-prefrontal responses during suspenseful movie-watching. This was in the inverse direction to which I predicted: positive associations between trait anxiety and amygdala-prefrontal responding were greatest when suspense was low.

Chapter 4

One interpretation of chapter 3's findings was that positive associations between trait anxiety and amygdala-prefrontal connectivity during low suspense scenes could reflect differences in connectivity while at rest. In order to aid interpretation of chapter 3's findings, I subsequently analyzed resting-state data from the same participants as chapter 3, using the same anxiety scores and regions of interest. I failed to find any associations between self-reported trait anxiety and resting-state 'intrinsic' functional connectivity, though did report an association between a behavioral measures of threat vigilance and amygdala-periaqueductal gray connectivity.

Chapters 5-6

My findings from chapters 2-4 highlight that movie-watching can be used to elicit anxiety-relevant idiosyncratic responses in neural circuitry. However, whether said brain activity might manifest in physiological (autonomic) responses and subjective experiences of state anxiety remained unclear. In order to test this, I first developed a pipeline for extracting heart rate, a proxy for autonomic balance, from cardiac recordings in chapter 5. I then tested whether *trait* anxiety was associated with differences in cardiac activity (i.e., heart rate) and subjective responding (i.e., continuous *state* anxiety ratings) to the same movie stimuli as chapter 3. I

demonstrated that *trait* anxiety symptoms were associated with continuous *state* anxiety during suspenseful movie-watching, but not heart rate.

Chapter 7

In chapter 5, I failed to find evidence of an association between trait anxiety and autonomic responses to a suspenseful movie clip. This initially suggested that the idiosyncratic neural responses I observed in chapter 3 were not associated with engagement of peripheral physiology. However, this did not rule out the possibility of general alterations in the communication between the brain and the autonomic nervous system. In chapter 7, I therefore explored the extent to which the 'defensive response network' may be responsible for communication with the autonomic nervous system while under states of anxiety. I predicted that anxiety-inducing movie clips would increase communication between the 'defensive response network' (amygdala, dmPFC, and insula in particular) and the autonomic nervous system. Here, I compared heart rate and neural (BOLD) responses in subjects watching an anxiogenic, suspenseful movie clip and a non-suspenseful, control movie clip to produce measures of neural-autonomic coherence (the similarity of heart rate and BOLD responses). I predicted that the anxiety would be associated with increased neural-autonomic coherence in the suspenseful condition compared to the non-suspenseful condition. My results provide preliminary evidence for altered coherence between amygdala-prefrontal responding and autonomic activity during suspenseful movies. However, results were in the inverse direction to my prediction. There was a positive association between heart rate and amygdala-prefrontal responses in the non-suspenseful control condition, but this was reduced to negative or non-significant in the suspense condition. This indicated that communication between the brain and the autonomic nervous system may be greater during non-anxiogenic movies compared to anxiogenic movies.

8.2 Implications

The overarching question throughout my thesis was: do our findings from the anxiety literature generalize to more naturalistic settings? The preliminary answer: in some cases, yes; in others, no; but—most often—neurobiological responses may diverge to

previous observations from traditional paradigms. I studied anxiety using a range of approaches, investigating effects of trait and state anxiety on the brain, peripheral physiology, and subjective responses. I found mixed evidence. Some predicted effects, such as an association between trait anxiety and amygdala-dmPFC responses to faces, were not apparent during movie-watching. Some relationships, such as that between anxiety and neural-autonomic communication, emerged in the opposite direction to results from previous task-based studies. These findings warrant potential re-evaluation/contextualization of prior anxiety research; specifically, calling into question whether such results represent the manifestation and impact of anxiety on neurobiological responses outside specific laboratory settings and in daily life. In this section of the thesis, I discuss such re-evaluations primarily in relation to the neural and autonomic underpinnings of anxiety. I then discuss more broadly the utility of movie-watching for anxiety research.

Neural Responses to Faces

A wealth of literature indicates that both state and trait anxiety impact the processing of faces. For instance, the speed, accuracy, and intensity in which emotional facial expressions are perceived appears to be facilitated by anxiety (Arrais et al., 2010; Bradley et al., 1999; Doty et al., 2013; Kavcıoğlu et al., 2021; Robinson et al., 2011). A bias toward categorizing faces as fearful appears associated with increased engagement of amygdala-dmPFC circuitry, the degree to which varies with individual differences in trait anxiety (Robinson et al., 2012, 2014). As such, I predicted that individuals scoring higher in trait anxiety would demonstrate increased amygdala-dmPFC responses to faces during movies. I observed overall increased amygdala-dmPFC responses to faces in the whole sample, but this effect did not vary as a function of trait anxiety scores.

This could represent a true null, but theoretical interpretation needs to be grounded within the analytical approach. Here, I failed to find an association between trait anxiety scores and amygdala-dmPFC responses to faces *in general*. Responses to faces of all emotional expressions was assessed due to the implication that anxiety biases processing across all emotional expressions, including neutral faces (Kavcıoğlu et al., 2021). I did not detect a significant association between amygdala-

prefrontal responses to faces in general and trait anxiety (though there were associations with superior parietal activation). Thus, I found tentative evidence against the notion that anxiety is associated with amygdala-prefrontal responses to all faces in more naturalistic settings. However, some studies have implicated bias perception appears primarily for threat-relevant faces, fearful expressions in particular (Doty et al., 2013; Robinson et al., 2012). In chapter 2, responses to specific facial expressions (i.e., fearful) were not evaluated due to concerns regarding the ability of the face recognition software to accurately decode emotional expressions. This leaves open the possibility that trait anxiety may still shape amygdala-dmPFC responses to faces, but only to specific emotional expressions.

It is also import to consider context. I adopted an approach typically employed in the task-based literature, feature based general linear modelling, which tested for average responses to faces across entire movies. This modelling was time-invariant: it assumed brain responses would be consistent throughout movies. While the association between trait anxiety and amygdala-prefrontal activity may potentially be observable in minimal contexts (such as resting-state; Kim et al., 2011), this association is likely greatest in anxiogenic contexts, congruent with diathesis-stress models of anxiety (Zuckerman, 1999). Therefore, the findings from chapter 2 indicate that trait anxiety may not be associated with altered amygdala-prefrontal responsiveness to faces in general, but it is plausible that such effects could emerge dependent on wider emotional context.

Neural Responses to Suspense

I next took to investigating anxiety while taking into account the overall emotional dynamics of the movie. Specifically, I extracted dynamic measures of brain connectivity and embedded these features within ratings of ongoing suspense, which served as a naturalistic anxiety induction. Findings from the threat of shock literature implicate that as general anxiety increases, amygdala-prefrontal connectivity increases, and this is greatest for those scoring high in trait anxiety (Robinson et al., 2012, 2014). As such, I predicted positive associations between trait anxiety and amygdala-prefrontal responses would be greatest during highly suspenseful scenes. Yet, my findings indicated the associations were greatest during low suspense scenes.

Put simply, trait anxiety was associated with relatively increased amygdala-dmPFC responding during low suspense scenes and reduced amygdala-dmPFC engagement during high suspense scenes.

One interpretation is that the positive association between trait anxiety and amygdala-prefrontal connectivity during low suspense scenes could arise as a function of difference in 'intrinsic' connectivity at rest. My follow-up study using resting-state scanning revealed no such associations between trait anxiety and 'intrinsic connectivity'. As such, there is evidence to suggest effects were driven by the content of the movie. My visualizations of neural time series indicated idiosyncratic responses were far more nuanced and dynamic than findings from the threat of shock literature would imply. That is, anxiety-relevant idiosyncratic responses to suspense may not arise simply as a linear function of suspense.

One perspective for contextualizing these results comes from research in media psychology literature. There is evidence to suggest that the initial experience of anxiogenic movie scenes may be aversive to all, but there is large individual variations in subjective experiences preceding/following such scenes. Some may experience positive emotions, while others feel anxiety (Clasen et al., 2018). Indeed, neuroticism, which strongly correlates with trait anxiety (r = ~.7, Muris et al., 2005), tends to be associated with reduced seeking of anxiogenic media, as well as greater self-reported anxiety in the aftermath of such content (Clasen et al., 2018). This is congruent with neuroimaging studies which find anxiety is associated with earlier amplified anticipation as well as slowed habituation of amygdala-prefrontal responses to aversive events (Blackford et al., 2013; Campbell et al., 2014; McMenamin et al., 2014; Najafi et al., 2017; Protopopescu et al., 2005). I therefore suggest that the effects observed associations between trait anxiety and amygdala-dmPFC engagement in chapter 3 are arising due to suspense, but these dynamics might relate to early anticipation of- and slowed disengagement following suspenseful scenes.

The key findings here are that associations between trait anxiety and responses in a fundamental threat circuit (amygdala-dmPFC) do emerge during suspenseful movie-watching. However, incongruent with threat of shock paradigms, the association between trait anxiety and amygdala-dmPFC engagement may not simply be linearly modulated as a function of anxiogenic features of the environment. Rather,

there are nuanced dynamics relating to anxiety (potentially earlier anticipation of and/or slowed disengagement to threat) which drive idiosyncratic responding. However, what exact processes these effects may reflect remained unclear. Could this brain activity relate to engagement of peripheral physiology? Are these individual differences in neural responses manifesting in altered subjective experiences of suspenseful movies? In my remaining chapters, I sought to address these questions.

Autonomic Responses to Suspense

Even at the outset of modern psychology, peripheral physiological responses have always been considered as playing a role in affective states (Dewey, 1894; James, 1894). In relation to anxiety, it has been theorized that peripheral physiology is engaged to facilitate defensive behaviors in response to threat, namely fighting or fleeing (Cannon, 1929). Activity across the autonomic nervous system may aid such behaviors; for instance, by providing increased blood flow to support the body for physical exertion (Critchley, 2009; McCorry, 2007). Experimental work does suggest state and trait anxiety are be associated with autonomic responding, as reflected in cardiac activity (Beatty & Behnke, 1991; Kantor et al., 2001; Levine et al., 2016). In this thesis, I failed to replicate such effects in suspenseful movie-watching.

I tested the extent to which trait anxiety symptoms were associated with heart rate during a suspenseful movie clip (the same as used in chapter 3). No associations with heart rate were apparent. This is in spite of trait anxiety being associated with states of anxiety and engagement of the 'defensive response network' to the same suspenseful movie. If this represents a true null, this should be considered in discussions of anxiety in relation to threat responding. The framework motivating these studies was based on findings from literature on responding to personally-directed threats; for instance, responses to uncertain threat of shock. However, in recent years, it has been emphasized that such threat responding may not be necessary for eliciting subjective experiences of anxiety, and vice versa (LeDoux & Pine, 2016; Taschereau-Dumouchel et al., 2022). My results do not invalidate previously observed associations between self-reported trait anxiety and physiological responses to threatening stimuli. Rather, in line with recent calls, we cannot presume that autonomic responses are always associated with traits/states of anxiety. This may be especially apparent in

conditions outside traditional paradigms, which have typically framed anxiety around personally-directed threat. This seems particularly the case for movies, which do not pose a direct, physical threat toward an individual.

Subjective Responses to Suspense

When assessing how *trait* anxiety may shape *states* of anxiety during suspenseful movie-watching, I decided to employ an approach which might encapsulate symptom-level information, intersubject representational similarity analysis. This was because prior work has indicated that *types* of trait anxiety symptoms play a key role in the relationship between anxiety and autonomic responding (Pittig et al., 2013). Accordingly, I compared individual differences in trait anxiety symptomatology. This was operationalized as pairwise similarities (comparisons between pairs of subjects) in responses across questionnaire items (which assess different types of anxiety symptoms, such as worry and somatic symptoms). Put simply, each pair of subjects was compared as to how similar they were in terms of the *type* of anxiety symptoms they experienced. This is in contrast to my previous analyses which used summated responses across all items to engage overall *intensity* of trait anxiety symptoms.

Using this approach, I detected a significant association between trait anxiety symptom profiles and subjective responses to a suspenseful movie (i.e., continuous ratings of state anxiety). In other words, different *types* of trait anxiety symptoms were associated with differences in *when* individuals felt anxious during suspenseful movie-watching. The association between trait anxiety and continuous ratings of state anxiety was not apparent when using sum scores (*intensity* of trait anxiety). Taken together, this indicated that the *type*—but not overall *intensity*—of trait anxiety symptoms is associated with subjectively-experienced states of anxiety during suspenseful movies.

If these differences are underpinned by brain responses to suspense, this holds implications for neuroimaging analyses of movie data. For instance, in chapter 2 I tested the association between trait anxiety summary scores and neural responses to faces, but failed to detect a significant relationship. Given that symptom types appear to show a stronger mapping onto subjective experiences than general symptom intensity (at least, as measured by self-report questionnaires), linking neural responses to movies with trait anxiety symptom *types* (rather than sum scores) might

prove a more sensitive approach. Calls for such transdiagnostic approaches in psychiatry, which go beyond diagnostic categories and look at underlying dimensions of pathology, have been made in recent years for both task and resting-state neuroimaging research (Parkes et al., 2020). Due to the use of adaptive questionnaires or lack of item-level data, such a symptom-level analysis could not be implemented in my neuroimaging chapters. Nonetheless, my findings motivate future movie studies to consider transdiagnostic/symptom-level analyses when studying the neurobiology of anxiety.

Neural-Autonomic Responses to Suspense

Although I previously failed to detect effects of anxiety on autonomic responses in general, this led me to my final line of inquiry. Even in the absence of observable increases/decreases in overall autonomic activity, it is possible that reciprocal communication between anxiety circuitry and the autonomic nervous system could nonetheless change as a function of anxiogenic movie-watching. Compared to work which studies anxiety's impact on central and peripheral nervous systems as separate lines of inquiry, there is scant literature directly investigating neural-autonomic communication in the context of anxiety. Some work has implicated increased communication between the 'defensive response network' and autonomic nervous system during states of anxiety (Abend et al., 2022; Makovac, Meeten, Watson, Herman, et al., 2016). In this thesis, I sought to test whether these effects were apparent during movie-watching. I predicted that suspenseful movie-watching would elicit increased communication between the 'defensive response network' and autonomic nervous system. To test this, I assessed the similarity between neural and heart rate time series during suspenseful vs non-suspenseful movie clips to produce measures of neural-autonomic 'coherence'. I found significant associations, but in the inverse direction to predicated. Neural-autonomic coherence was stronger (and positive) in the non-suspenseful condition compared to the anxiogenic, suspenseful condition (which was non-significant or negative).

Given the neuroimaging method (fMRI), sample size (n = 29), and proxy for autonomic responding (heart rate), it is difficult to draw strong inferences from these effects. Nevertheless, for shaping future hypotheses and methodological choices, it is

worth considering the implications of these findings, especially given their divergence from the prior literature. Anxiety has been often been discussed in regard to elicitation of increased sympathetic activation (Friedman & Thayer, 1998a). As such, I predicted that as activation/connectivity across the 'defensive response network' increases (in particular, amygdala, dmPFC, and insula), so would sympathetic activation (and consequently, heart rate). However, anxiety also appears to dampen the parasympathetic branch of the autonomic nervous system. Notably, parasympathetic withdrawal may occur more rapidly than increases in sympathetic activity (Critchley, 2009). One interpretation, is thus that decreased associations between neural responding and heart rate could arise due to withdrawal of parasympathetic control. In the control condition, amygdala-prefrontal circuitry may be associated with parasympathetic slowing of the heart. Conversely, in the anxiogenic condition, there may be a 'releasing of the brake'; amygdala-prefrontal circuitry may no longer instructing the parasympathetic branch to attenuate cardiac responses. Yet, this would still not explain the discrepancy with the prior literature.

An alternative interpretation is that effects were not necessarily driven by the suspenseful condition, but instead by the control condition. The non-suspenseful control condition, 'Partly Cloudy', contained positively valenced content. Positively valenced stimuli (e.g., happy faces) can promote cardiac acceleration, albeit this is of a typically weaker magnitude than negative valenced stimuli (Critchley, 2005). Nonetheless, an alternative interpretation is that these effects do not pertain to anxiety, but rather the impact of positive mood on increasing sympathetic activation (and conversely, heart rate).

My final interpretation is that these effects were relevant to anxiety, but did not arise due to top-down entrainment of autonomic responding from the brain. Instead, these results could have arisen as a function of bottom-up, interoceptive processes. This is especially important to consider given my findings (chapter 6) that did not provide evidence of the same suspenseful movie being associated with altered autonomic responses. It is thus possible that suspenseful movie watching reduces peoples' attention to internal bodily signals. For instance, one prior task-based study indicates that promoting attention to bodily signals (i.e., respiration) engages amygdala-prefrontal regions (Doll et al., 2016). It is therefore possible that the presented neural-autonomic coherence measures may be sensitive to such

interoceptive awareness. If a key purpose of anxiety to attend to potential threats in the environment (Mobbs et al., 2015), engagement with the stimulus in the anxiogenic condition could have detracted individuals awareness of their own bodily signals, such as heart rate. In order to disentangle these effects, future research should seek to generalize these effects to other movies, use other measures of neural/autonomic responding, and consider active manipulations of autonomic balance in order to draw stronger causal inference.

I have now embedded my findings within the prior literature. However, the implications of this thesis are not limited to specific neurobiological models of anxiety. I now discuss broader implications from this work in relation to the framing and conceptualization of anxiety, how movies might be useful for development of prediction-oriented research, and its implications for clinical settings.

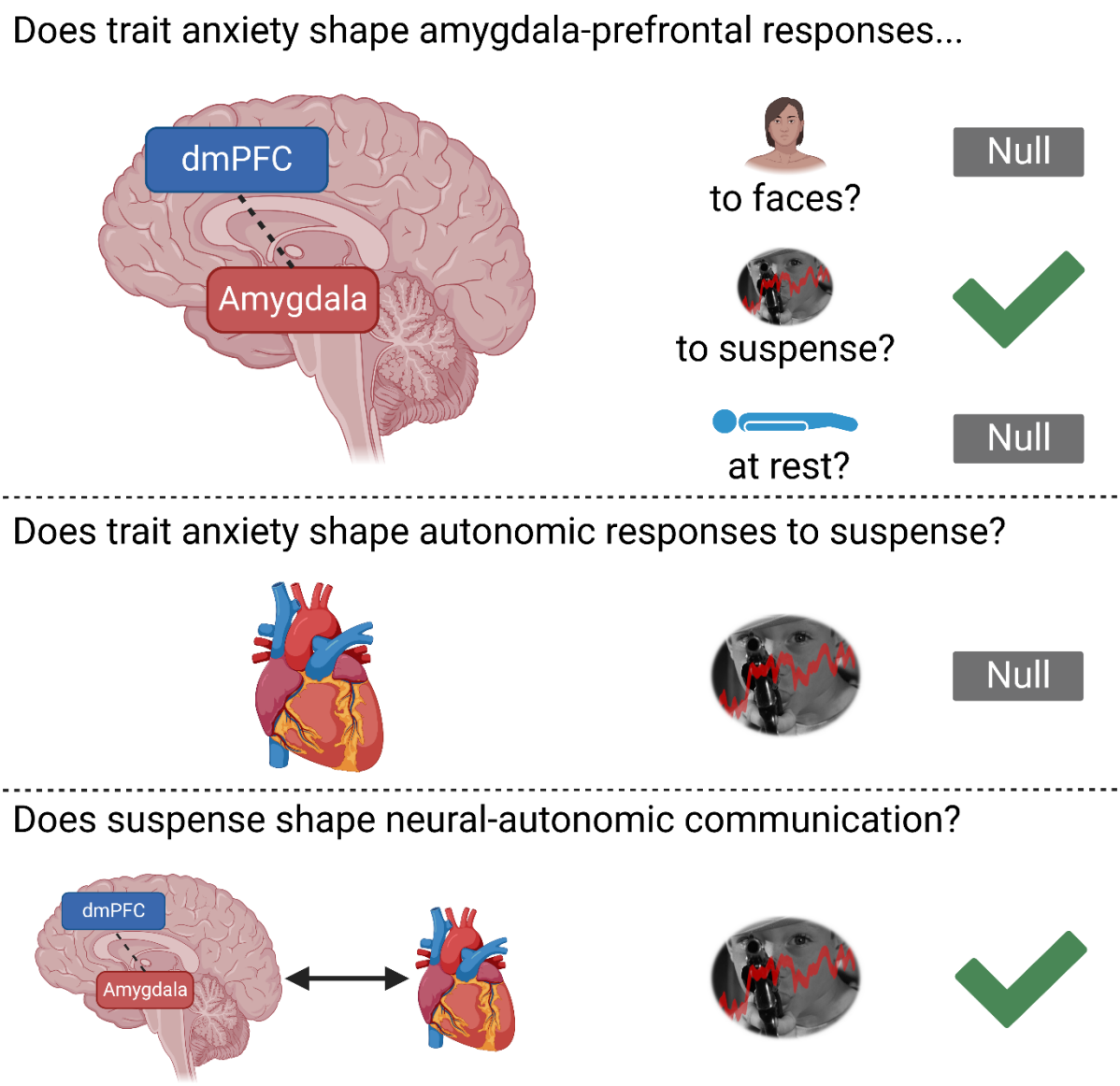

Figure 8.1. Illustrative summary of chapter findings. I tested associations between trait anxiety and amygdala-prefrontal connectivity. I failed to find an association in the context of face perception during movies (chapter 2), I did find evidence for an association in regard to suspense during movies (chapter 3), and these effects were not apparent at rest (chapter 4). I also failed to find an evidence of trait anxiety impacting autonomic responses to suspenseful movies (chapter 6), but did find suspenseful movies impact neural-autonomic communication (chapter 7). dmPFC = dorsomedial prefrontal cortex.

The Source of Threat Matters

In this thesis, I have discussed specific discrepancies between findings when using different state anxiety manipulations. Between traditional experimental paradigms, such as threat of shock vs CO2 challenges, there are inconsistencies; for example, in how trait anxiety biases the perception of faces (Doty et al., 2013; Dyer et al., 2022; Robinson et al., 2012). When comparing traditional paradigms to this thesis, there are also clear discrepancies. As highlighted previously, there are suggestions that states of anxiety (using traditional paradigms) are associated with *increased* communication between the brain and the autonomic nervous system (Abend et al., 2022; Makovac, Meeten, Watson, Herman, et al., 2016); yet, in suspenseful movies, I noted *decreased* communication. In addition to how ecologically-rich experimental procedures are, this speaks to a much broader point regarding how anxiety is induced and studied. Anxiety has typically been discussed as a general response to uncertain threat. However, different sources of threat might evoke different neurobiological responses.

Is suspense actually a naturalistic analogue to threat of shock? Threat of shock is not typically thought of as a desirable experience, while many people actively seek out anxiogenic media such as horror movies (Bantinaki, 2012). Threat of shock poses a direct, personal threat to an individual. Suspense-elicited anxiety is driven by potential harm to characters in a movie. Distinctions such as self vs other threat (which could also relate to empathy) may be one of the core factors underlying potential inconsistencies between threat of shock studies and the findings in the present thesis. For instance, when threat is personally directed, there could be a tuning of attention toward one's own bodily signals in order to monitor physical health. When threat is directed at others, perhaps such interoceptive awareness is not as necessary. It is therefore plausible that different modalities of anxiety induction elicit different neurobiological responses.

There is no doubt that threat of shock effectively induces anxiety, as has been repeatedly validated with self-report measures (Robinson et al., 2012). However, perhaps there needs to be a push toward more refined contextualization of findings in regard to specific modalities of anxiety induction. That is, moving the focus away from general states of anxiety, toward more refined discussions of how specific sources of anxiety impact the brain and behavior. For instance, personally directed physical threat

may interact with trait anxiety *during* the anticipation of the threat. However, as I discuss in chapter 3, perhaps trait anxiety is more strongly associated with responses in the aftermath of suspenseful scenes. This may relate to divergences in how some experience positive affect following such scenes, while others remain in a state of anxiety (Clasen et al., 2018). This would also be in line with different diagnostic categories of pathological anxiety; for instance, some being more general (generalized anxiety disorder) while others are more social (social anxiety disorder). Future research should seek to explicitly test discrepancies between sources of anxiety. One possible avenue could even be to explore this *within* movies; for instance, by testing the impact of anxiety as induced by uncertain physical vs social harm to characters.

Brain-Behavior Prediction

The present thesis was theoretically-motivated. For example, much of my hypothesis-testing was centered on an 'aversive amplification' hypothesis of amygdala-dmPFC function that posited this circuit as driving attentional biases toward threat. In order to investigate whether this was engaged in more naturalistic contexts (compared to threat of shock), I tested whether anxiety was associated with amygdala-dmPFC responses in movie-watching. Not all anxiety research is necessarily aligned with said goals of informing specific neurobiological models of anxiety per se. Another avenue of neuroscience research has been focusing on phenotypic prediction, which might confer benefits to the diagnosis and guidance of treatment for psychopathology (Dhamala et al., 2023; Parkes et al., 2020). These studies are often predicated on data-driven analyses of resting-state data to predict individual phenotypes, such as trait anxiety. A frequent, primary goal of said studies is to improve the accuracy of prediction algorithms to detect psychiatric symptoms (Dhamala et al., 2023; Parkes et al., 2020). Yet, resting-state derived predictive modelling of anxiety has yet to reveal successful, replicable results (Boeke et al., 2020).

Movie fMRI-derived connectivity measures have been demonstrated as outperforming resting-state based prediction of cognitive function (Finn & Bandettini, 2021). In relation to anxiety, I demonstrated that suspenseful movie-watching can elicit neurobiological individual differences in trait anxiety that are not apparent at rest. In this thesis, I did not analyze the data with prediction as a goal. This is because

multivariate, data-driven analyses of fMRI data can result in model parameters being difficult to interpretable in regard to brain activation/connectivity (Haufe et al., 2014). This could have hindered theoretical interpretation. At present, I am not aware of any research using movie fMRI data to target prediction of trait/pathological anxiety specifically. Given findings from this thesis, a useful first step for future studies could be to compare the relative utility of resting-state vs suspenseful movie fMRI data in the prediction of trait/pathological anxiety.

Clinical Evaluations and Interventions for Anxiety

If one goal of research into anxiety is to understand the biological and psychological processes anxious individuals engage with in day to day life, it is of utmost importance that experimental findings are tested in contexts that represent such daily experiences. Not just for our own theoretical interpretations, but because these studies hold impact outside of academia. In the context of clinical applications, the need for ecologically-rich anxiety research holds implications in both the assessment of pathological anxiety, as well the development of interventions.

There is currently a move in psychiatry research toward clinical translation of biological and cognitive measures to assess the impact of anxiety on the brain and cognition (in line with Research Domain Criteria, Cuthbert & Insel, 2013; Insel et al., 2010). Traditional task-based fMRI designs, such as face perception paradigms, are being used as biomarkers in clinical research to assess the efficacy of anxiolytic medication and psychological therapy (Gingnell et al., 2016). Yet, the present results highlight such tasks may not fully encapsulate the ways in which anxiety is manifesting and impacting an individual outside of these specific tasks. Therefore, there is a need to re-evaluate neuroimaging measures which assess the neural correlates and impact of anxiety on brain function in clinical contexts. Movie fMRI seems like a distant procedure for use in clinical assessment, but its development for clinical settings is already underway in other domains, such as presurgical language mapping (Yao et al., 2022). This thesis posits that piloting movie fMRI in future clinical trials is a novel and potentially useful endeavor that may yield clinically meaningful insights into the underlying mechanisms of anxiety disorders.

The motivation for validating findings from the anxiety literature in ecologically-rich settings also extends to the development of treatments. Therapeutic interventions are designed to alleviate anxiety in people's daily lives. Yet, only approximately half of those with anxiety disorders will respond to initial psychological/pharmacological treatment (Ansara, 2020; Clark, 2018; NHS Digital, 2022). These interventions are selected and developed based on our current evidence base. For instance, in recent decades, pharmacological agents (i.e., anxiolytic medication) have been synthesized with specific biological targets in mind that are derived from findings in basic research (Sartori & Singewald, 2019). In turn, traditional methods, such as fMRI-measured brain responses to static faces, are then employed in clinical trials to test whether anxiolytic medication is acting on hypothesized neural responses (Gingnell et al., 2016). If the research used to form the basis of therapeutic development does not fully or appropriately encapsulate the biological substrates of anxiety (i.e., due to a lack of ecological validity), this indicates there may be a need for potential re-evaluation in regard to the paradigms used to identify novel treatment targets for clinical interventions in anxiety. It may be fruitful that—alongside traditional cognitive paradigms—movie-watching is formally considered in developmental pipelines for therapeutic intervention (e.g., anxiolytic medication).

8.3 Limitations and Considerations

How Naturalistic are Movies?

This thesis was motivated with the need for extending anxiety research to more naturalistic settings. As such, I used a relatively more ecologically-rich procedure, movie-watching. However, this fundamental assumption of ecological validity should be scrutinized: are movies actually 'naturalistic'? Movie paradigms have only started to gain popular attention in recent years. There have been multiple calls for its widespread use to provide ecologically-rich settings in which to study mental processes more representative of everyday life (Eickhoff et al., 2020; Sonkusare et al., 2019; Vanderwal et al., 2019; Vigliocco et al., 2023). On the other hand, some have argued that movies should not by default be considered representative of everyday life (Grall & Finn, 2022). Movie scenes are built on the foundation of finely curated, artistic script content, lighting, set designs, camera angles/distance, video

editing, acting, sound, and more. Dependent on the purpose and goal of a movie, these directing decisions are made to engage and entertain audiences, but were not explicitly designed to represent everyday experiences per se. As such, the type of content relating to dialogue, sets, acting, and more cannot automatically be assumed to be representative of how people view the world in their everyday lives.

The present thesis contained a range of movie stimuli, but a suspenseful grayscale clip from the 1960's, '*Bang! You're dead*', was used for 3 of the studies (chapters 3, 6, and 7). By asking participants to provide ongoing behavioral ratings of anxiety (chapter 6), I validated that this was useful for evoking states of anxiety. However, is this specific stimulus representative of conditions outside the laboratory? With currently available evidence and limitations on neuroimaging, there is not clear answer yet. It is important to understand that ecological validity occurs on multiple continuums. Movies are not a complete solution to ecological validity, but rather may serve as a stepping stone toward naturalistic generalizability. Only by combining evidence from movie-watching with other approaches, such as wearable neuroimaging devices employed outside the laboratory (e.g., OPM-MEG and fNIRS; Boto et al., 2018; Piper et al., 2014), can we start to build a better understanding of anxiety-dependent neurobiological responses that are consistent across contexts.

Finally, it is important to acknowledge that movie-watching is—arguably—a naturalistic behavior in and of itself. The average person in the UK consumes ~5 hours of TV/Video content a day (Ofcom, 2022). Given that so many people spend a significant proportion of their day consuming video media, it could still be considered a worthwhile naturalistic behavior to study in and of itself, even if perception of such stimuli does not generalize to other settings. All things considered, it is important for researchers to consider the extent to which their stimuli may be representative of other environments, and to discuss findings accordingly. Careful consideration should be given to how and why a movie stimulus may be useful and ecologically-valid for addressing a research question.

Analytical Considerations for Movie-Based Anxiety Research

An advantage of movie paradigms is that they offer a platform in which data can be flexibly analyzed (more so than task and rest) to evaluate a broad range of mental

process, from fundamental sensory systems to emotion. Moreover, the data can be approached from a variety of analytic perspectives. My conclusions throughout this thesis need to be contextualized within methodological decisions. Such analytical choices typically entail a degree of compromising either model sensitivity or interpretability. This is of course not specific to movie data. However, movies are socially/emotionally rich, complex, multisensory stimuli. Employing interpretable models to capture such complex stimuli is challenging and can make inferences toward the null especially difficult. Below, I review three key techniques (feature-based GLM, seed-based functional connectivity, and intersubject representational similarity analysis) in light of my findings.

Feature-based GLM has been a popular tool in cognitive neuroscience research since it was one of the first approaches to be widely adopted as a tool for analyzing task-based fMRI data (Friston et al., 1994). This approach relies on numerous assumptions, such as when and how the brain responds to stimuli. When tasks are purposefully curated, there can be greater confidence in such assumptions. For instance, by interleaving stimuli with a blank screen for several seconds, hemodynamic responses can be more confidently attributed to processing of specific stimuli. However, when applying this to movie data, such an approach is challenging as stimulus-specific properties in movies (e.g., nuanced fluctuations in socio-emotional context) can be difficult to model with confidence (Vanderwal et al., 2019). In chapter 2, I report that this approach revealed consistent main effects of expected brain activations in response to faces (fusiform gyrus) and spoken language (primary auditory cortex). Therefore, this approach does seem to offer utility for capturing within-subject responses to visual/auditory features of movies. While I did observe some between-subjects interactions for activation to stimuli and trait anxiety, these were small clusters of activation outside my hypothesized regions. This does not discount the implementation of feature-based modelling to study anxiety with movie data, but my results must be understood within model assumptions; in this instance, invariance of responses over time (e.g., activation to faces across a movie). Such an assumption may result in false negatives, as the modelling may fail to capture anxiety-relevant, contextual modulations of brain responses. Indeed, chapter 3 outlined there may well be interactions between trait anxiety, ongoing suspense, and brain responses. With this in mind, I encourage future studies to continue using such an

approach, especially when investigating within-subject responses to stimuli. However, careful consideration of the content of movie stimuli (e.g., fluctuations in suspense, specific emotional expressions, language) should be made when adopting such an approach for investigating the impact of trait anxiety on brain responses.

I also made use of seed-based functional connectivity. Unlike feature-based GLM, this approach makes fewer assumptions about neural time courses as the modelling is driven by the brain data (rather than by assumed hemodynamic responses to stimuli). This was employed as such a data-driven approach might have been more sensitive to detecting brain-anxiety relationships. I failed to find evidence of robust associations between brain connectivity and self-reported trait anxiety (though did report one correlation with a behavioral measure threat vigilance). One possibility is that the very fact that it relies on minimal assumptions means statistical sensitivity is lost. This approach essentially ignores time-locked content and concatenates data across the movie into connectivity metrics for each subject. Consequently, different subjects' connectivity measures could be driven by different stimuli/timings in the movie, making functional connectivity measures less sensitive to associations with trait anxiety. This may be appropriate for short video clips which consistently elicit specific affective states, but less appropriate when dealing with full-length movies which vary in the types of emotions they evoke. An alternative, is the use of dynamic connectivity measures, though these require further processing/modelling (e.g., implemented as part of intersubject correlation, as in chapter 3). Because of this, and in light of the findings from the present thesis, I encourage anxiety researchers to be particularly careful when using seed-based functional connectivity for movie fMRI, ensuring there is adequate justification for such an approach.

Lastly, I adopted a hybrid approach, intersubject representational similarity analysis, for the study of individual differences in trait anxiety. This analysis operates by comparing pairwise similarities in trait anxiety measures with pairwise similarities in neural, physiological, and/or behavioral time series. This can be applied to movie data due to the time-locked nature of the stimuli (participants are watching the same movie, so time series can be compared). This can confer a benefit to sensitivity, as it does not make assumptions about what specific features are associated with individual differences in trait anxiety. In chapter 6, this approach proved fruitful in

detecting associations between trait anxiety symptomatology and subjective responses to a suspenseful movie (continuous ratings of state anxiety); however, this effect was not apparent when using standard trait anxiety measures (sum of responses to an anxiety questionnaire).

Future movie fMRI anxiety research using intersubject representational similarity analysis should consider approaches which don't assume linear (or monotonic) increases in brain activation alongside trait anxiety sum scores. Specifically, when constructing psychological similarity matrices, symptom-level data should be a key consideration. The key advantage for implementing this as part of intersubject representational similarity analysis (as opposed to within-subject modelling) is that it does not require dimensionality reduction (e.g., transdiagnostic factor analyses; Wise et al., 2023), as participants symptom-level data can be directly contrasted between participants.

A disadvantage of intersubject representational similarity analysis is that there can be a loss to theoretical specificity (compared to feature-based GLM). It can be inferred that trait anxiety-relevant idiosyncrasies do arise during movie-watching. However, which features of the movie may be driving these effects is unknown (at least, with how it was implemented within my thesis). Moreover, the lack of feature-based modelling could, in some instances, also obscure effects. Exactly because intersubject representational similarity analysis does not make assumptions regarding time points that drive effects, it weights every data point in a time series equally. If a movie is only eliciting anxiety-relevant responses for a small period of time (e.g., 10% of the movie), it is possible that the remainder of irrelevant data points (e.g., 90% of the movie) may mask effects. This is especially important context for null results. In chapter 3, I failed to find an association between trait anxiety and movie-wide brain similarity measures. Any inferences toward the null need to be contextualized in the fact that brain time series are compared across the entirety of movie stimuli and does not exclude the possibility of shorter, discrete idiosyncratic responses (which may arise in response to suspense, as reported in the TR by TR analysis of chapter 3).

I have outlined some of the advantages and disadvantages of three methods for analyzing movie data in light of findings from my experimental chapters. Choice of analysis should be primarily dependent on the research question of interest and relies

on some compromise between model interpretability vs sensitivity. Given the lack of movie studies investigating anxiety, I believe it is too early a stage whereby strong inferences should be drawn from singular analyses of movie data. Instead, I believe it will be beneficial for future studies to implement multiple techniques in parallel.

Data-driven approaches may help provide a basic characterizations of brain responses to movies (e.g., whole-brain connectivity measures for different scenes) which can then be used to guide further hypothesis-testing in independent datasets/different movies (e.g., selecting a subset of functional connections we think show strongest responses to anxiogenic scenes). Additionally, employing multiple techniques in tandem may help to curtail specific assumptions that constrain analyses when relying on single techniques. For instance, in chapter 7, I employed three different techniques for approximating neural-autonomic communication. This was due to a lack of strong priors as to the temporal relationship between central and autonomic nervous systems. By using complimentary techniques alongside each other, we can guide future research to refine such methods (e.g., what assumptions to make regarding lags between neural and cardiac responses).

Open Movie Data for Anxiety Research

The study of individual differences in brain functions necessitates particularly large sample sizes (Marek et al., 2022), which require significant financing and time. So I could comprehensively address a range of questions within funding/time constraints, I made use of appropriate, open datasets to test my predictions in all but one of my chapters. However, the use of open datasets imposes certain restrictions and considerations, some specific to anxiety research and some more general. Below, I discuss the implications of these restrictions on measures of trait anxiety and neuroimaging, as well as the utility of preregistering secondary analyses.

Trait Anxiety Measures

In chapters 2-4, I investigated associations between trait anxiety and brain responses. By using these open datasets, I was not able to select specific trait anxiety measures used. Chapter 2's datasets (Naturalistic Neuroimaging Database and Human

Connectome Project; Aliko et al., 2020; Van Essen et al., 2013) made use of the NIH Toolbox (*NIH Toolbox*, n.d.). The NIH toolbox is a proprietary software that delivers a battery of assessments to measures cognition and trait affect. It makes use of adaptive questionnaires that typically comprise fewer questions than more standardized questionnaires of trait anxiety that are employed in clinical research, such as the hospital anxiety and depression scale or state-trait anxiety inventory (Spielberger, 1983; Zigmond & Snaith, 1983). There has been some validation of the affect scales (Salsman et al., 2013), but not nearly as much as standardizes clinical questionnaires which are used in the diagnosis of pathological anxiety. Therefore, the trait anxiety scales used chapters 2 and 3 might hold poor psychometric properties which gave rise to null findings. Because this was openly available data, I was not able to guide design and so there were no alternatives which may have provided more reliable and valid measures of trait anxiety.

In chapter 6 (a dataset I collected in-house), I demonstrated that specific trait anxiety symptoms correspond more strongly to subjective states of anxiety than do overall trait anxiety sum scores on questionnaires. A re-analysis of the data in chapters 2-4 might have revealed whether such symptom-specific dimensions significantly correlate with brain activity in instances wherein I used trait anxiety summary scores. Yet, the adaptive questionnaires employed in chapter 2 meant that symptom-data could not be integrated due to different participants having different questions, making their item-level responses not directly comparable. The dataset used in chapters 3 and 4 (Cam-CAN; Shafto et al., 2014; Taylor et al., 2017) did make use of a standardized self-report anxiety questionnaire (hospital anxiety and depression scale, (Zigmond & Snaith, 1983). However, the publicly available version of the dataset only contains summary scores. This highlights that such restrictions imposed by open data can hinder the ability to employ certain techniques, such as symptom-level analyses.

A similar limitation was also case for measures of threat vigilance in chapters 2 and 3. Threat vigilance measures were derived from drift-diffusion modelling (DDM) of accuracy/reaction times to fearful facial expressions. However, the only available data were summary measures collapsed across trials (average accuracy/reaction times to facial expressions). Consequently, I employed a simplified version of drift-diffusion modelling that deals with trial averages, E-Z DDM (Wagenmakers et al., 2007). Drift rate parameters generated by EZ-DDM have been demonstrated to correlate highly

with drift rates derived from trial-level DDM (*r* = ~.9; Wagenmakers et al., 2007). However, the availability of complete trial-level data may have given slightly greater sensitivity to detecting associations with brain connectivity. This might be especially important in the case of chapter 4 which only detected resting-state functional connectivity to correlate with one measure of threat vigilance (amygdala-periaqueductal gray connectivity).

Given that my hypotheses in chapters 2-6 were centered on trait anxiety, it is also important to consider how these datasets recruited participants. All of the datasets I used did not have trait anxiety as a primary focus for data collection. In many instances of cognitive function, such as working memory ability, this may potentially not matter in the context of recruitment, as random sampling will help recruit a heterogeneous sample. On the other hand, individuals demonstrating particularly high levels of trait anxiety have been shown to often avoid volunteering for fMRI studies (Charpentier et al., 2021). This is exemplified in chapter 4, wherein only 42 out of the 639 participants in chapter 4 scored in the moderate-to-severe category of trait anxiety (HADS-A >= 11; Stern, 2014). If trait anxiety is a key measure of interest, one approach is to purposefully recruit participants scoring high in trait anxiety which can help generate a wide distribution of scores on trait anxiety measures. This can improve statistical sensitivity and allows inferences to be made that generalize to higher levels of trait anxiety. For addressing my research questions, using primary datasets which purposefully recruited more participants at the moderate-to-severe end of trait anxiety scales could have provided greater sensitivity and allowed generalizability to higher levels of trait anxiety. Therefore, a key limitation of using open datasets for the study of trait anxiety can be the lack of appropriate sampling.

Neuroimaging Measures

The constraints imposed by the use of open data also extend to fMRI measurement. The four open fMRI datasets I used contained large variability in scanner strength, ranging from 1.5 to 7 Tesla. The data from chapters 3 and 4 was collected on a 3T scanner and had relatively large voxel sizes (3 x 3 x 4.4mm). The use of a higher scanner strength (e.g., 7T) may have allowed for more precise measurement of small, subcortical regions in the ‘defensive response network’, such as the periaqueductal

gray (Huggins et al., 2021). Moreover, scanner strength does not just influence spatial/temporal resolution of echo planar imaging (EPI), but also shapes how factors such as susceptibility artifacts, physiological/thermal noise, and field inhomogeneities impact images (Fera et al., 2004; Moser et al., 2012; Triantafyllou et al., 2005; van der Zwaag et al., 2009). This can make direct comparisons between images collected at different field strengths difficult due to inconsistencies in signal-to-noise ratios. In chapter 2 (figure 2.4), I noted significant differences in general amygdala-whole brain functional connectivity profiles. This, in part, may have been driven by differences in field strength.

Likewise, the EPI sequence parameters varied significantly between and within datasets. For instance, the HCP and Caltech Conte Center (chapter 2 and 7) datasets used multiband accelerations of 5 and 6 respectively, while the Cam-CAN dataset (chapters 3 and 4) did not make use of any multi-slice acquisition. Multiband acceleration may hold detrimental impacts on signal-to-noise in subcortex (Bouyagoub et al., 2021; Todd et al., 2016). Given that the amygdala formed a key region for my hypothesis-testing, this could have resulted in a loss of statistical sensitivity and might be one explanation for null findings in some chapters. The impact of EPI sequence parameters is especially illustrated in chapter 2, wherein post-hoc exploratory analyses indicated that trait anxiety correlated with amygdala connectivity in runs which used anterior-posterior phase encoding, but not posterior-anterior phase encoding.

The differences in scanner strength and sequences exemplify that, in general, comparisons of results between chapters that used different datasets should be tentative. Moreover, they outline how the datasets used were not optimized especially for my regions of interest. If a primary dataset had been collected in-house for studies, this might have enabled more precise comparisons between chapters and potential increases in sensitivity. For instance, consistent use of multi-echo sequences (as used in the Cam-CAN) appears to improves BOLD sensitivity in the amygdala (Posse, 2012). Had I collected primary datasets for this thesis, implementation of multi-echo imaging would have been a key consideration.

Preregistration and Open Movie Data

Open datasets enable data mining from a range of theoretical and analytical perspectives. However, repeated analyses on open data hold the potential to inflate error rates (Thompson et al., 2020). This might pose a particular problem for movie data, wherein analytic flexibility is much higher than rest and task data. This is one of the reasons I preregistered every experimental chapter. That is, I uploaded a document to the Open Science Foundation (Foster & Deardorff, 2017) detailing hypotheses and analysis pipelines online a prior to running the experiments. If studies are conducted within a theoretical framework that drives specific predictions, this may help to reduce false positive rates (Scheel et al., 2021).

Movie paradigms are relatively more novel, especially so in anxiety research. Could such constraints on analyses hinder our understanding of how anxiety shapes responses to movies? In my experience throughout this thesis, my general answer is no. Nevertheless, this thesis has demonstrated the utility of conducting post-hoc exploratory analyses that deviate from preregistered tests. One example is in chapter 3. My planned hypothesis-testing was constrained to amygdala-dmPFC circuitry. However, in post-hoc exploratory analyses, I noted a broad range of significant associations between trait anxiety, suspense, and connectivity within the 'defensive response network' which motivated the continued use of this network in further studies (like in chapters 4 and 7). Therefore, preregistration may be a useful tool for reducing false positive rates but it should not let researchers hinder exploration, especially when using such unexplored paradigms like movie-watching.

Mirroring the analytical discussion in chapter 8.3.2, perhaps a hybrid approach might be also useful when multiple, appropriate datasets are available. Non-preregistered, exploratory, data-driven characterizations of how anxiety impacts responses can be established in a single, discovery sample to generate specific predictions that can be preregistered for analyses in a separate dataset. One potential issue is of course the aforementioned concerns regarding cross-dataset comparisons in anxiety and neuroimaging measures. However, this is when datasets which use differing movie stimuli are particularly useful (like in the Naturalistic Neuroimaging Database and Human Connectome Project). A discovery, then hypothesis-testing approach can be employed *within* datasets. For instance, the Naturalistic

Neuroimaging Database showed 10 different movies to participants. Future research could look at anxiety and brain activity during movie-watching in non-preregistered analyses for a subset of movies. Based on these findings, preregistration can then be submitted to test the extent to which effects replicate in other movies. Taken together, I strongly recommend future anxiety research does consider the implementation of preregistration, while at the same time not allowing this to restrain the implementation of discovery and post-hoc exploratory analyses.

8.4 Directions for Future Research

Mitigating Low-Level Confounds

As highlighted previously, movies contain purposeful directing and editing choices. To a cognitive neuroscientist, these may be considered as inducing low-level perceptual confounds. For instance, in chapter 3, I noted that suspenseful dynamics correlated with measures of volume, brightness, and faces present on the screen in 'Bang! You're Dead'. As such, it may be difficult to elucidate the unique contributions of suspense compared to features such as overall brightness. However, to a director, this collinearity is likely a purposeful choice to elicit a certain response (Grall & Finn, 2022). Suspense might be considered a property emerging from the combination of these features. Indeed, darkness does appear to facilitate feelings of anxiety and associated behaviors (Grillon et al., 1997; Mühlberger et al., 2008). Simply regressing these features out may then detract from anxiety-relevant biological/behavioral signals. There are two approaches which may be suitable for mitigating such low-level confounds.

Firstly, an added benefit of different movies is that they vary in low-level features (and consequently, the collinearity between features). Like my analysis in chapter 2, future studies should seek to test the generalizability of results across a range of different movies. However, in chapter 7, the suspenseful clip was grayscale, while the non-suspenseful control clip had color. In this instance, future studies could seek to test whether anxiety impacts neural-autonomic communication after controlling for these effects, by testing this relationship across a variety of movies stimuli. Secondly, neuroscientists are starting to overcome certain challenges through the creation of their own media to probe specific mental processes (Vanderwal et al.,

2015). To my knowledge, no such media exists for eliciting anxiety specifically. One future direction could be the curation of anxiogenic media that attempts to orthogonalize sensory features so as to mitigate concerns regarding perceptual confounds.

Automated Feature Extraction

In light of analytical considerations, it may be fruitful for future research to consider methods of automated feature extraction. Traditional analytical techniques such as feature-based general linear modelling require numerous assumptions about movie data. However, movies are incredibly, rich, complex, multisensory stimuli. As such, I leaned toward more data-driven approach, which come at a cost of interpretability (e.g., which specific features of the movie are driving anxiety-relevant idiosyncrasies). I did attempt to capture overarching emotional context by comparing neural responses to behavioral ratings of suspense collected from an independent sample. This was for a single ~8 minute video clip. However, such an approach will be less practical when dealing with longer movies, as well as studies which collect data across a range of movies (such as the NNDb; Aliko et al., 2020).

There are developments in affective computing which attempt to automate the extraction of emotional features such as ongoing dynamics of arousal and valence using visual and auditory signals from movies (Kuhnke et al., 2020). By using automated approaches to characterize emotionally-salient features of movie stimuli, future research may be able to rely more so on techniques such as feature-based general lineal modelling to derive more precise theoretical interpretations. In the context of anxiety, one avenue for future work would be to test whether the relationship between trait anxiety and responses to faces in movies may be modulated as a function of overall emotional context.

Toward Causal Inference

Although I have contrasted movie-watching conditions in this thesis, my observations are still based on correlations. For instance, this thesis predominantly focused on cross-sectional comparisons of trait anxiety. Such designs can be prone to confounds.

For instance, in the Cambridge Center for Aging and Neuroscience dataset, I noted collinearity of trait anxiety with age (ρ = -.23). This hinders the ability to disentangle the unique contributions of these factors in shaping neurobiological responses to movies. One approach to tackle this could be through the implementation of longitudinal designs. That is, collecting movie-watching data for the same individuals across multiple points in time. By using these designs, potential confounds (such as demographics) are held more constant. Therefore, by assessing within-subject longitudinal trajectories in anxiety, there is stronger confidence as to whether neurobiological responses to movies are arising due to anxiety specifically. To my knowledge, no studies have collected such data.

There is of course a potential constraint here; repeated exposure to the same movies could bias interpretations. Using mixed designs which counterbalance different movie stimuli could help curtail this issue while also increasing confidence in generalizability across stimuli. Alternatively, given the associations between trait anxiety and (lack of) habituation to stimuli (Blackford et al., 2013; Campbell et al., 2014; Protopopescu et al., 2005), repeated exposure to the same movies could also be an interesting avenue of research in and of itself. Irrespective of design specifics, future work implementing longitudinal designs could help validate the extent to which findings (especially cross-sectional ones) from the present thesis reflect true mechanisms underlying anxiety.

A complimentary method could also be the implementation of active manipulations within movie-watching designs. For instance, cognitive behavioral therapy techniques (e.g., mindfulness) could be used to try and dampen states of anxiety in response to suspenseful movies, which can then be contrasted with neurobiological responses. Another possibility is the use of pharmacological manipulation. For instance, sympatholytic medications (e.g., beta-blockers) could be administered to help discern the extent to which amygdala-prefrontal circuitry might be related to interoceptive awareness during suspenseful movie-watching. Only by implementing such manipulations within movie paradigms, a stronger inference can be made regarding the causal contributions of certain circuits and physiology to anxiety.

Pathological Anxiety

Lastly, the findings throughout the thesis need to be contextualized in terms of subclinical anxiety. All studies either investigated subclinical variation in trait anxiety or induced state anxiety in healthy individuals. If a core purpose of anxiety research is to provide better identification and treatment of relevant disorders, it is important that findings always be contrasted to individuals with pathological levels of anxiety. Although we can investigate subclinical variation in anxiety, which may hold implications for pathological anxiety, translation cannot be assumed; the impact of psychopathology on neurocognitive processes can manifest in a qualitatively different manner to subclinical symptoms (Ingram & Siegle, 2009).

There is some research starting to contrast healthy control participants with patients. Some movie fMRI data has been collected in participants with diagnoses of schizophrenia (Patel et al., 2022; Rikandi et al., 2017), ADHD (Tansey et al., 2022), and autism (T. A. W. Bolton et al., 2020). Yet, to my knowledge, there is currently no available movie fMRI data for participants with anxiety diagnoses. Therefore, future movie fMRI work which explicitly studies patients with anxiety disorders will be needed before we can consider whether the present results may be illustrative of pathologically severe levels of anxiety. Based on the findings in the present thesis, I particularly encourage future research to test whether pathological anxiety manifests in: 1) altered amygdala-prefrontal responses to suspenseful dynamics; and 2) reduced neural-autonomic communication during movies.

8.5 Conclusions

There is a clear gap in the anxiety literature: we have not ascertained whether our current understanding of the neurobiology of anxiety holds true in ecologically-valid settings. At the intersection of traditional experimental tasks and completely naturalistic studies is movie-watching, which may offer a useful platform for extending the research focus of anxiety toward naturalistic settings. As such, I focused on how trait and state anxiety may shape neural, subjective, and autonomic responses to movies. I provided evidence that trait anxiety does shape neural and subjective responses to movies. However, these effects often manifested in a manner inconsistent with the prior task-based literature. For instance, trait anxiety was not

associated linear increases in amygdala-prefrontal responses to suspense; rather, anxiety was associated with greatest responses during low suspense scenes. Likewise, I also demonstrated that communication between the brain and the autonomic nervous system may be impacted as a function of anxiety during movies. However, inconsistent with the prior literature, my results pointed to reduced (instead of increased) neural-autonomic communication as a function of anxiety. I have demonstrated in this thesis that movie-watching in general offers a useful platform for naturalistic anxiety research. Moreover, my results indicate that a potential re-evaluation of our neurobiological models of anxiety are needed. Traditional tasks may not be encapsulating the way in which anxiety impacts peoples' responses to the world outside of such settings. Therefore, my thesis encourages the continued use of movie-watching as relatively naturalistic platform for studying the neurobiology of anxiety.

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