ELUCIDATING THE NATURE AND DEVELOPMENT OF NEURAL MECHANISMS ASSOCIATED WITH ANXIOUS APPREHENSION AND ANXIOUS AROUSAL ACROSS ADOLESCENCE

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ABSTRACT

Paul B. Sharp: Elucidating the nature and development of neural mechanisms associated with anxious apprehension and anxious arousal across adolescence
(Under the direction of Eva H. Telzer)

Work on adult anxiety has found that anxious apprehension, marked by chronic worry, and anxious arousal, marked by elevated sympathetic hyperarousal, are instantiated in different neurobiological systems and involve different information processing dysfunctions. However, little is known regarding how these transdiagnostic types of anxiety develop. The present dissertation seeks to apply this transdiagnostic approach to anxiety to the study of adolescent neurodevelopment. Study 1 established that anxious arousal and anxious apprehension are distinguishable via self-report, supporting that these traits are meaningfully different as early as 11 years old. Neurobiologically, anxious arousal positively correlated with dmPFC-amygdala structural connectivity, interpreted as an elevated propensity to amplify anxiety responses, whereas anxious apprehension positively correlated with right iFG structural connectivity, interpreted as reflecting elevated inhibition of immediate threat processing. Evidence was not found for neural correlates of anxiolytic dysfunction in anxious arousal, nor for neural correlates of increased internal mental rehearsal in anxious apprehension. Study 2 built on Study 1 by examining how intrinsic connectivity was related to types of anxiety both cross-sectionally and longitudinally. Study 2 found no evidence that *a priori* defined functional pathways mapped onto types of anxiety. In contrast, a data-driven approach revealed that functional amygdala connectivity can predict variation in anxious arousal at both waves, whereas functional iFG

connectivity can predict variation in anxious apprehension in wave 2. Taken together, the present dissertation establishes that anxious arousal and anxious apprehension emerge in early adolescence, and may be marked by different kinds of information processing dysfunctions. Future work needs to more rigorously test if inferences about information processing associated with neural correlates found here are valid

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It has been a privilege to intellectually pursue what I would have otherwise pursued as a hobby over the past 6 years in graduate school. I tell my friends that are outside of academics that my life is like a treasure hunt. I get to seek, each day, for hidden answers to questions that have as much to do with elucidating my own humanity as they do with explaining extreme forms of mental suffering. It is a gift that I can pursue this exhilarating quest for my career.

However, passion alone didn't get me to my current state of knowledge and achievement. The countless questions, critiques and advice from other curious minds were essential to my growth as a scientist, scholar, and human being. Another phrase I bring up when I describe what I do to friends and family is that I live in "the world of ideas." I visited this world for the first time in Illinois. Each week in our clinical seminar we would engage a big question: where is the line between dysfunction and function? What is the logic of proposing and supporting the existence of a diagnostic taxon? What is the difference between neurobiological and psychological phenomena, and does one cause or underlie the other? It was here, with Wendy Heller as my guide, that I first experienced such an immersive, enriching, and stimulating space for exploring ideas. Sitting in a room with seven motivated scholars, challenging each other to defend our beliefs about fundamental concepts in the study of the human mind, was unlike anything I'd experienced before.

Wendy has been a source of support in every sense of that word. She has continually nurtured my intellectual development, and has been both a mentor and partner in advancing our shared ideas around types of anxiety. She was there to listen and guide me when I found out my

dad was diagnosed with pancreatic cancer in my first year. She supported me to leave the clinical program and her lab to pursue my research interests with Eva because she knew it would be advantageous for my career aspirations. And the greatest gift is that, even though I made that transition from her lab, we remain close personally and intellectually. It is pure fun to explore the world of ideas with Wendy, and I know we will continue to explore together for the rest of my career.

When I made the transition to developmental science, I was so fortunate to work with Eva Telzer. From my first meeting with Eva, I felt her deep enthusiasm for pursuing questions in developmental neuroscience, and I knew we would work well together. That said, when I first started off in her lab, I was unsure how my interests in clinical phenomena would blend with Eva's interests and those of my other new lab members. But over the past three years, I've gotten much closer with Eva and other students in the lab, and am so excited each time we meet and write feverishly on Eva's beautiful glass board. I've learned so much from her, and have gained an appreciation of the connection between her work and my own. One of the best feelings I've experienced with Eva over the past 6 years is what I'd call intellectual surprise. It's the feeling when you connect dots between seemingly disparate and disconnected topics, and are genuinely surprised to stumble on those insights. Working with Eva has deepened and widened my understanding of anxiety by exposing me to areas I know little about, and inspires me to engage with others outside of my area of expertise.

As I've explored the world of ideas, I've been lucky to forge many important collaborations. I owe a ton to my extended intellectual family, that includes Joel Thomas, Kathy Do, Greg Miller, Brad Sutton, & Peter Ornstein. Greg's mentoring cannot be overstated. I've learned so much from him that has helped shape my meta-theoretical views, which I hold to be

the foundation on which all of my current and future work rests. His willingness to engage my many questions as a young scholar in my first year was a sign of his devotion to guiding young scientists, and his genuine interest and curiosity in science. Writing papers with Greg has been a joy, too. Much of my method of meticulously combing through other colleagues' writing borrows much from Greg's style. The long exchanges of comments in the margins of papers have been rich debates; I think of them like modern-day, Renaissance-esque salons. These kinds of exchanges propelled me to tenaciously reach out to other great minds in our field, many of which are listed above. My conversations with Peter, for instance, remind me of those I've had with Greg, and I've been grateful for them. Moreover, my conversations about the limits and promises of neuroimaging with Brad in the domain of methodology mirror those I've had with Greg in the domain of philosophy of science.

In addition to introducing me to many mentors, graduate school brought me in touch with fellow students that I'm thankful to call my friends. Joel and Kathy feel like my adopted brother and sister. My love for them is beyond words. Of course, outside of all the moments of joy, fun, sadness and love we've shared together, I've also learned so much from them. The many hours of discussing philosophy on Joel's couch will forever remain a sacred place in my mind and heart. And the many long talks in our office between Kathy and me taught me so much about developmental methodology, social behavior in adolescence, and many other fascinating phenomena. Cultivating deep bonds with these two brilliant and caring individuals has been a true blessing.

Of course, this love I share with Kathy and Joel is only possible because of what I learned about love from my mom and dad. My parents' love reminds me of this really large cargo net that was suspended high up in the trees at summer camp. When I was about seven or so, my

counselors helped me climb up to this suspended cargo net in the forest. At first, I was apprehensive, and felt quite scared to move too much. Then, my counselors urged me to jump, because no matter what I did, they said, the cargo net would catch me. I've never been scared to jump and reach for my dreams because of my parents' love. No matter the outcome, I always know they'll be there to catch me.

As I approach my dissertation defense, I'm also deeply grateful for my committee members' willingness and capacity to provide such clear and thoughtful directions to sharpen my ideas. Indeed, the present document is the culmination of all of the thinking that is a product of my own development, hard work, and the many individuals that have scaffolded such growth.

As my mentors and friends know, I'm quite excited for the next steps of my career. Going to London to study at the Max Planck Centre for Computational Psychiatry is a dream come true. The opportunity to learn how to formalize and experimentally test my incipient ideas of why individuals chronically worry is a gift, and I am both nervous and eager for the challenges that lie ahead. What gives me a sense of comfort is knowing that as I embark on this new adventure in the world of ideas, I bring with me the collective knowledge and support from all of my colleagues, mentors, friends and family. Not only do I hope they know and feel the deep sense of gratitude I have for each of them, but also my commitment and intent to continue to cultivate our relationships in the future. Although I feel the tension surrounding this moment of change and uncertainty, the relationships I have with all those I've mentioned here extend beyond academic and professional boundaries. Each of you is, and will always be, a part of my story.

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Chapter 1: Introduction

How to advance developmental mechanistic explanations of the etiology of anxiety

Pathological anxiety is the most ubiquitous form of mental illness, affecting over 1 in 10 individuals globally (Steel et al., 2014) and manifesting in various syndromes, such as generalized anxiety disorder and panic disorder. There is growing consensus that most forms of pathological anxiety arise from disruptions in normative mechanisms to varying degrees, can be thought of as being dimensionally related to rather than categorically separate from adaptive anxiety, and may be more related to etiological factors than DSM-defined anxiety and fear disorders (Dillon et al., 2014). As such, it is vital to understand how anxiety arises normatively and how such processes go awry across both classically-defined clinical disorders (e.g., generalized anxiety disorder) and subclinical but elevated levels of dispositional anxiety (e.g., anxious apprehension; Sharp, Miller & Heller, 2015).

Given that these disorders tend to emerge early in development, it is essential to study anxiety during periods when the internal systems instantiating various types of anxiety undergo changes that precede and may portend dysfunction. Several factors predict the emergence of a range of types of pathological anxiety. For instance, an early marker of reacting negatively to novel stimuli as measured in infancy, termed behavioral inhibition, predicts more complex forms of social anxiety later in life (Fox et al., 2005). Moreover, behavioral inhibition predicts increases in the likelihood of a range of DSM-defined anxiety disorders arising in childhood (Biederman et al., 2001). In addition, twin studies and family studies reveal that both genetics and shared environment are predictive of adult forms of anxiety disorders (Pine, 2007).

However, *how* these etiological factors shift normative processes (accurate threat detection and/or planning to avert negative future threats) into pathological states (perceiving benign or ambiguous stimuli as highly threatening and/or excessive and repetitive worry), remains largely unresolved.

Answering 'how' questions in psychology requires mechanistic models (Thomas and Sharp, 2019). How, for instance, inconsistent parenting leads to anxiety in children requires an explanation of how that environmental input (inconsistent parenting) shapes internal processing (aspects of perception, emotion and cognition) to result in a pattern of behaviors and feelings we identify as elevated anxiety. In the present dissertation, I will seek to advance an understanding of the internal processing involved in two types of transdiagnostic anxiety traits and how such processing changes over time by taking a developmental neuroscience perspective. More specifically, I will test hypotheses regarding which aspects of brain structure and function relate to both cross-sectional and longitudinal changes in different types of anxiety. Indeed, this approach of identifying neural correlates of psychological phenomena has been a useful first step in elucidating how internal processing is implemented[1] in human biological systems for a range of psychological phenomena (Berridge & Robinson, 1998; Livingstone & Hubel, 1984).

[1] I use the term "implement" to describe the relationship between psychological functions and biological systems given that I adopt Marr's levels of analysis (Marr, 1982) to explain psychological mechanisms. Marr's framework changed the way scientists went about investigating visual processing and has since been applied to psychological phenomena broadly in fields such as computational neuroscience, cognitive science, and cognitive neuroscience (Thomas & Sharp, 2019). In the framework, the computational level defines the information processing problem or goal the mind must 'solve', and what constraints there are on the problem imposed by the environment (e.g., the goal of anxiety is to alert the organism of impending danger; how does the environment send such signals? How does the human body impose constraints on how it could detect such signals?). The algorithmic level describes the steps taken by a complex system to solve the problem (analyzing information to infer the likelihood and risk of threats and computations on these assessments to behaviorally prepare the organism to avoid the threat). Finally, the implementational level describes how that algorithm is carried out in the relevant biological components (e.g., amygdala initially detects threat and sends signals to prefrontal cortex to compare these initial assessments with background goals and memories).

If we better understand the biology involved in anxiety, and how it develops, we can learn about how cognitive functions work. Indeed, this is exactly what was done for vision: scientists investigated what kinds of stimuli parts of occipital cortex responded to, and then inductively theorized what types of information processing (e.g., edge detection, color detection) were carried out by those brain regions. Although the effort to reason about information processing underlying emotional episodes is undoubtedly more difficult than what was done for vision, in principle, the same can be done. As such, the present dissertation will seek to understand what neurobiological components are related to forms of anxiety in youth, and how these components change over time.

Despite anxiety disorders being among the most commonly diagnosed disorders in childhood and adolescence, Albano, Chorpita and Barlow (2003) noted the lack of progress on understanding anxiety disorders in children relative to elucidating adult forms of anxiety. Indeed, the recent increase in studies of anxiety in youth in the developmental neuroscience literature (e.g., Caouette & Guyer, 2014) is yet to be well-integrated with theoretical developments in adult psychopathology and non-human animal work on anxiety. The goal of the Introduction is to review what the field has learned about two distinct forms of trait anxiety, anxious apprehension and anxious arousal, to inform the larger enterprise to better understand the mechanisms of anxiety and how they develop early in life. Much of the material presented below inspires the studies comprising the present dissertation, but do not motivate the experimental design or the specific predictions contained therein. As such, I will endeavor to highlight what material below is directly germane to the logic of the studies I will carry out, and what ideas need to be tested in future experiments.

I will first highlight incipient theories about the biological components and information processing functions involved in these types of anxiety from work primarily advanced in adults and non-human animal work. Next, I will demonstrate how applying this transdiagnostic anxiety framework the study of child and adolescent anxiety literature. can begin to make sense of seemingly unexplained findings. Ultimately, I will describe the logic behind two studies that will advance an understanding of how anxious apprehension and anxious arousal develop in adolescence. Specifically, these two studies will test hypotheses regarding how neurobiological markers are related to anxious apprehension and anxious arousal cross-sectionally and longitudinally.

Evidence that anxiety is not a unitary phenomenon

Although we have parsed various anxiety disorders in clinical psychology and psychiatry (e.g., specific phobias vs. generalized anxiety disorder), most basic research on anxiety tends to treat anxiety as a unitary construct. For instance, one of the most prominent models of anxiety, the tripartite model (Clark and Watson, 1991), differentiates anxiety from depression in that anxiety involves high physiological arousal and depression is *not* marked by hyperarousal. As such, it is common to think of physiological hyperarousal as part of the essence of anxiety.

The notion of anxiety as including physiological hyperarousal, however, contrasts with a history of findings in the adult literature that established a lack of such hyperarousal in chronic worriers (Mathews, 1990). Indeed, worry involves cognitive resources that have been shown to attenuate physiological responding, perhaps as a temporary strategy to avoid more difficult emotions and physiological correlates related to confronting or accepting the presence of aversive environmental stimuli (e.g., Spielberg et al., 2013). Recognizing that this form of anxiety fundamentally differed from more somatic forms of anxiety, Heller, Miller and

colleagues (1997; 1999; 2000) proposed a framework to differentiate these two dimensional forms of anxiety, now known as anxious apprehension and anxious arousal. A primary goal of Study 1 is to demonstrate that these dimensions are separable in adolescence, and to investigate their neurobiological correlates.

Anxious Apprehension

Anxious apprehension, which is marked by excessive worry, involves increased activation in left inferior frontal gyrus in adults (e.g., Heller et al., 1997, Engels et al., 2007). This finding is important, as this part of the brain contains Broca's region, which is known from lesion studies to be crucially involved in verbal production (Blank et al., 2002). Indeed, given that most forms of worry involve internal verbal rehearsal of potential negative outcomes, it makes sense that this area of the brain would be overactive in individuals who chronically worry.

More recently, it has been shown that *right* inferior frontal gyrus is also hyperactive when individuals prone to worry are instructed to worry about versus accept various hypothetical negative outcomes in an fMRI paradigm (Ellard et al., 2017). Theorists have interpreted this activation as being related to an over-engaging of inhibitory functions to compensate perhaps for an initial orienting bias to threat, which is known to occur in high rates in chronic worriers (e.g., Monk et al., 2006). As such, it has been shown in adults that chronic worriers disengage from bottom-up threat signals and instead engage worry to avoid the immediate and intense feelings associated with confronting imminent threat (e.g., Spielberg et al., 2013). Moreover, chronic worriers are also perceptually sensitive to potential threats in their environment, and in particular, to verbally-written threats (Goodwin, Yiend, and Hirsch, 2017). It makes sense that threat-sensitivity to written words appears greatest for chronic worriers given that verbal content frequently comprises worries that are highly conceptual and hypothetical in nature.

Additional neural correlates of this threat-sensitivity in worriers have been examined in fMRI tasks that require attentional control. Activation in regions involved in attentional control, including left dorsolateral prefrontal cortex and anterior cingulate cortex, are altered for those high in anxious apprehension (Engels et al., 2007). The time-course of activity in these regions suggests that there is an initial failure of cognitive control due to the distracting nature of task-irrelevant threats that is then compensated for by later-engaging of control regions (Sharp, Miller and Heller, 2015).

Anxious Arousal

In contrast to anxious apprehension, anxious arousal is associated with sympathetic hyperarousal and hypervigilance in the presence of mild stressors (Heller et al., 1997; Nitschke et al., 2000). Anxious arousal as measured by the Mood and Anxiety Symptom Questionnaire Anxious Arousal subscale (MASQ-AA; Watson and Clark, 1991) is associated with elevated right hemisphere activity in resting-state EEG, primarily in lateral frontal areas (Nitschke et al., 1999). While state fear engages right posterior regions and networks specialized for monitoring the environment and responding to threat, the biological components of anxious arousal include frontal, temporal, and parietal regions, which are thought to instantiate hypervigilance, attentional biases, and dispositional tendencies (Burdwood et al., 2016; Compton et al., 2003; Engels et al., 2007; Nitschke et al., 1999).

Subsequent studies have replicated these findings, identifying more specific brain regions implicated in anxious arousal using EEG (e.g., O'Hare and Dien, 2008) and fMRI (e.g., Engels et al., 2007). Anxious arousal was associated with activity in right inferior temporal gyrus (ITG) and middle temporal gyrus (MTG), which are two nodes in a neural system thought to instantiate threat detection (Engels et al., 2007; Nitschke et al., 2000; Spielberg et al., 2013). These studies

used Stroop-like paradigms, in which those with high levels of anxious arousal showed hyperactivity in these temporal regions while attempting to ignore negatively-valenced words. Such findings support the argument that anxious arousal as measured by the MASQ-AA is a trait marked by a lower threshold to engage a fear response when exposed to mildly threatening stimuli.

Non-human animal work refines an understanding of anxious arousal

It is difficult to translate research with non-human animals to our developing understanding of all types of human anxiety. Two reasons largely justify the claim that such translational work is best-suited for studying anxious arousal and not anxious apprehension. First, anxious apprehension involves complex functions such as imagining future scenarios (e.g., "what will happen if I fail my test") and creating counterfactual realities (e.g., "if I make a bad joke at the party, then I will be ridiculed"), which are both thought to be uniquely human functions, especially when they involve (as they frequently do) *linguistic* representations (Mathews, 1990; Sharp, Miller & Heller, 2015; Pearl and Mackenzie, 2018). Second, anxiety is operationalized in non-human animals as elevated sympathetic nervous system (SNS) activity or defensive behavior (e.g., freezing) that indicate elevated SNS activity, both of which, again, are *not* elevated in anxious apprehension. Work, however, needs to refine how SNS activity for those with anxious apprehension may differ when threats *are* imminent, as they may experience *phasic* instances of hyperarousal.

By contrast, anxious arousal is thought to be generated by the inference that aversive consequences are looming (e.g., a rat believing it will get shocked if it turns the corner in the maze, or humans thinking that they will have a heart attack if they work out too much). Indeed,

such computations need not involve the higher-order cognitive functions that are central to anxious apprehension.

Recent work in rodent optogenetics (using different colored light to activate or inhibit genetically-modified neural circuits *in vivo*) has made significant strides in identifying neural pathways that can produce and mitigate anxious states. In this work, two basic psychological processes have been theorized to occur sequentially. The first process, called "interpretation", labels an external stimulus as threatening or innocuous (Calhoon and Tye, 2015). Major nodes of the system implementing this process include the amygdala, the bed nucleus of the stria terminalis, the ventral hippocampus, and the prefrontal cortex (primarily medial regions). Specific dynamics within the amygdala and its several nuclei have been associated with different facets of this interpretation subprocess. For instance, activity within basolateral amygdala is thought to realize associations between neutral stimuli (i.e., conditioned stimuli) and subsequent threats and rewards (Janak and Tye, 2015; Maren and Quirk, 2004).

The second process is called "evaluation". The evaluative subprocess weighs the evidence signaled by the initial interpretation subprocess in order to either amplify or scale-back the anxious state (Calhoon and Tye, 2015). Medial prefrontal cortex is thought to be a central node in this system, in which the prelimbic, infralimbic, and cingulate cortices and their projections to nodes in the interpretation system implement different functions such as integrating current goals and retrieving relevant memories to modulate anxious states (Adhikari et al., 2015; Sirota et al., 2008). Conclusions from such work are that infralimbic to basomedial amygdala circuits suppress anxiety responses whereas prelimbic to central amygdala facilitates anxious states (Livneh and Paz, 2012; Senn et al., 2014). It will be tested in Study 1 whether or

not homologous circuits in humans high in anxious arousal implement these anxiogenic and anxiolytic functions.

The combination of cellular-resolution and causal manipulation that is possible in rodents but impossible in humans can help the effort to infer exactly what biological dynamics might underlie the spatially and temporally coarse signals we glean from fMRI. Using optogenetics, it has been possible to induce facets of anxiety-related behavior (e.g., freezing) while leaving other behaviors (e.g., avoidance) non-engaged by targeting separable circuit projections (Calhoon & Tye, 2015). Indeed, many neuroimaging and behavioral measures smooth over these differences, as their fine-grained details are either not taken into account a priori (e.g., Jalbrzikowski et al., 2017) or are not detectable when simply correlating behavior with self-reported instances of anxiety (e.g., Robinson, 2012).

Existing data and theory support that the many relationships between neural circuits and the various informational processes and behaviors involved in anxiety reviewed above in non-humans are partially conserved in human adults. In healthy adult humans, the structural connectivity between amygdala and dorsal anterior cingulate cortex (part of dmPFC) has been shown to positively covary with trait anxiety (Greening and Mitchell, 2015), whereas the structural connectivity between amygdala and medial OFC (part of vmPFC) negatively covaries with trait anxiety (Greening & Mitchell, 2015; Kim & Whalen, 2009). Moreover, functional MRI studies of human adults show that amygdala-dmPFC functional connectivity increases as state anxiety is induced (Robinson et al., 2012, 2013, 2014).

That said, there are conflicting findings in the literature on the relationship of the structural and functional neural correlates of anxious apprehension and anxious arousal. Within structural connectivity, some have found a negative correlation between the structural

connectivity of the medial OFC-amygdala pathway and trait anxiety (e.g., Greening and Mitchell, 2015), whereas others found a positive correlation between the structural connectivity of the ventral prefrontal cortex-amygdala pathway and trait anxiety (Clewett, Bachman, and Mather, 2014). Moreover, data-driven studies on trait anxiety differ from structural connectivity findings. For instance Liu et al. (2015) found that neither amygdala nor dorsal medial prefrontal cortex connectivity contributed significantly to the classification of trait levels of social anxiety. A goal of studies 1 and 2 is to compare and contrast how structural and functional neural correlates of different types of anxiety present in early adolescence.

How do these forms of anxiety develop?

Anxiety disorders comprise a class of highly prevalent disorders in childhood and adolescence, with estimates that up to 10% of children and adolescents are diagnosed with a form of pathological anxiety (Chavira, Stein, Bailey, & Stein, 2004; Costello at al., 2005). Moreover, high levels of anxiety early in life are predictive of several forms of psychopathology later in life, such as depression and substance use problems (Pine et al., 1998). Adolescence, specifically, is considered a "tipping point" during which the rates of initial onset of pathological anxiety precipitously increase (Dahl and Hariri, 2005).

Several questions, however, remain regarding how anxious apprehension and anxious arousal differentially develop. Treating anxiety as a unitary construct may obscure divergent developmental pathways across anxious apprehension and anxious arousal that bear on diagnosis and treatment. Subsequent sections will thus review the genetic and environmental contributions to the development of anxiety. Although these causal theories will not directly be tested in the present dissertation, they can be integrated with hypotheses about proximal neurobiological mechanisms investigated here in future longitudinal work.

Genetics of anxiety

There is mounting evidence that a meaningful portion of variation in anxiety is attributable to genetic differences (Muris, 2006). Indeed, it has been shown that the frequency of reported fears has a heritability estimate of .29 in children ages 8-16 (reflecting the increased phenotypic similarity in monozygotic twins relative to dyzogotic twins; Stevenson, Batten, and Cherner, 1992). Moreover, trait anxiety has been shown to have heritability estimates between 0.3 and 0.65 (Hettema et al, 2001; Kendler, 1999; Scaini et al, 2012; Smoller et al, 2009). That said, the specific genes associated with types of trait anxiety share variance with other stress-related disorders such as post-traumatic stress disorder and depression (Smoller, 2016). This calls into question whether or not part of the set of genes associated with risk for anxiety is indicative of a broader risk factor for internalizing forms of psychopathology (e.g., Kreuger & Markon, 2006).

Temperament as a precursor to dimensional traits: Behavioral inhibition

Some consider the path between genetic diatheses and later-emerging psychopathology to be mediated by a feature of temperament, known more commonly in the human literature as behavioral inhibition, and in the non-human literature as fearful temperament (e.g., Shackman et al., 2013). Children deemed to be high in behavioral inhibition have a fourfold increase in the likelihood of eventually developing an anxiety disorder (Chronis-Tuscano et al., 2009). One of the only longitudinal studies on behavioral inhibition early in life (mean age 6 years, range 5-8 years) found that it predicted social anxiety emerging in childhood (Muris et al., 2011), supporting prior cross-sectional findings (Biederman et al., 2001). More recently, a meta-analysis showed that there is a sevenfold increase in developing social anxiety disorder when having elevated behavioral inhibition (Clauss and Blackford, 2012).

However, the relationship between genetic diatheses, behavioral inhibition, and later-emerging anxiety disorders is complex and varies widely across individuals. Evidence suggests that behavioral inhibition does not have a *sine qua non* (i.e., necessary) genetic diathesis; indeed, behavioral inhibition has been shown to vary meaningfully as a function of environmental input such as parenting (Turner, Beidel & Wolff, 1996). As such, one can think of behavioral inhibition and its genetic antecedents as influencing baseline risk for problems in anxiety that is then sensitive to environmental input throughout development. Moreover, although the relationship between behavioral inhibition and social anxiety appears strong early in life, it becomes quite malleable in late adolescence (e.g., 50% of individuals in college who previously endorsed behavioral inhibition earlier in life no longer endorsed it at the time of measurement; Poole, Tang & Schmidt, 2018). The present dissertation will not examine the relationship between behavioral inhibition and the development of types of anxiety. However, the present dissertation may reveal overlap in the neural correlates and information processing functions between behavioral inhibition and types of anxiety in adolescence that can inform future longitudinal projects.

Environmental contributors to the development of anxiety

Myriad environmental factors shape the expression of genetic diatheses, temperament, and other psychological functions that contribute to problems in anxiety throughout development. Chorpita and Barlow (1998) developed a rich theory integrating cognitive theories on the perception of uncertainty and the belief of having a lack of control with both attachment theory and theories of parenting style (e.g., overprotectiveness; Parker, 1983). Their theory contends that various kinds of parent-child relationship can influence children's perceptions of a lack of control over their environment, leading to pathological anxiety. For instance, pulling on

work from Gunnar (1994), Chorpita and Barlow (1998) highlight that experimentally manipulating separation between parents and children elevates stress-responding in relevant hypothalamic circuitry, which over time can increase stress and promote beliefs about the world as uncontrollable. Thus, parents who fail to appropriately attend to their children's needs (e.g., when they're distressed) lead children to experience elevated anxiety and a sense of uncontrollability (Parker, 1983; Sroufe, 1990). This developmental theory comports with modern theories of psychological functions and neural mechanisms of anxiety in adults that pinpoint how various computations about uncertain outcomes (probability of risk, magnitude of risk) are central parts of the mechanism that explains pathological anxiety (Grupe & Nitschke, 2013). Indeed, if one has a core belief that the world is inherently uncontrollable, then estimates of risk probabilities on average, at the very least, will be elevated relative to individuals that conceive of the world as more stable. This developmental theory of uncontrollability as a precursor to anxiety, however, may be more important for understanding the development of anxious apprehension than anxious arousal. Indeed, other theories of the development of phobias (high in anxious arousal) focus on observational learning from parents who display signs of anxious arousal in the presence of acute stressors (Gerull & Rapee 2002). Thus, types of learning histories may be central to understanding how anxious arousal and anxious apprehension differentially develop.

Anxiety can also be exacerbated by proximal family and social stressors during adolescence. For instance, both the frequency of interacting positively with peers and school adjustment throughout late childhood and adolescence prospectively predict rates of pathological anxiety (Buss and McDoniel, 2016). Moreover, family stressors such as conflict and family identity (i.e., if the adolescent feels valued by their family) that shift in adolescence also greatly

contribute to both broad risk for psychopathology and specific risk for anxiety disorders (Kerns & Brumariu, 2014; Sharp, Heller, & Telzer, 2019). Although the present dissertation will not examine these environmental factors, they will be essential in explaining how mechanisms of anxiety are shaped across development.

Neurodevelopment of anxiety

Both genetic and environmental factors shape the development of internal processing involved in types of anxiety. One lens into how such internal processing develops is via an emerging field, developmental neuroimaging (e.g., Telzer et al., 2018). Elucidating the neurobiological systems involved in anxiety via neuroimaging has produced promising findings that require further investigation. For instance, a consistent finding in the literature is that reduced amygdala-vmPFC functional connectivity is associated with trait anxiety (Burghy et al., 2012; Hahn et al., 2011; Roy et al., 2013) in adolescence. Additionally, it has been shown that elevated right vlPFC activation, attentional bias to threat, and elevated striatal activation, thought to implement greater sensitization to social reward and punishment, are associated with social anxiety (Guyer et al., 2008). Moreover, we know that generally, adolescence is a period in which prefrontal cortex increasingly shows signs of regulation over subcortical activity, which is interpreted as adolescents gaining a greater capacity for intrinsic emotion regulation (Gee et al., 2013).

Much more work needs to be done in the domain of developmental neuroimaging to further elucidate how pathological anxiety develops in adolescence. Future work needs to investigate within- and between-subject changes in the functional and structural neural correlates of anxiety across adolescence. Moreover, such work should be motivated by integrating an

understanding of the neural circuits involved in anxiety from non-human and adult human literature with nascent efforts to delineate neural correlates of anxiety in adolescent humans.

Explaining the neural systems implementing types of anxiety requires integrating findings regarding the function and structure of neural circuits

A major goal of the present dissertation is to compare structural and functional neural correlates of types of anxiety in adolescence. Indeed, to understand how a neural system carries out a psychological phenomenon requires characterizing both the neural system's structure and function. Neural structure can refer to many things about the physical structure of the brain, such as gray matter volume, cortical thickness or how brain regions are interconnected via bundles of axons. For present purposes, I will use neural structure or "structural connectivity" to refer to the axonal pathways, and the strengths of those pathways, that connect neural regions to each other. For instance, if one neural region implements cognitive control[2], and another, fear processing, the strength of the connection between these regions may relate to how well one can regulate fear responding. Indeed this has been shown to be the case in humans using correlational (e.g., Greening & Mitchell, 2015) and experimental designs (e.g., Sharp et al., 2018), albeit using coarse-grained techniques such as fMRI relative to more invasive methods in non-human and post-mortem studies that can directly measure neuronal strengths (Miller, McNab, Jbabdi, & Douaud, 2012).

Function, on the other hand, refers to how active (relative to some baseline level of activation) a single region is (e.g., measured with MRI that ostensibly indicates a latent amalgam of neuronal firing of action potentials) *or* in a more recent neurobiological framework called "connectomics", to what degree neural activation in one region covaries with neural activation in

[2] I use this facile phrenological characterization of the modularity of neural regions for the sake of simplifying the example, even though in reality any complex psychological phenomenon like cognitive control is likely implemented in a distributed network of macro-defined neural regions.

another region. Since most functions in the brain are thought to involve the collaboration of multiple regions, the field is starting to transition more towards the connectomics framework (Park & Friston, 2013).

Compelling mathematical models of how neural systems are organized and function require both functional and structural information. These models, called neural network models (e.g., Lecun, Bengio & Hinton), involve numbers called "weights" that determine the strength of structural connections between regions. However, the pattern of neuronal firing, or the functional dynamics of the system, is only partly constrained by the weights and organization of structural connections. Indeed, biological evidence of real neural networks have demonstrated that the same structural map of neurons can produce different functional dynamics[3] (due to e.g., the difference between gap junctions and chemical synapses, or the number and kinds of membrane currents; Bargmann & Marder, 2013). Ultimately, it is thought that the pattern of firing, constrained by structure, is how information is represented and processed in the brain (and abstractions of the brain in neural networks models).

A major first-step in understanding how these complex nonlinear neural systems can carry out psychological processes is through using data-driven, machine learning analyses, which in some implementations are trained on data in order to fit weights to a given neural network. Indeed, this has led to demonstrations of neural networks carrying out mental functions (although arguably dissimilar to how humans implement them; see Lake, Ullman, Tenenbaum &

[3] Bargmann and Marder, pioneers of elucidating "simple" (relative to humans; here, *C. Elegans*) nervous systems stated the following about the relationship between structure (which she calls a wiring diagram, or "connectome" in contemporary neuroimaging parlance) and neural function: "A given wiring diagram can produce widely different dynamics with different sets of circuit parameters, and conversely, different circuit mechanisms can give rise to similar oscillation dynamics. Without knowing the strength and time course of the synaptic connections as well as the numbers and kinds of membrane currents in each of the neurons, it would not be possible to simply go from the wiring diagram to the dynamics of even two neurons. Synaptic connectivity alone does not sufficiently constrain a system." (Bargmann & Marder, 2014, p.487)

Gershman, 2017), such as visual and speech recognition, to similar or even greater degrees of accuracy than humans (Graves, Mohamed, & Hinton, 2015; Yamins & DiCarlo, 2016). It is then possible to investigate how the structural weights, organization, and functional dynamics of these neural systems gave rise to the output of the system (e.g., accurately identifying objects). Work on anxiety is just beginning to elucidate the structural and functional connectivity correlates of types of anxiety (e.g., Greening & Mitchell, 2015). Future work must endeavor to integrate findings across functional and structural domains in further elucidate mechanistic models about the underlying structural weights and functional dynamics of neural systems implementing types of anxiey.

Directly comparing structural connectivity to functional connectivity may offer avenues for understanding the neural dynamics involved in anxiety. Unlike structural connectivity that refers to underlying physical connection strengths between two regions, functional connectivity between two regions is influenced by direct *and indirect* paths between two regions. For instance, the functional connectivity between amygdala and prefrontal cortex is influenced not only by the structural connectivity between these regions, but also by several longer paths (e.g., amygdala to parietal to prefrontal cortex; Rubinov & Sporns, 2009), as well as potentially by other physiological mechanisms that give rise to synchronous firing (e.g., volume transmission; Anderson, 2014). Knowing what circuit dynamics give rise to functional connectivity measures is a challenge for several reasons (see Mehler & Kording, 2018), yet, this effort is essential to understanding why for instance functional connectivity measures might be better at distinguishing types of anxiety than structural connectivity measures.

Moreover, as is true in artificial intelligence work mentioned above, one of the most powerful ways to investigate the relationship between structure and function is to use machine

learning tools in a data-driven way. If one does not have strong *a priori* hypotheses, or insufficient models of neural mechanisms, machine learning can be used to explore how neural structure, function and their combination can predict variation in anxiety. Thus, one goal of the present dissertation is to integrate knowledge of functional and structural connectivity correlates of types of anxiety in early adolescence, using both a priori hypotheses and data-driven approaches.

Why parsing anxiety into separable dimensions can improve our understanding of the development of anxiety

The literature review above provides a trove of findings in cognitive, biological and developmental domains regarding what pathological anxiety is, what its causes are, and how they develop over time. Several theoretical and empirical reasons linked to these prior findings illustrate why parsing anxiety into anxious apprehension and anxious arousal will help to refine our understanding of the development of anxiety disorders.

Perhaps most important, the mechanisms giving rise to anxious apprehension and anxious arousal differ significantly (Sharp, Miller & Heller, 2015), and as such, it is imperative to improve our understanding of how these types of anxiety emerge developmentally. Even if children show similar levels of behavioral inhibition and threat-sensitivity early in life, why do some end up repetitively worrying while others experience problems in physiological hyperarousal and defensive preparedness? Given the rise in the incidence of anxiety disorders in adolescence, it is fruitful to explore how and why different forms of anxiety shift throughout adolescence (Beesdo et al., 2007; Roza et al., 2003).

Second, several points of confusion in the literature can be resolved by the present dual-trait framework which recognizes that anxiety is multidimensional. For instance, Dieleman et al. (2015) found that, among youth having either generalized anxiety disorder, separation anxiety

disorder, social anxiety disorder or specific phobia, only those with specific phobia experienced heightened levels of sympathetic nervous activity. Although the authors attempted to explain this unexpected finding by referring to the fact that specific phobia is a "more differentiated taxon" than the other anxiety disorders they studied, their results may be more directly accounted for by the fact that specific phobia comprises higher levels of anxious arousal, whereas the other categories of disorders possess higher levels of anxious apprehension.

This is not an isolated finding in this small body of literature on mechanisms of child anxiety. For example, Albano, Chorpita and Barlow (2003) mention that panic disorder has the highest heritability, whereas GAD has the lowest, but claim that this is best-explained by the contention that "the heritability at the level of disorders is unlikely" (p.308). However, the dual-trait framework of anxiety that differentiates anxious arousal and anxious apprehension may better account for this meaningful difference in heritability. It is known that panic disorder involves high levels of anxious arousal, whereas generalized anxiety disorder involves high levels of anxious apprehension (Sharp, Miller & Heller, 2015). Even if heritability is not explained at the level of disorders, heritability could very-well be explained at the level of transdiagnostic constructs (i.e., cutting across traditional disorder criteria), which in this case include anxious apprehension and anxious arousal. The import of transdiagnostic constructs is garnering empirical support from accumulating evidence within the National Institute of Mental Health's Research Domain Criteria initiative (Insel, 2014).

A recent longitudinal model of how chronic worrying develops lends support to the claim that anxious apprehension and anxious arousal likely develop differently. Muris et al. (2002) showed that both age and cognitive development positively predicted worry elaboration, which in turn predicted personal worries (worries about my or my family's life, as opposed to e.g.,

worrying about climate change). Importantly, the frequency of personal worries is an important outcome given that the overwhelming majority of instances of pathological worry comprise personal worries (Mathews, 1990). The hypothesis that cognitive development is part of the etiology of pathological worry is further supported by language-dependent neural regions being crucially involved in anxious apprehension (e.g., Heller et al., 1997), as well as the finding of a small but positive correlation between IQ and worry (Penney Miedema, & Mazmanian, 2015). Theorists currently working on a computational model of chronic worrying have proposed that the breadth and depth of mentally simulating the future, a function likely associated with intelligence, is central to worrying (Bulley, Henry & Suddendorf, 2017; Sharp & Eldar, 2019). It remains unclear, however, at what age worry emerges. It has only been demonstrated that the frequency of worries increases significantly from ages 6 and up, whereas more immediate fears and phobias (high in anxious arousal) dominate in ages 5 and below (Muris & Broeren, 2009). However, there still is likely a range of diversity of the content, scope and functional dynamics of worry across childhood, adolescence and adulthood.

This nascent picture of the development of anxious apprehension differs from what is known about the developmental trajectory of anxious arousal, which may emerge earlier in life. Indeed, behavioral inhibition, which can emerge early in toddlerhood, is associated with elevated sympathetic arousal in response to novelty, a central feature of anxious arousal (Heponiemi, Keltikangas-Järvinen, Kettunen, Puttonen, & Ravaja, 2004; Marshall & Stevenson-Hinde, 1998; Stevenson-Hinde & Marshall, 1999). Moreover, this physiological marker early in childhood prospectively predicts similar differences in adulthood (Bell et al., 1993), suggesting it may be fairly stable over time.

Two empirical studies to investigate structural and functional neural correlates of anxious arousal and anxious apprehension in early adolescence, and how they change over time

The three goals of the present dissertation are as follows. First, to examine if current biomarkers and psychological indicators of types of anxiety can distinguish anxious arousal and anxious apprehension in adolescence. Second, to extend our understanding of the relationship between structural and functional neural connectivity of both anxious apprehension and anxious arousal in early adolescence. Third, to investigate within-subject changes in types of anxiety by examining longitudinal changes in the neural correlates of anxious apprehension and anxious arousal across 1 year of development in adolescents.

Study 1 is designed to meet goal (1) by investigating the neural and psychological correlates of anxious apprehension and anxious arousal in a sample of early adolescents. The biological correlates of anxious arousal that were tested were informed by non-human optogenetic studies (a method that can causally manipulate specific neural circuits). Translating these circuit-specific markers of anxiety in non-human animals to the study of human anxiety adds to the growing body of knowledge regarding the neural correlates of anxious arousal. For anxious apprehension, I investigated two hypotheses regarding neural correlates of separable processes involved in anxious apprehension: internal simulation of negative consequences via internal rehearsal (e.g., repeating negative consequences that would happen if I make a faux pas at a party; instantiated in left inferior frontal gyrus) and inhibiting extended processing of mild threat signals (e.g., inhibiting processing of task-irrelevant threatening words such as “death”; instantiated in part in right inferior frontal gyrus). Knowing which (or both) of these processes is most strongly associated with anxious apprehension in early adolescence can add to our knowledge of how these types of anxiety develop. Such developmental evidence could help identify more precise targets for intervention/prevention that precede clinically-relevant pathological levels of anxious apprehension.

Study 2 will address goals (2) and (3) outlined above. Goal (2), to extend knowledge of the relationship between structural and functional correlates of anxiety in youth, will be addressed both in hypothesis-driven and data-driven ways. Hypotheses are directly related to those delineated in Study 1, and address whether the structural markers found in Study 1 predict the same relationships in the functional connectivity domain. Given that structure only partly constrains function (see section *Explaining the neural systems implementing types of anxiety requires integrating findings regarding the function and structure of neural circuits*), Study 2 will also use a data-driven approach to explore what other connections influence the functional connectivity of key regions involved in anxiety types, and to what degree those additional connections improve prediction in the variation of self-reported anxiety.

Supplementing a structural connectivity analysis with a functional connectivity analysis can also yield more detail regarding the mechanisms realizing anxiety. For instance, if I establish in Study 1 that the strength of the structural connection between amygdala and subgenual anterior cingulate cortex (sgACC) positively predicts anxious arousal and I test for the same connection functionally, I can make certain inferences based on the joint results. In Figure 1, the top row, A, represents the situation in which the structural connectivity of a single circuit (denoted as the 'direct connection') is positively associated with anxious arousal. If I find that the functional connectivity of the aforementioned pair of regions is also associated with anxiety, this provides evidence that the functional dynamic is strongly constrained by the underlying structural weights. However, this is not always the case. In the bottom row, B, many functional connectivity relationships could exist without direct connections.

Alternatively, there are potentially several meaningful reasons that could explain why I find evidence that structural connectivity predicts anxiety, but functional connectivity does not. One reason, depicted in Figure 1-B, is that *indirect* pathways between amygdala and sgACC influence functional connectivity between those regions in various ways (some amplifying, some diminishing communication between the two regions), accounting for the overall net communication pattern being uncorrelated. Moreover, the time-course of activity in each region is potentially also influenced by many other connections (Fig 1-C) e.g., amygdala has with other regions, and with non-axonal functional dynamics (Fig 1-D; e.g., gap junctions and volume transmission; Borroto-Escuela et al., 2015).

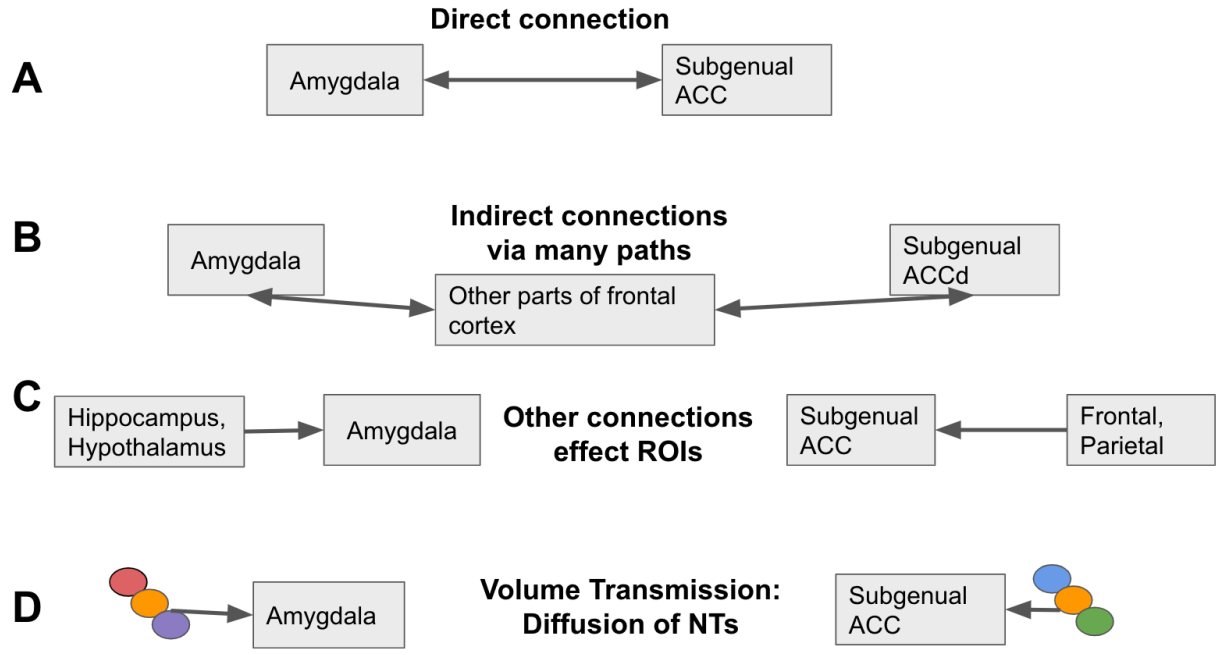

Figure 1. Potential biological reasons that can account for functional connectivity between amygdala and subgenual anterior cingulate cortex (ACC). (A) A direct structural connection produces increased functional connectivity between amygdala and subgenual ACC. (B) other parts of frontal cortex structurally mediate amygdala and subgenual ACC, and account for their elevated functional connectivity. (C) Regions affect both amygdala and subgenual ACC timecourses, which can diminish the functional connectivity of amygdala and subgenual ACC if these influences are uncorrelated. (D) Neurotransmitter (NT) diffusion can create functional connections that need are separate from direct or indirect axonal connections between amygdala and subgenual ACC.

Thus, because there could be many possible pathways between the regions that I single out *a priori*, I supplemented my hypothesis-driven tests involving connections between parts of frontal cortex and amygdala with a data-driven approach. In this analysis, I explored how anxious apprehension and anxious arousal can be predicted when including all amygdala connections in a single modified regression function. The results of this data-driven approach can add information regarding how circuits other than the direct structural connection between the regions identified *a priori* might better predict better the relationship between functional connectivity involving key brain regions and types of anxiety. Additionally, this data-driven approach uses a process called cross-validation, in which part of the sample is used to estimate the function relating amygdala connections to anxiety levels, and an entirely independent sample is used to 'test' the fit of this function. This method has been demonstrated to be less susceptible to over-fitting one's data (noise affecting one's beta estimates, which results in poor generalization).

Asking longitudinal questions in two ways

Finally, Study 2 will address goal (3) by examining longitudinal relationships between changes in functional connectivity within a subject with changes in self-reported anxiety across one year of development. This is vital to understanding how development impacts the relationship between anxiety and intrinsic functional connectivity.

One way will be to see if the population-level relationship between anxiety and functional neural connectivity shifts across time. Molenaar and Campbell (2009) refer to this as the stationarity criterion: that population-level relations gleaned in a single time-point will hold across time. Indeed, the present study will endeavor to demonstrate if that is the case across one year of adolescent neuropsychological development.

A second way to test longitudinal predictions is to see if the relationship between functional connectivity and anxiety types within subjects is different than that found at the between-subjects level. Molenaar and Cambpell refer to this as the homogeneity criterion: that population level statistics are *the same* as within-individual statistics. When this does not hold, this is evidence that longitudinal analyses are required to estimate individual-level variation, as it may reveal dynamics quite different than the group-level statistics. A standard way to test the homogeneity criterion cannot be executed in the present study, because *multiple* timepoints must be sampled to fit a random-effects multilevel model. However, as is detailed in Study 2, there may be certain inferences I can make using a method of mapping within-subject changes in the brain to within-subject changes in self-reported anxiety using two timepoints. If brain-behavior changes *within* individuals differs from single timepoint relations between brain and anxiety measures *across* individuals in the population, it suggests that individuals differ significantly from each other in terms of how anxiety is implemented and shifts across time.

Concluding remarks

The ultimate goal of my dissertation is to inform an understanding of the mechanisms giving rise to types of human anxiety and their development. As has been recently articulated, mechanisms in psychology are explained through delineating the information processing steps underlying a given function, and demonstrating how such processing is implemented biologically (Montague, Dolan, Friston and Dayan, 2012; Thomas and Sharp, 2018). Together, this enables scientists to grasp structure-function relationships that deviate from 1:1 (which most psychological functions do; Pessoa, 2017; Anderson, 2014) as well as leverage psychology and neuroscience findings to potentially create more precise and effective interventions (Sharp & Eldar, 2019). The present set of studies also has the potential to inform what targets to consider

when mapping parental, social, genetic and hormonal factors onto changes in anxiety that might account for *why* neurobiological changes occur in adolescence.

Chapter 2: HOW TYPES OF TRAIT ANXIETY ARE INSTANTIATED BIOLOGICALLY IN ADOLESCENCE

Introduction

The endeavor to explain how pathological anxiety develops has seen significant progress over the past few decades in the fields of developmental psychology (Buss & Mcdoniel, 2013) and developmental neuroscience (Pine, 2007). However, much of this work is predicated on conceiving of anxiety as a unitary construct, which contrasts with recent research in adult human neuroscience investigations of anxiety (Moser, 2013; Sharp, Miller & Heller, 2015). Indeed, it has been demonstrated that adult anxiety can be parsed into at least two distinct, transdiagnostic traits, known as anxious apprehension and anxious arousal, that have separate neural correlates and share little variance in their behavioral symptoms. Anxious apprehension is characterized by excessive worry whereas anxious arousal is marked by elevated fear and sympathetic hyperarousal.

Past work on the development of anxiety is likely biased due to researchers not taking into account these meaningfully different types of anxiety. Indeed, previous unexplained findings in the literature on youth anxiety can be addressed by acknowledging that anxiety is multidimemsional along the two aforementioned axes. For instance, panic disorder has been shown to have a much higher rate of heritability relative to generalized anxiety disorder (Albano, Chorpita & Barlow 2003). Although this finding has been interpreted as disorders not being well-circumscribed by the Diagnostic and Statistical Manual, this disparity might be better accounted for by panic disorder comprising high levels of anxious arousal, whereas generalized

anxiety disorder comprises high levels of anxious apprehension. Moreover, a recent neurophysiological study of children with various anxiety disorders (generalized, social, separation) and specific phobias showed that only those with specific phobia displayed elevated sympathetic hyperarousal (Dieleman et al. 2015). This unexpected disparity similarly could be explained by specific phobia comprising high levels of anxious arousal, whereas the other anxiety disorders involving high levels of anxious apprehension.

As such, the present study sought to test hypotheses regarding the self-reported indicators and neurobiological markers of anxious apprehension and anxious arousal in early adolescence, a time-period that has been shown to be a tipping-point in the development of pathological anxiety (e.g., Dahl and Hariri, 2005). I selected *a priori* defined structural connections from both human and non-human neuroscience research that has begun to elucidate how these forms of anxiety are implemented biologically. I first developed hypotheses about the structure of particular neural circuits for anxious arousal by pulling from non-human rodent neuroscience work on fear and anxiety (e.g., Adhikari et al., 2015). Indeed, the psychological functions thought to be involved in anxious arousal are likely shared, in part, with simpler organisms (Sharp, Miller, & Heller, 2015), which include computations regarding the likelihood of threats in the immediate environment and preparing the body to handle such threats via elevated and preparatory sympathetic arousal. I specifically focused on optogenetic studies within non-human work on anxiety because optogenetics is particularly powerful in its ability to infer causal relations among neural regions. Indeed, optogenetics allows researchers to directly control information flow in the brain between regions using light stimulation.

Two pathways between amygdala and regions in prefrontal cortex were identified using optogenetics as playing different roles in anxiety-related behavior in rodents. The downstream

connection from ventral medial prefrontal cortex (vmPFC) to basomedial amygdala was found to be anxiolytic, whereas the connection between basolateral amygdala and dorsal medial prefrontal cortex (dmPFC) promoted freezing behavior under certain conditions (Adhikari et al., 2015). These findings from rodent optogenetics comport with a rich body of literature on the relationship between these two amygdalar pathways and elevated state and trait anxiety in both rodents and human adults. Previous molecular neuroscience work in rodents identified anterior cingulate-amygdala structural connections as necessary for instantiating anxiety (Bissiere et al., 2008; Malin, Ibrahim, Tu and McGaugh, 2007). In healthy adult humans, the structural connectivity between amygdala and dorsal anterior cingulate cortex (part of dmPFC) has been shown to positively covary with trait anxiety (Greening and Mitchell, 2015), whereas the structural connectivity between amygdala and medial OFC (part of vmPFC) negatively covaries with trait anxiety (Greening and Mitchell, 2015; Kim and Whalen, 2009). Moreover, functional MRI studies of human adults have found that amygdala-dmPFC functional connectivity increases as state anxiety is induced (Robinson et al., 2012, 2013, 2014). Taken together, there is support that in both rodents and human adults, the amygdala-dmPFC pathway is involved in anxiogenesis whereas the amygdala-vmPFC pathway is involved in anxiolysis.

By contrast, anxious apprehension is characterized by verbal rumination and worry, two phenomena that qualitatively differ from the more rudimentary anxious phenomenology rodents engage in. Moreover, anxious apprehension includes states marked by rich verbal content, can be about temporally or conceptually distal threats, and engages higher-order cognitive functions (Sharp, Miller and Heller, 2015). Two pieces of evidence of how worrying is implemented in humans are (1) the association of anxious apprehension with language-dependent neural processing in left inferior frontal gyrus (iFG) and (2) the association of anxious apprehension

with inhibiting immediate threat processing brought on by an orienting bias to mild threats, a process involved right iFG (MacLeod, Mathews & Tata, 1986). A set of findings indicate that chronic worriers engage both left iFG at-rest (e.g., Heller et al., 1997) and right iFG when instructed to worry (Ellard et a., 2017).

It is vital to test whether the neurobiological correlates of anxious apprehension and anxious arousal manifest in adolescence prior to the emergence of clinically-relevant psychopathology, as the results of such studies can help identify biomarkers of psychological dysfunction that precede disease onset and can inform theories regarding the pathophysiology of the disorder. In the present study, I leveraged diffusion-weighted MRI data to examine how different types of trait anxiety, anxious arousal and anxious apprehension, are related to changes in structural connectivity in a sample of nonclinical adolescents.

I first sought to demonstrate that different types of trait anxiety, anxious arousal and anxious apprehension, are distinguishable in an early adolescent sample. Toward that end, I predicted that these distinct traits will share variance to a similar magnitude as is found in adults ($r < .2$). Next, I sought to adapt recent findings on the structural connectivity of anxiety that have either come from studying adult humans or non-human animals to the present early adolescent sample. In line with animal work in rodents (e.g., Adhikari et al., 2015), I predict that anxious arousal would be positively related to structural connectivity between rostral anterior cingulate cortex (rACC) and amygdala and would be negatively correlated with structural connectivity between medial orbitofrontal cortex (OFC) and amygdala. I focused on rostral anterior cingulate as a homolog of rodent dmPFC (which is defined as the rodent cingulate cortex; Adhikari, 2015) due to a convergence across histological, ethological and human neuroscience work in regards to its association with (1) anxiety behavior and phenomenology and (2) connectivity with amygdala

(Greening & Mitchell, 2015; Vogt and Paxinos, 2014). Indeed, rACC is positioned between limbic and cortical connections and is critical for amygdala-dependent learning (Bissiere et al., 2008). Because of their more precise anatomical designation and because of their nomenclature in the atlas from which I extracted such regions, I will refer to rACC and medial OFC instead of dmPFC and vmPFC, respectively.

For anxious apprehension, instead of measuring the structural connectivity of specific circuits, I instead averaged all connections (called the "weighted degree" of a key region; Rubniov & Sporns, 2009) involving inferior frontal gyrus (iFG), a key region implicated in anxious apprehension in adult neuroimaging work (e.g., Engels et al., 2007). I chose this measure because I did not have pathway-specific information (e.g., amygdala-vmPFC) to pull from, like I did with anxious arousal, and so chose a region-level measure of structural connectivity. Specifically, I hypothesized that the weighted degree of left and right iFG would be positively correlated with anxious apprehension. These predictions tested two the two hypotheses: (1) that anxious apprehension is positively associated with internal verbal rehearsal of worries (left iFG; e.g., Engels et al., 2007) and (2) that anxious apprehension is positively associated with inhibiting bottom-up threat signaling (right iFG; e.g., Ellard et al., 2017).

To maximize the sensitivity of our structural connectivity analyses, I employed a model to estimate multiple fibers within each voxel (Behrens et al., 2008). This method is superior to traditional diffusion tensor imaging (DTI) analyses given that DTI studies that derive fractional anisotropy assume each voxel contains one single major fiber direction, which is not the case, as over 90% of voxels contain more than one fiber orientation (Jeurissen et al., 2010). Thus, it is essential to use models that do not assume a predominant single fiber direction within a given voxel.

Methods

Participants

54 adolescents participated in the present study. 14 adolescents were excluded from analyses due to corrupted diffusion weighted data (see Quality Control section below). Our final sample included 40 adolescents (21 females; mean age= 13.49 years, range = 12.16–14.78 years). Child ethnicity was reported by parents, and the questionnaire allowed for parents to report being part of more than one ethnicity (i.e., percentages will exceed 100 due to children belonging to more than one group). 10% identified their children as Hispanic (4), 25% (10) identified their children as African American, 70% (28) identified their children as Caucasian, 10% identified their children as Asian (4), 2.5% identified their children as Native American (1), and 2.5% identified their children as "other" (1). Household income was also reported by parents (3 parents chose not to report). The range of annual household income was between 18,000 and 192,000 dollars. The inner quartile range, defined by the 25^{th}, 50^{th} and 75^{th} percentiles, were 35,500, 69,000 and 120,000 dollars, respectively. These are fairly representative numbers of the positively skewed distribution that exists in the general population, with a slightly elevated median income (median income in US = $61,000; Census.gov, 2017). All participants completed a phone screen, during which parents confirmed their child had no history of a clinical diagnosis of mental health disorders, were not taking any psychotropic medications, did not have a learning or developmental disability, and were free of all MR contraindications. All participants provided written informed assent and parents provided informed consent which were approved, along with the entire study protocol, by the Institutional Review Board.

Measures

<u>Self-reported anxious arousal</u>

Participants filled out the mini version of the Mood and Anxiety Questionnaire (Casillas and Clark, 2000), which asks participants to report about their mood and anxiety symptoms experienced over the past two weeks on a 5-point Likert scale. The anxious arousal scale consists of 10 questions measuring sympathetic hyperarousal symptomatology, such as, "my hands were shaky" or "I felt dizzy and lightheaded". The scale had good internal consistency (Cronbach's a = 0.82). To ensure findings were specific to anxiety, I controlled for depression as measured by the 8-question depression subscale (Cronbach's a= 0.70) on this same measure.

Self-Reported Anxious Apprehension

Typically, anxious apprehension is measured using the Penn State Worry Questionnaire (Meyer et al., 1990). The present dataset used a single item from the Strengths and Difficulties Questionnaire (Goodman, 1997), "I worry a lot." Although there are known limitations of using single-item measures, a recent large-scale study (n=9,040; Schroder, Clark & Moser, 2017) designed a single-item measure for anxious apprehension for time-limited contexts (large-scale studies, hospital settings). Indeed, Schroder, Clark and Moser (2017) found the item 15 on the PSWQ, "I worry all the time", was correlated highly with the total PSWQ (r=.82), had a high 1-year test-retest reliability (r=.82). This question has three possible answers: 0 = Not True, 1 = Somewhat true and 2 = Certainly true.

Imaging protocol

Diffusion-weighted imaging (DWI) data were collected using a Siemens 3T Trio MRI scanner and a 32-channel head coil. The acquisitions consisted of 30-direction DTI data with a b-value of 1000 s/mm2 and 2 b= 0 s/mm2 images acquired at the beginning of the run. The imaging consisted of 72-slices, 2 mm thick acquired with 1.9 mm× 1.9 mm in-plane resolution. A single-shot, spin-echo EPI acquisition was used with TE of 100 ms, TR of 5 s, an SMS

multiband factor of 2 (Auerbach et al., 2013; Setsompop et al., 2012a; Setsompop et al., 2012b; Xu et al., 2013) using the CMRR sequence, and a GRAPPA factor of 2 for parallel imaging (Griswold et al., 2002). In addition to the DTI scan, a structural T1-weighted magnetization-prepared rapid acquisition of gradient echo (MPRAGE) acquisition was acquired with 0.9 mm isotropic resolution, TE of 2.32 ms, TR of 1.9 s, and a magnetization preparation pulse with an inversion time, TI, of 900 ms. Participants lied still in the scanner and watched clips of movies so as to prevent them from falling asleep during the scan. The purpose of this scan was to measure the relatively stable structural connectivity of the brain in key circuits that does not change during the scan. Thus, unlike functional scans, participants could be thinking about anything during this scan.

Quality control

Prior to transforming data and conducting any analyses, each raw DWI file was manually checked to determine if there were artifacts that corrupted> 3 volumes in order to yield high-fidelity tractography results (Oguz et al., 2014).

Preprocessing

Prior to connectome reconstruction, diffusion weighted data were preprocessed by converting DICOM files to NIFTI format, followed by eddy current correction using an affine registration to the b= 0 image (i.e. without gradients). Finally, in preparation for probabilistic tractography, FSL's bedpostx (Behrens et al., 2007) was run, which estimates a probability distribution of primary fiber orientations at each voxel using Markov chain Monte Carlo sampling.

Cortical parcellation

Freesurfer's recon-all (Fischl et al., 1999) were run on each subject's high-resolution T1-weighted structural image. This outputted a cortical parcellation from which regions were defined for subsequent probabilistic tractography. The present analysis used the 68 cortical regions defined by Freesurfer that cover the entire cortex, and 14 from subcortical regions, which comprise an 82-region connectome (Desikan et al., 2006). To prepare these regions for tractography, each region was registered to diffusion-weighted space, first using Freesurfer's bbregistertool (using FSL's FLIRT initialization) to compute the transformation matrix from diffusion-weighted space to T1 space. This was followed by Freesurfer's mri_vol2vol to bring the Freesurfer parcellations into diffusion-weighted space using the inverse of the previously computed transformation matrix. Bbregister has been shown to improve registration beyond more traditional methods, in which the cost function examines gradient directions and magnitudes across tissue boundaries (Greve and Fischl, 2009).

Probabilistic tractography and connectome construction

FSL's probtrackx2 (Behrens et al., 2007) was used to carry out probabilistic tractography. Each entry in the connectome was normalized by the average volume of each ROI comprising the pathway.

Connection of interest analyses

Two a priori connections of interest, comprising amygdala to rostral ACC and amygdala to medial OFC, were predicted to implement anxious arousal. Each connection was explored in both left and right hemispheres of the brain. These connections were extracted from the overall connectome matrix, in which each of these connections comprised the connection density between the aforementioned pair of cortical or subcortical regions. I also predicted that both left and right iFG weighted degree will be associated with levels of anxious apprehension. The

weighted degree, as mentioned in the Introduction, is the average of all connection densities involving here left and right iFG. Each of these regions was defined by the Freesurfer parcellation according to the Desikan et al. (2006) atlas.

I chose not to control for gender in the regression analyses because I did not have strong *a priori* reasons for thinking gender would be a confounding variable of the relationship of interest. A confounding variable must exert a causal effect on both the independent and dependent variable in a regression scheme. Indeed, although I have evidence that gender influences anxiety (e.g., Lewinsohn et al., 1998) existing literature suggests that the relationship between gender and connectivity depends on the circuits under consideration (Ingalhalikar et al., 2014). Moreover, much of the literature on effects of gender on intrinsic connectiviy comprised unreliable neural data (e.g., 5 minutes of resting-state data; Elliott et al., 2019). Moreover, if neural correlates mediate the relation between gender and behavioral symptoms of anxiety, controlling for gender could remove meaningful variance in the relationship between brain and behavior.

Results

Table 1
Descriptive Statistics Study 1

Measure	N	Range	Min	Max	Mean	Std. Deviation	Skewness
Anxious Arousal	40	25	0	25	6.325	6.149	1.253
Anxious Apprehension	40	2	0	2	.73	.847	.574
Depression	40	23	3	26	16.7	5.743	-.303

Descriptive findings

Although anxious apprehension and anxious arousal were skewed, I chose not to log-transform their values, as the residuals from the multiple regressions below were roughly normal and the sampling distribution with a sufficiently large sample size of the test statistics of interest

(beta weights) were roughly normal. Indeed, simulation studies have shown that samples as low as 15 from skewed data generate p-values almost identical to those emanating from normal data (Habeck & Brickman, 2018).

Self-report findings

As measured via self-report anxious arousal and anxious apprehension were very weakly associated (r=.052, p=.75). Depression was correlated with anxious arousal (r =.442, p =.004), and negatively related to anxious apprehension, although not statistically significantly (r = -.118, p = .47). There were gender differences in anxious apprehension, in which females had average higher levels than males (t(38)=2.27, p=.02) but not for anxious arousal (t(38)= -.45, p=.65).

Neurobiological Findings

I conducted multiple regressions predicting types of anxiety from brain measures. In each regression, I controlled for depression and concurrent types of anxiety, given that we wanted to infer unique effects unrelated to potential covariates. I extracted 4 pathways comprising amygdala to sub-regions of frontal cortex that I expected to covary with anxious arousal and extracted the weighted degree of left and right iFG that I expected to covary with anxious apprehension. For each structural pathway used to predict types of anxiety, see Table 2.

For each regression, if a subject's structural connectivity was greater or less than 2.5 standard deviations from the mean, their data was removed from that regression model. This resulted in removing two subjects from the regression involving Anxious Arousal and left amygdala to left rACC. Controlling for the family-wise error-rate, given that each test was directional, and that there were 12 simultaneous regressions, yields a family-wise alpha level of a=.004. Only two meaningful relationships emerged from 12 regressions. Anxious arousal was associated with left rACC to amygdala (β=.597, p=.001). For anxious apprehension, there was a

trending relationship between anxious apprehension and right iFG degree (β=.314, p=.056). See Table 3 and 4 for full results of all 12 regressions.

Table 2
Pearson correlations between selected pathways and types of trait anxiety

Measure	L Amyg – L mOFC	R Amyg – R mOFC	L Amyg – L rACC	R Amyg – R rACC	L iFG degree	R iFG degree
Anxious Arousal	.058	-.152	.466**	-.031	.035	-.070
Anxious Apprehension	-.107	-.194	-.092	-.23	.057	.28

Note. * <.05 ** <.01

Table 3

Regression of anxiety types and depression against a priori defined amygdala circuits

	L Amyg – L mOFC			R Amyg – R mOFC			L Amyg – L rACC			R Amyg – R rACC		
Variable	*B*	*SE(B)*	*β*	*B*	*SE(B)*	*β*	*B*	*SE(B)*	*β*	*B*	*SE(B)*	*β*
Intercept	33.53	10.80		9.80	3.16		0.12	0.79		1.42	1.253	
Anx Arousal	.63	.59	.19	-0.04	0.17	-.038	0.17	0.43	.597**	-.01	0.069	-.030
Anx App	-3.58	3.88	-.15	-1.56	1.140	-.219	0.18	0.28	.09	-.62	0.451	-.226
Depression	-.99	.638	-.28	-.24	.19	-.233	-.019	.05	-.06	.01	.074	.026

Note: Family-wise error corrected *$p < .05$. **$p < .01$.

Table 4

Regression of anxiety types and depression against a priori defined iFG circuits

	L iFG weighted degree			R iFG Weighted Degree		
Variable	*B*	*SE B*	*β*	*B*	*SE B*	*β*
Intercept	2823.09	191.70		2340.22	228.53	
Anxious Arousal	4.50	10.52	0.08	-12.75	12.54	-.179
Anxious Apprehension	16.82	68.97	.04	162.70	82.23	.314
Depression	-6.40	11.32	-.11	16.00	13.50	.21

Note: Family-wise error corrected *$p < .05$. **$p < .01$.

Discussion

The present study establishes that anxious apprehension and anxious arousal are distinguishable via self-report and features of the structural connectome in early adolescence. Firstly, anxious arousal and anxious apprehension are weakly associated in the present adolescent sample (r=.05) as measured by self-report, indicating that they are differentiable, perhaps even to a larger degree than in adulthood (e.g., Engels et al., 2007). Secondly, the present study provides preliminary evidence that anxious arousal and anxious apprehension have separable patterns of structural neural connectivity in early adolescence. Specifically, anxious arousal was positively related to connection strength between left amygdala to left rostral ACC. In contrast, there was a tentative relationship between anxious apprehension was related to right iFG weighted connectivity. Moreover, anxious arousal was not associated with iFG connectivity, and apprehension was not associated left amygdala to rostral ACC connectivity, thus supporting that effects are unique to each type of anxiety. Overall, results here should be considered preliminary given the small sample size, number of effects probed, and weak effects especially between anxious apprehension and structural connectivity.

The effort here to map structural connectivity metrics to anxious arousal demonstrates the utility in translating basic findings on circuit dynamics in non-humans to the study of adolescents with predisposing emotional traits. Moreover, that left amygdala circuits were predictive of anxious arousal comports with a recent study that sought to predict anxious arousal symptomatology in youth (Qin et al., 2014), in which left basolateral amygdala functional connectivity was the strongest predictor among many connectivity metrics in their model.

Present results suggest the medial OFC to amygdala pathway was not related to anxious arousal, which reflects that anxiety in early adolescence may be in part due to hyperactivity in

anxiogenic mechanisms as opposed to a failure of top-down regulation in anxiolytic mechanisms. This may be an explanation for why the medial OFC-amygdala pathway, which has been found in adults to be significantly related to trait anxiety, may not present as early in the development of anxious arousal (Clewett, Bachman, & Mather 2014; Greening and Mitchell, 2015; Kim and Whalen, 2009).

Present results regarding anxious apprehension are tentative. We found a trending relationship between right iFG connectivity and anxious apprehension, suggesting that inhibiting and avoiding the propensity to process threat presents early in life. Further work should seek to replicate this finding in a larger sample that is powered adequately for detecting smaller effect sizes that likely characterize these brain-behavior relations. Moreover, additional work is required to demonstrate how the developmental course of avoiding or inhibiting immediate threat relates to the course of other functions associated with anxious apprehension. Here, we found no evidence that structural correlates of internally simulating future negative consequences in left iFG was related to anxious apprehension. This null result could be explained by at least two reasons. First, repetitively internally simulating possible future negative consequences may only become maladaptive later in life. Second, it is possible that this psychological function may be detectable with other MRI metrics, such as functional connectivity. Thus, future work on the neurodevelopment of anxiety in adolescence should seek to integrate both functional and structural neural connectivity measures.

The study has its limitations. First, the diffusion-weighted acquisition paradigm was not optimized for tractography, although it was satisfactory for estimating many tracts. While 30 directions is common for diffusion tensor imaging, it is not optimal for probabilistic

tractography. Thus, it will be essential to test the present hypotheses using acquisition protocols that yield data capable of estimating complex crossing of fibers along pathways of interest. Second, as is true of most studies in connectomics, results are dependent on the parcellation scheme one uses. Future studies should use the most functionally-specific parcellation; for instance, ones that segment the amygdala into its distinct nuclei, if possible with the resolution of one's data. This is evident in some extant contradictory findings in which some have found a negative correlation between the structural connectivity of the medial OFC-amygdala pathway and trait anxiety (e.g., Greening and Mitchell, 2015), whereas others found a positive correlation between the structural connectivity of the ventral prefrontal cortex-amygdala pathway and trait anxiety (Clewett, Bachman, and Mather, 2014). Because the ventral prefrontal cortex in the latter study covers a large swath of cortex, it included parts of rostral cingulate cortex that are likely functionally separable from medial OFC as evidenced by the present study. Moreover, an issue of cross-study comparisons is the disjunction between the nomenclature (e.g., vmPFC) and the structural boundaries defining such anatomical regions (Roy et al., 2012), in which different public or manually-drawn atlases differ in their definitions of regions. Advances in spatial resolution of neuroimaging are centrally important to translate findings from non-human optogenetics and other more fine-grained work in ethology to the study of human neurodevelopment.

Second, I did not conduct clinical interviews, and only excluded participants at screening if they had a history of clinically diagnosed mental health problems, were currently taking psychotropic mediations, or had developmental disorders or learning disabilities. Thus, it may be the case that on the dimensional measure used in the present study of trait anxiety, those at the high end may have met criteria for clinical anxiety but were not yet diagnosed. Alternatively, a

strength of the study is that it is based on a community sample, which may be more generalizable than clinical studies in regards to sampling a wider spectrum of adolescents with varying levels of trait anxiety.

In sum, findings here support that anxious apprehension and anxious arousal are separable traits in early adolescence, and have initiated the difficult endeavor of elucidating how they are implemented biologically. Evidence here suggests that anxious arousal is implemented in bottom-up mechanisms supporting anxiogenesis rather than a weakening of top-down anxiolytic mechanisms. Moreover, for anxious apprehension, preliminary evidence here supports the notion that correlates of inhibiting or avoiding bottom-up threat signals (implemented in right iFG) may appear earlier than mechanisms supporting elaborate and typically linguistic simulations of future scenarios (implemented in left iFG). In order to better contextualize results, future work should endeavor to flesh out a more mechanistic understanding of how such structural correlates of anxiety are related to functional neural dynamics, as well as a more precise computational explanation of the information processing involved in various forms of anxiety.

Chapter 3: STUDYING CROSS-SECTIONAL AND LONGITUDINAL FUNCTIONAL DYNAMICS IN ANXIOUS APPREHENSION AND ANXIOUS AROUSAL

Introduction

Over the past decade, work on the biological systems involved in psychopathology has been spurred by investigating a marker of individual cognitive variation known as intrinsic neural connectivity (e.g., Buckholtz & Meyer-Lindenberg, 2012). Intrinsic connectivity refers to the correlational patterns of activity across brain regions that indicate communication among and between stable subnetworks of the brain (Yeo et al., 2011). Intrinsic connectivity is thus a type of functional connectivity that is stable across certain networks of the brain. Emerging evidence suggests that such patterns of intrinsic connectivity networks are more heritable than task-elicited activation patterns (Elliott et al., 2019; Ge et al., 2017; Glahn et al., 2010) and can identify meaningful differences in brain organization with respect to development and psychopathology (Fox, 2010).

The present study seeks to understand how reliable estimates of intrinsic connectivity can predict cross-sectional and longitudinal changes in transdiagnostic dimensions of anxiety in adolescence. Existing literature on the relationship between intrinsic connectivity and anxiety in adolescence has mostly conceived of trait anxiety as a unitary construct. A consistent finding in this small body of work is that reduced amygdala-vmPFC connectivity negatively covaries with trait anxiety (Burghy et al., 2012; Hahn et al., 2011; Roy et al., 2013). Indeed, this overlaps with work in non-human animals that has shown that activating amygdala-vmPFC circuits is sufficient to produce anxiolysis as inferred from behavior (Adhikari et al., 2015). Only one study

to date has investigated how anxious arousal and anxious apprehension might be differentiated in terms of intrinsic brain connectivity (Burdwood et al., 2016), but has two major limitations that diminish its generalizability and validity. First, it used a typical amount of data in resting-state studies, which has been shown to have very poor test-retest reliability likely due to measurement noise (Elliott et al., 2019). Second, it was conducted on a cross-sectional adult sample.

Seeking to compare and contrast patterns of structural (investigated in Study 1) and functional connectivity (investigated in Study 2) can improve inferences about the biological processes underlying MRI signals. For instance, if one strengthens the structural connection between two regions (e.g., via increased myelination), their functional connectivity may concomitantly increase. To investigate this possibility, I will map the same *a priori* defined connections detailed in Study 1 to types of anxiety, except here, I will investigate their *functional* connectivity.

Functional connectivity between two regions may be influenced by many other factors other than their direct structural connection, and thus, structure may only partly constrain functional connectivity. For instance, several indirect pathways connecting a given pair of brain regions can result in the two brain regions being highly functionally connected. Indeed, a host of other confounders including temporal and spatial smoothing, and patterns of neurotransmitter diffusion (Anderson, 2014) may account for functional connectivity (Mehler & Kording, 2018). The biological details of how circuits are organized are vital to understanding how information processing involved in anxious apprehension and anxious arousal are carried out in the brain. Assuming the regions involved are reciprocally connected (which they most likely are), negative functional connectivity may be a sign of negative feedback dynamics (e.g., an increase in activation projecting from amygdala to prefrontal cortex results in prefrontal cortex diminishing

amygdala activation) whereas positive functional connectivity may be a sign of positive feedback (e.g., Gee et al., 2013). Such information is not available to scientists operating from a purely structural brain perspective. For this reason, it is essential to compare functional connectivity findings with structural connectivity findings in the effort to understand how neural systems implement forms of anxiety.

The present study will test both *a priori* defined hypotheses regarding the relationship between intrinsic brain connectivity and types of anxiety, as well as data-driven analyses mapping more complex relationships between intrinsic connectivity and types of anxiety. Relationships between the brain and self-reported anxiety will be estimated cross-sectionally and longitudinally. Data-driven analyses will supplement the aforementioned hypothesis-driven tests for two reasons. First, mapping several connections involving key brain regions (e.g., amygdala) to types of anxiety can better capture the various reasons listed above that influence functional connectivity (e.g., a functional connection due to indirect pathways without a direct structural connection influence). Second, this discovery-oriented approach can start the effort to parse the more complex structure of circuits involved in anxiety beyond single or pairs of neural connections (e.g., Thomas & Sharp, 2019).

In addition to estimating functional connectivity cross-sectionally, the present study will be the first to estimate how longitudinal changes in functional connectivity are predictive of changes in anxious apprehension and anxious arousal. Because developmental neuroimaging studies are just beginning to be undertaken (e.g., Telzer et al., 2018), we know little about how intrinsic connectivity develops throughout adolescence. Only recently have large scale studies been conducted to examine intrinsic connectivity in youth, typically using cross-sectional analyses across different age groups (e.g., Fair et al., 2010; Fareri et al., 2015; van

Duijvenvoorde et al., 2016). For anxiety specifically, very few studies have explored longitudinal changes in anxiety, and if investigated, have not differentiated how different types of anxiety might have different neurodevelopmental courses. For instance, it has been shown that early life stress in childhood predicts decreased amygdala-vmPFC connectivity in mid adolescence, which is negatively associated with trait anxiety (Burghy et al., 2012). Moreover, it has been demonstrated that functional connectivity between amygdala and rostral ACC positively correlates with increased anxiety in early adulthood, whereas structural connectivity between amygdala and vmPFC positively correlates with anxiety in late childhood (Jalbrzikowski et al., 2017). Although these may inform longitudinal hypotheses, it is necessary to carry out longitudinal designs (Telzer et al., 2018) as within-subject relations between brain metrics and psychological development might vary widely across individuals.

Two types of analyses will be used to explore longitudinal relations between anxiety and intrinsic connectivity. If cross-sectionally, increases in amygdala-frontal intrinsic connectivity positively covary with anxiety, one might assume that this relationship would hold for individuals as they get older. Molenaar and Campbell (2009) refer to this as the "stationarity" assumption: that lawful relations in the population hold over time. However, this likely is not the case for most phenomena across development, as meaningful changes (e.g., puberty, changing social roles) exert effects on neurobiology that fundamentally shift how anxiety is implemented in various neural systems. This will be probed in the present study by investigating how the between-subjects relationship between anxiety and intrinsic connectivity differs across one year of development in adolescence.

Individuals may also differ *from each other* in terms of how changes in their connectivity relate to changes in their levels of anxiety across time. Molenaar and Campbell (2009) assert that

if subjects are different from each other in terms of how predictors relate to outcomes over time, it violates the "homogeneity" criterion that allows one to adapt single time-point between-subjects trends to within-subject trends over time. In their seminal review, Molenaar and Campbell (2009) cite a study in which a factor structure of personality derived from a single time-point between-subjects differed greatly from the variety of subject-specific factor structures derived from within-subject variability (Borkenau & Ostendorf, 1998).

Although the present study is not optimized to estimate the *degree* to which change across time differs across individuals (typically via random slope effects in multilevel modeling) the present design can provide initial evidence as to whether or not within-subject effects relative to between-subject effects will be important in explaining the relationship between anxiety and intrinsic connectivity. If, for example, within-subject changes in a given intrinsic connectivity measure predict within-subject changes in anxiety, but cross-sectionally there is no relationship between that brain connectivity measure and anxiety, this indicates that estimating within-subject variability is essential to elucidating how anxiety is implemented differentially implemented across individuals.[4] The present study will employ a longitudinal analysis referred to as the "method of first difference" (Liker, Augustyniak, & Duncan. 1985) to assess within-subject relations between anxiety and intrinsic neural connectivity by mapping changes in connectivity to changes in anxiety within subjects.

Methods

Participants

[4] An analogy to drive home this point is the following. Imagine you have three different kinds of computers, each with their own very different hardware, and you want to correlate how activity in RAM relates to CPU activity. At the population level, there may be no correlation between RAM as predictor and CPU as outcome, because the machines are entirely different from each other. However, when assessing how the computers change, there may be consistent within-computer relations that hold across computers.

Participants were involved in a longitudinal study that was focused on studying emotional, cognitive and social development in adolescents using a range of behavioral, neuroimaging, and self-report measures. As such, the present study was not designed to study anxiety specifically. Limitations of using this existing dataset are explored in the discussion. The present sample comprises two waves of data, in which the first wave comprises 6th and 7th graders, and the second wave, 7th and 8th graders collected one year later, ages ranging from 11-14 years old at wave 1 (average age=12.8 years old), and at wave 2, 12-15 years old (average age=13.75 years old). Overall, the larger sample from which we selected participants totaled 148 participants at wave 1, and 115 participants at wave 2. 104 participants (52 Female) were included in the present study as they completed both waves of data collection and had the requisite amount of data to estimate intrinsic connectivity (11 subjects had too little data; see Quality Control section). The average time between scan was 348.36 days with a standard deviation of 27.11 days. In terms of participant ethnicity, 29% identified as Hispanic or Latino (30), 23% identified as African American (24), 28% percent identified White (29), 2% percent identified as Native American (2), and 18% identified as mixed ethnicity (19). SES was determined by self-reporting of ranges of family income in 10 tiers (1: 0-15k in 15k dollar increments, except for 7: 90 – 99k, 8:100-120k, 9: 120-150k, and 10: over 150k), Median annual household income was between 45,000-60,000 dollars, and the distribution was positively skewed. 10% of the sample had a total household income of extreme poverty (0-15,000 dollars annual income).

Measures

Scanning protocol

All scans were acquired on a 3T Siemens PRiSMA scanner. High-resolution T1 scans were .8 mm isotropic (slice thickness .8 mm), TR=2400 ms, TE=2.22 ms, FOV=256 mm. T2 structural images were acquired to improve registration, and were acquired with 1.2 mm x 1.22 mm resolution, slice thickness = 3mm, TR = 5700 ms, TE = 65 ms, FOV = 230 mm. For all BOLD scans, voxel size was 2.5 mm x 2.5 mm, Slice Thickness = 3mm, TR = 2000 ms, TE = 25 ms, FOV = 230 mm.

General functional connectivity acquisition

General functional connectivity estimates intrinsic connectivity by concatenating time-series data across all BOLD scans (both resting-state and task-based). Tasks used in wave 1 and wave 2 are briefly described below. Again, although task-based data are being used, the present analysis treats it as resting-state data; it does not estimate stimulus-induced activation as is typically done in classical univariate generalized linear modeling approaches to task-based fMRI data. Rather, GFC estimates stable intrinsic connectivity patterns that tend to be coactive both at rest and doing various cognitive tasks.

Preprocessing

Motion was removed by using FSL MCLFIRT algorithm as well as via a rigorous independent component analysis to remove both physiological noise and noise (custom implementation of FSL's MELODIC algorithm). Specifically, ICA was trained on an independent set of adolescent neural data in which relevant patterns of noise were classified. Subsequently I used the results of this classifier to automatically identify components of noise in the present dataset, and removed those from analysis. Motion was handled in the same way as Lee, Miernicki and Telzer (2018), in which ICA denoising was used to remove noise components that were not suppressed using FSL's MCLFIRT algorithm. Indeed, this has been

shown to be superior to motion censoring and using motion regressors in a general linear model approach for estimating intrinsic functional connectivity (Pruim et al., 2015). Images were then registered to standard space by a combination of linear and nonlinear transforms, first by registering BOLD images to high resolution T1 space via T2 images, and then to standard 2mm MNI space. Images were also smoothed at 6 mm full-width at half-maximum.

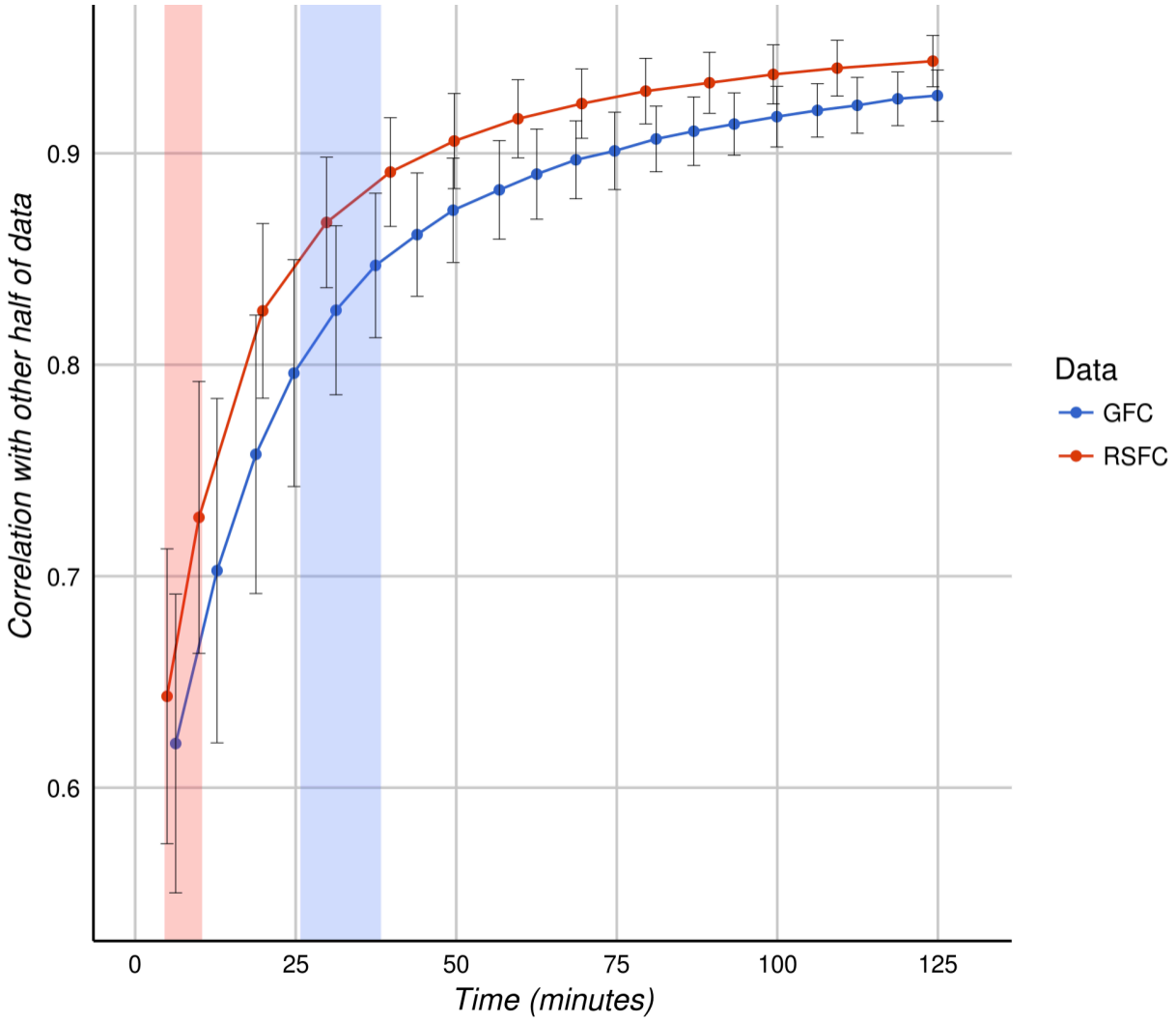

Figure 2. A comparison of resting state and GFC estimates of intrinsic connectivity. The red region represents the amount of time used in typical resting state scans used to estimate intrinsic connectivity, which results in very poor reliability (0.6). Reliability here is defined as the correlation between estimates of intrinsic connectivity derived from data across two separate measurement periods taken close together. For instance, if 10 minutes of fMRI data are

collected, and the first 5 minutes are used to estimate intrinsic connectivity, the reliability coefficient represents the correlation between that estimate and the estimate derived from the final 5 minutes of the scan. Given that intrinsic connectivity should not shift over the course of a single scan, reliability theoretically should be close to 1. In the blue region, the reliability of intrinsic connectivity increases to tolerable levels (0.8) using GFC on 25 or greater minutes of data. The reason for imperfect connectivity is due to measurement error and other sources of noise in the data.

fMRI data used to estimate intrinsic connectivity: resting-state + multiple tasks

General functional connectivity estimates intrinsic connectivity metrics from common functional scans across waves of data. Because Elliott et al. (2019) and Gratton et al. (2018) found (and replicated) that variance in intrinsic connectivity was not well-explained by variation due to task (See Figure 2, borrowed from Elliott et al., 2019), I used all tasks that were common across both waves and resting state data. Total time of tasks + resting state data is 52 minutes, which, per Figure 2, yields much greater reliability using GFC in non-longitudinal contexts than typical 8-10 minute resting-state scans and analyses.

Resting-state scan acquisition

Resting state scans were 8 minutes long. Participants were instructed to keep their eyes open and simply gaze at an image that did not change over the duration of the scan.

Social Incentive Delay Task (wave 1 and 2; 2 rounds, 13 minutes total)

The SID is designed to measure neural sensitivity to anticipation of and receipt of social rewards and punishments. In the task, teens saw a circle, a square, or a diamond. Then, they saw a white square. They are trained (prior to entering the scanner) to press their right index finger as fast as they can after seeing the white square, but not before. During the training completed prior to the scan, they learned that each shape is a cue, which indicates whether or not they will see a happy, angry, or blurred face depending on how quickly they press.

Ratings Task (wave 1 and 2; 2 rounds, 16 minutes total)

This task involved participants rating what they think their parents or peers their age think of various risky behaviors, using a 1-9 likert scale. For example, participants rated how good or bad their parents felt about failing an examination in school. 1 refers to participants asserting the proposed behavior is very bad, whereas 9 is very good.

Cups Task (wave 1 and 2; 3 rounds, 15 minutes total). The Cups task measures risk-taking in the context of monetary rewards. The task has been used in prior studies of adolescent risk taking (e.g., Galvan & McGlennen, 2012), during which adolescents make decisions in the context of a sure outcome or unsure outcome.

Quality control

An intensive set of quality control steps were taken to ensure that subjects' data was not biased by artifacts or loss of data. First, I created a custom algorithm to ensure that all subjects had at least 80% of data in each sphere used in the connectome construction (due to parts of the brain being cut off or due to signal loss, which primarily affects ventral, frontal regions and parts of the top of parietal cortex). If more than 90% of the final sample had less than 80% of data in any region, I excluded the region from all connectomes. For all other data loss on a subject-specific basis, I excluded all spheres that had less than 80% coverage, and imputed the data when computing regressions. I also only included participants that had at least 25 minutes of data, as the reliability of intrinsic connectivity is 80% at this level. This resulted in excluding 11 participants, leading to a final sample of 104 participants with the requisite amount of data to ensure intrinsic connectivity estimates were reliable

Connectome construction

We investigated whole brain intrinsic connectivity using 273 brain regions from a parcellation scheme derived in a large independent dataset (Seitzman et al., 2018). BOLD data

was averaged within 5mm spheres surrounding each of the 273 coordinates (defined in standard 2mm MNI space) in the parcellation. Specific ROIs for hypothesis driven tests were selected to match closely the anatomical definitions of structural ROIs in Study 1. Moreover, when picking multiple spheres to define ROIs, the time-series within these spheres all came from the same functional networks defined in Seitzman et al., 2018, and were averaged. These average time-series were then correlated with each other according to a priori hypotheses. All connectivity estimates were derived by computing Pearson correlations on each pair of regional timeseries. Due to data-loss and lack of hypotheses regarding the cerebellum, all cerebellar connections were excluded from present analyses.

Self-reported anxious apprehension

Typically, anxious arousal is measured using the Penn State Worry Questionnaire (Meyer et al., 1990). The present dataset used a single-item measure, which is consistent with how it was measured in Study 1, using here the Child Behavior Checklist – Youth Self Report (Achenbach, 1991), "I worry a lot." Note, however, the wording and scale are identical across this item in the SDQ (used in Study 1) and the CBCL as used here. Although there are known limitations of using single-item measures, there was recently a large-scale effort (n=9,040; Schroder, Clark & Moser, 2017) to design a single-item measure for anxious apprehension for time-limited contexts (large-scale studies, hospital settings). Indeed, Schroder, Clark and Moser (2017) found the item 15 on the PSWQ, "I worry all the time", was correlated highly with the total PSWQ (r=.82) and had a high 1-year test-retest reliability (r=.8). This question has three possible answers: 0 = Not True, 1 = Somewhat or sometimes true and 2 = Very true or often true. Change scores on the measure could thus range between -2 and +2.

Self-reported anxious arousal

Participants filled out the Child Behavior Checklist (Achenbach, 1991), which includes items that overlap with the anxious arousal scale used in Study 1. I selected items, mostly from the somatic complaints subscale, that have been shown in youth to indicate anxious arousal (referred to as 'trait fear') and overlap with criteria in the gold-standard self-report measure used for anxious arousal, the Mood and Anxiety Symptom questionnaire (Chorpita, Albano, & Barlow, 1998). These items include reporting how often children experience the following physiological symptoms:

Dizzy
Overtired
Nausea
Stomach aches
Vomiting
Problem with Eyes
Headaches
Rashes
Aches or Pains

This 9-item measure was derived by taking the sum of all items had acceptable internal reliability in the present sample (α=0.72).

Self-reported depression

Participants filled out the Short Mood and Feelings Questionnaire (SMFQ; Angold, Costello, & Messer, 1995). This questionnaire was designed to measure depression in children and adolescents and has 85% specificity for diagnosing major depression in youth (Angold, Costello, & Messer, 1995). Questions were answered on a 3-point likert scale (0=Not True 1=Sometime True, 2=Mostly True) for items related to mood (e.g., "I felt miserable or unhappy").

A Priori Hypotheses

I computed Pearson correlations for the following brain-psychological relationships. For anxious apprehension, I hypothesized that the weighted degree of left and right iFG would be positively correlated with anxious apprehension. For anxious arousal, I predicted that amygdala to ventromedial prefrontal connectivity would be negatively correlated with anxious arousal, and that amygdala to dorsomedial prefrontal cortex would be positively correlated with anxious arousal. These regressions tested hypotheses regarding how these types of anxiety are implemented in the brain.[5]

Data-driven analyses: Nested Cross-validated Ridge Regression

I supplemented *a priori* analyses with a data-driven approach to explore how intrinsic intrinsic connectivity across all amygdala and iFG connections predict anxious arousal and anxious apprehension. As an example, the data-driven algorithm optimized a function mapping all amygdala connections (i.e., how functionally connected left amygdala is with all other regions in the brain) to anxious arousal. If relevant information is contained in these connections, there will be a high correlation between predicted anxious arousal levels derived from the model and actual anxious arousal levels.

Because many features (i.e., independent variables) are included in the regression algorithm that map brain changes to psychological changes, I used ridge regression, which minimizes collinearity among features and reduces overfitting. I also used a nested 8-fold cross-validation procedure similar to the design implemented in Greening and Mitchell (2015). Here, a training subset of the data was used to (1) estimate a parameter to reduce multicollinearity among predictors and (2) estimate the function relating intrinsic connectivity to levels of anxiety.

[5] Although regression weights can indicate causal relations, here, they can only indicate the implementation of anxiety in biological circuits. For this reason, we did not predict e.g., anxiety at wave 2 from the brain at wave 1, controlling for anxiety at wave 1, given that this regression would remove the meaningful covariance between anxiety and brain at wave 1 (i.e., evidence for implementation).

The scheme uses a leave-one-out cross-validation procedure on the training subsamples to estimate these parameters (i.e., fit regression on all of training except one participant, test function on the participant left out, and repeat leaving a different participant out each time). The average best-fitting regularization parameter and beta-weights relating brain measures to self-reported anxiety types were then applied to independent data-points (comprising 13 left-out "test" participants; "left-out" here means these subjects were not used in the sample used to estimate regression coefficients). This was done 8 times on 8 different training and testing sample pairs, which created a vector comprising predicted anxiety scores for the entire sample size. This vector was compared to the actual vector of anxiety scores via a Pearson correlation, which indicates how predictive the collective set of brain connectivity measures was of a given type of anxiety.

When I refer to "iFG connectivity" or "amygdala connectivity" in the context of interpreting data-driven analyses, these terms refer to the many connections involving either iFG or amygdala in the modified regression schemes that comprise the data-driven analyses. For instance, amygdala connectivity refers to the predictive success of regressions that fit beta weights to the intrinsic connectivity (scaled from 0-1 via Pearson correlation) of amygdala-vmPFC, amygdala-dmPFC, etc. for *all* amygdala-involving connections in the brain.

Results

Table 1
Descriptive Statistics

Measure	N	Range	Min	Max	Mean	Std. Dev.
Anxious Apprehension Wave 1	104	2.00	.00	2.00	.442	.621
Anxious Apprehension Wave 2	104	2.00	.00	2.00	.548	.667
Anxious Apprehension Change	104	3.00	-2.00	1.00	.106	.652
Anxious Arousal Wave 1	104	13.00	.00	13.00	3.673	2.934
Anxious Arousal Wave 2	104	15.00	.00	15.00	3.644	3.247
Anxious Arousal Change	104	17.00	-8.00	9.00	-.029	2.881

Note. Change measures were computed by subtracting anxiety at wave 2 from anxiety at wave 1, resulting in higher numbers reflecting an exacerbation of anxiety.

Descriptive statistics

The present sample had a positively skewed distribution across both anxiety measures, which is to be expected given the use of an adolescent community sample (e.g., Beesdo et al., 2007). Despite this skewness, the non-normality of residuals is buffered by large sample sizes, which via simulation has been shown to result in normally-distributed sampling distributions of statistics of interest (here Pearson correlations), and allows for valid inferential tests (i.e., p-values being reliable across differing levels of how non-normal the residuals are). Mean levels of anxious apprehension rose across adolescence (although statistically non-significant, $t(104)=1.22$, $p=.22$), whereas mean levels of anxious arousal stayed relatively stable. In terms of how frequent it was to change anxious apprehension across time, 68 remained the same, 25 got worse, and 11 improved. For anxious arousal, the range of change spans a decrease in 8 points on the scale to an increase in 9 points on the scale. The correlation was slightly higher between anxious apprehension and anxious arousal in the present sample than is typically found in adults (wave 1: $r=.43$, wave2: $r=.49$). That said, prior findings in samples over-sampling for high levels of anxious apprehension and anxious arousal have found the correlation to increase to around 0.5 (Engels et al., 2010). Levels of anxious apprehension (mean levels females=.62, males=.27, $t(103)=2.95$, $p=.004$) and anxious arousal (mean levels females=4.17, males=3.17, $t(103)=1.76$, $p=.08$) at wave 1 were higher in females and males. This pattern held at wave 2 for anxious apprehension (females=.83, males=.27, $t(103)=4.68$, $p=.000$) and anxious arousal (females=4.57, males=2.71, $t(103)=3.04$, $p=.003$).

We expected the test-retest reliability of neural and psychological measures to be significantly lower than 1 given that we hypothesize types of anxiety, and their neural implementation, will change over the course of a year. Anxious arousal was correlated with itself across year of development at r=.35, whereas anxious apprehension was at r=.49. Brain measures were weakly correlated across 1 year of development in adolescence, ranging from r=.06 to r=.24.

A priori hypotheses: Does structure in Study 1 predict function in Study 2?

Amygdala-Frontal Cortex connections and nxious rousal

No *a priori* defined connections, spanning amygdala-dmPFC and amygdala-vmPFC at either waves or longitudinally, significantly correlated with anxious arousal. As was done in Study 1, we controlled for depression and concurrent levels the additional dimension of anxiety. No regressions revealed significant effects of amygdala-dmPFC or amygdala-vmPFC connectivity on anxious arousal. The strongest effect was found between anxious arousal and right vmPFC-amygdala connectivity, albeit a weak effect (β =.095) and large p-value (p=.29).

iFG connectivity and anxious apprehension

Anxious apprehension, at either wave or longitudinally, was not correlated with the weighted degree of all iFG connections (correlation= -0.08, between anxious apprehension and right iFG at wave 2). As was done in Study 1, we controlled for depression and concurrent levels the additional dimension of anxiety. No regressions revealed significant effects of iFG metrics on anxious apprehension.

Table 2
Pearson correlations between intrinsic connectivity and types of trait anxiety

	Anx App Wave 1	Anx App Wave 2	Anxious App Change	Anxious Aro Wave 1	Anxious Aro Wave 2	Anxious Aro Change
Left iFG Wave 1	0.000	-0.032	-0.034	0.042	0.032	-0.007
Right iFG Wave 1	-0.009	0.008	0.018	0.053	0.05	0.002

Left iFG Wave 2	-0.040	-0.043	-0.006	-0.006	-0.048	-0.049
Right iFG Wave 2	0.022	-0.078	-0.01	-0.028	0.003	0.032
Left iFG Change	-0.028	-0.005	0.022	-0.034	-0.057	-0.029
Right iFG Change	0.025	-0.070	-0.096	-0.068	-0.038	0.027

Note. Entries are Pearson correlation coefficients. All p-values are greater than .05. The critical correlation coefficient value to reach statistical significance in the present sample is r = 0.19.

Machine learning analyses: Cross-validation & Estimating degree of predictive power

The machine learning analysis comprised an 8-fold nested cross validation procedure. 8 different training subsamples of the data were used to map a function relating brain metrics to anxiety measures as well as determining the optimal regularization parameter for the function (this parameter reduces multicollinearity among independent variables). Subsequently, each function was applied to an independent testing subsample of the data that was not used to estimate the function (function here refers to the beta-weights in the regression). The model fit to the testing subsample generated predictions of anxiety levels that was compared to participants' actual anxiety levels using Pearson correlation coefficients.

Data-Driven analyses involving iFG connections

At wave 2, a weighted combination of left iFG intrinsic connectivity metrics was significantly associated with anxious apprehension levels (r=.23, p=.01). All other machine-learning analyses at different waves were non-significant for iFG (see Table 3). Importantly, only positive correlations are meaningful in all data-driven analyses used here, given that the correlation is referring to the relationship between predicted and actual anxiety levels (i.e., an inverse relationship between predictions and actual scores is non-interpretable). Each iteration of the machine learning analysis fits a function to predict anxiety scores, and these predicted scores are correlated against the actual scores. The greater the strength of the *positive* correlation, the greater the predictability is self-reported anxiety from intrinsic connectivity. Negative

correlations reflect that the predictive function is likely fitting noise and not signal, in that the function fit to predict anxiety does not work well on out-of-sample predictions (and happens, in certain cases, to be anticorrelated with actual anxiety scores). For this reason, I used one-sided significance tests to compute p-values. Moreover, the correlation at time 2 was significantly different than the correlation between left iFG and anxious apprehension at time 1 ($z(104)=1.88$, $p=.03$). Interestingly, I also found within-subject longitudinal changes in left-iFG was associated with anxious arousal ($r=.21$, $p=.015$), although this was not expected.

Table 3
Machine learning results for iFG Connections

Variables in correlation	Wave	Pearson Correlation (r, p-value)
Left iFG with Anxious Apprehension	1	(-0.03, 0.37)
Left iFG with Anxious Apprehension	**2**	**(0.23, 0.01)**
Right iFG with Anxious Apprehension	1	(-0.25, 0.005)
Right iFG with Anxious Apprehension	2	(-0.11, 0.13)
Left iFG with Anxious Apprehension	longitudinal	(-0.03, 0.37)
Right iFG with Anxious Apprehension	longitudinal	(-0.009, 0.46)
Left iFG with Anxious Arousal	1	(-0.02, 0.44)
Left iFG with Anxious Arousal	2	(-0.07, 0.24)
Right iFG with Anxious Arousal	1	(-0.012, 0.45)
Right iFG with Anxious Arousal	2	(-0.13, 0.10)
Left iFG with Anxious Arousal	**longitudinal**	**(0.21, 0.015)**
Right iFG with Anxious Arousal	longitudinal	(-0.06, 0.27)

Note. Negative correlations are meaningless in this scheme, given that it denotes that there was a negative relationship between predicted and actual anxiety measures.

Data-Driven analyses involving amygdala connections

Across all waves, both cross-sectionally and longitudinally, amygdala intrinsic connectivity did not predict anxious apprehension (largest magnitude of correlation between predicted and actual anxious apprehension levels was $r=.03$). For anxious arousal, left amygdala connections predicted levels of anxious arousal at wave 2 ($r=.193$, $p=.025$). A similar

relationship between left amygdala connectivity and anxious arousal was found in wave 1 (r=.11, p=.14), albeit smaller and thus non-significant. See Table 4 for all correlations.

I conducted a follow-up Steiger's Z-test on the correlation between anxious arousal and amygdala connectivity at Time 1 (r=.193) with the correlation between anxious arousal and amygdala connectivity at Time 2 (r=.11). Results demonstrated that these correlations were not significantly different from each other (z(104)=.58, p=.28).

Table 4
Machine learning results for amygdala connections

Variables in correlation	Wave	Pearson Correlation (r, p-value)
Left Amygdala with Anxious Apprehension	1	(-0.04, 0.35)
Left Amygdala with Anxious Apprehension	2	(-0.01, 0.44)
Right Amygdala with Anxious Apprehension	1	(-0.07, 0.24)
Right Amygdala with Anxious Apprehension	2	(0.03, 0.38)
Left Amygdala with Anxious Apprehension	longitudinal	(-0.16, 0.05)
Right Amygdala with Anxious Apprehension	longitudinal	(0.03, 0.40)
Left Amygdala with Anxious Arousal	1	(0.11, 0.14)
Left Amygdala with Anxious Arousal	**2**	**(0.19, 0.025)**
Right Amygdala with Anxious Arousal	1	(-0.2, 0.02)
Right Amygdala with Anxious Arousal	2	(0.07, 0.24)
Left Amygdala with Anxious Arousal	longitudinal	(0.07, 0.24)
Right Amygdala with Anxious Arousal	longitudinal	(-0.18, 0.04)

Note. Negative correlations are meaningless in this scheme, given that it denotes that there was a negative relationship between predicted and actual anxiety measures.

Discussion

The present study investigated how intrinsic connectivity, estimated using a cutting-edge method to generate reliable statistics called General Functional Connectivity (Elliott et al., 2019), was related to anxious apprehension and anxious arousal in a longitudinal sample of adolescents. I first tested if *a priori* defined functional pathways were associated with anxious arousal and anxious apprehension both cross-sectionally and longitudinally. In addition to these hypothesis-driven tests, I used data-driven analyses to more broadly characterize how various intrinsic functional connections involving key regions of the brain could predict cross-sectional and

longitudinal variation in anxious arousal and anxious apprehension. These data-driven analyses allowed me to more broadly sample the various circuits involving amygdala and iFG in an exploratory fashion, which can provide hypotheses for future studies.

Overall, all correlations between *a priori* defined functional connections and types of anxiety were of low magnitude and non-significant, both cross-sectionally and longitudinally. Correlations between these regions and anxiety could change, however, when the activity between two regions is highly constrained during certain, relevant contexts (e.g., when confronted with a potential threat, the correlation between structure and function in specific anxiogenic paths could be much higher; Gratton et al., 2018). Further work is needed to identify how specific, task-related connectivity might be more tightly linked to structural connectivity correlates of anxiety.

I then used a data-driven approach to explore the complex circuit dynamics of key regions (e.g., iFG for anxious apprehension) that might further explain how anxious apprehension and anxious arousal are implemented neurobiologically in adolescence. In this machine-learning framework, I found that left iFG connectivity was associated with anxious apprehension cross-sectionally, and left amygdala connectivity was associated with anxious arousal cross-sectionally. This adds to the effort to understand which key regions in adolescence are involved in anxious apprehension and anxious arousal, and necessitates further exploration into the specific types of connections and their organization that accounts for the ability to predict these types of anxiety. Moreover, data-driven findings here highlight that although amygdala connectivity has been associated with trait anxiety defined broadly in adolescence (e.g., Swartz et al., 2014), it may be specifically important for anxious arousal and not anxious

apprehension. However, it should be stressed that data-driven analyses are exploratory in nature, and require further replication via hypothesis-driven tests to confirm their reliability.

The gap between findings at wave 1 and wave 2 can inform when certain functions involved in anxiety emerge. A modest correlation was found between amygdala functional connectivity and anxious arousal at wave 1 that became stronger and thus significant at wave 2. Even though the prior finding was non-significant, the American Statistical Association (2015)[6] and more recent Bayesian alternatives to frequentists significance testing (e.g., Kruschke, 2018), argue against using statistical significance as the sole criterion for interpreting the actual meaningfulness of findings. For instance, both the magnitude and direction of the effect is important, which is separate than passing significance thresholds that are a function of sample size. To determine if there was a meaningful difference in these correlation values, we conducted a Steiger's z-test, which estimates if the difference in two bivariate correlation statistics is meaningfully different from 0. The difference between the correlations at wave 1 (r=.11) and wave 2 (r=.193) was not significantly different from 0, lending evidence to the notion that anxious arousal may be instantiated in amygdala connectivity throughout early adolescence.

By contrast, anxious apprehension was predicted from left iFG functional connectivity at wave 2, but not at wave 1. A Steiger's z-test was then conducted to determine if the correlation at wave 2 and wave 1 were different, and indeed it was. These findings suggest that disruptions in internal mental simulation involved in worrying may emerge later in adolescence. As such, self-

[6] "Practices that reduce data analysis or scientific inference to mechanical "bright-line" rules (such as '$p < 0.05$') for justifying scientific claims or conclusions can lead to erroneous beliefs and poor decision making. A conclusion does not immediately become "true" on one side of the divide and "false" on the other…P-values and related analyses should not be reported selectively. Conducting multiple analyses of the data and reporting only those with certain p-values (typically those passing a significance threshold) renders the reported p-values essentially uninterpretable."

Source: The ASA's Statement on p-Values: Context, Process, and Purpose. (2016) The American Statistician. 70: 129-133.

reporting of worry at earlier ages (wave 1 comprises 11-13 years old) may reflect less elaborative worry that requires less mental simulation. Prior work has provided preliminary support for this contention in that worry elaboration increases with age and cognitive development (Muris et al., 2002). Moreover, that right iFG connectivity did not predict anxious apprehension suggests the tendency to inhibit immediate threat responding is captured better by specific functional network dynamics. For instance, it may be more valuable to look at the degree of involvement of right iFG in the ventral attention network (which is especially important for threat detection; Corbetta, Patel & Shulman, 2008), instead of the entire brain network as was done in the present analysis.

I also sought to investigate how within-subject changes in anxiety could be predicted by within-subject changes in intrinsic connectivity across 1 year of development. Examining within-subject change is vital given that, unlike cross-sectional studies of the development of anxiety, subjects act as their own controls, diminishing confounders of effects of interest such as cohort effects (e.g., Louis et al., 1986). Only one unexpected finding emerged from the data-driven analyses on within-subject changes, which showed that left-iFG changes were predictive of changes in anxious arousal. Indeed, because left-iFG very likely implements many functions, future research should endeavor to investigate what kind of information processing it may be carrying out that is of relevance to understanding the development of anxious arousal.

In order to adequately test whether or not there is variability across subjects in how within-subject changes in functional connectivity relate to within-subject changes in anxiety, future work must include more repeated measures of brain connectivity at longer (e.g., annually) and smaller time-scales (e.g., daily). Both time-scales are needed to characterize how both more phasic fluctuations in anxiety, and more slower-moving developmental changes in anxiety types over years, can be partially explained by how intrinsic connectivity shifts within subjects. As

such, longitudinal neuroimaging paradigms are needed to understand individual, or sub-group, developmental trends in the relationship between brain development and changes in types of anxiety across adolescence. Incipient efforts in other domains not related to anxiety have found that for basic cognitive functions (cognitive control) there is wide variation across subjects in the relationship between brain functional dynamics and psychological performance (Braga & Buckner, 2017).

In addition to a lack of sampling relevant longer and shorter timescales of brain and behavior changes related to types of anxiety, future studies also should endeavor to use multiple converging measures of anxious apprehension and anxious arousal. Indeed, given that Study 1 revealed structural connectivity correlates of these two types of anxiety, future work should measure both structural and functional connectivity on the same sample to be able to directly compare how the two types of brain measures are related. As stated in the Introduction, structure and function contribute meaningfully different and necessary information in the effort to elucidate how neural systems work (e.g., McLelland & Rumelhart, 1981). Thus, the present sample is limited in not directly comparing structural and functional neuroimaging modalities.

Moreover, a limitation of the present study was its use of a one-item measure for anxious apprehension and a non-gold-standard self-reported measure of anxious arousal. Future studies should seek to use the most psychometrically well-validated tools to measure self-reported levels of anxious apprehension and anxious arousal in adolescence (Flake & Fried, 2019). Moreover, the present sample had a much larger correlation between self-reported anxious apprehension and anxious arousal than that found in study 1. Many reasons might account for this disparity. For instance, the correlation estimate in any given sample could be influenced by sampling error, different ranges of psychopathology across both samples, sample size, and unknown factors that

influence the prevalence of co-occurrence of anxious apprehension and anxious arousal in adolescence. It may also be due to the questionnaire used in the present study to measure anxious arousal, which is not considered the gold standard measure (Watson and Clark, 1991).

Future work should also consider supplementing self-reported metrics of anxiety types by extracting latent variables from both self-report and peripheral physiological measures. Lastly, it should be noted that due to lower signal-to-noise ratio in amygdala relative to other regions in the brain, it may be required to garner much more data to estimate highly reliable intrinsic connectivity estimates involving amygdala (Elliott et al., 2019).

Overall, the present study advances an understanding of how anxious apprehension and anxious arousal are instantiated in part in intrinsic neural connectivity throughout adolescence. The results presented here provide promising hypotheses for future longitudinal and experimental work. Experimental work should test and validate the role left iFG plays in elaborative worry, and longitudinal work can better describe how chronic worry changes across childhood and adolescence in part via left iFG connectivity changes. Moreover, longitudinal studies can lend support to how stable and specific amygdala connectivity is in predicting anxious arousal and not anxious apprehension.

Chapter 4: CONCLUSIONS AND FUTURE DIRECTIONS

Synthesizing findings from Studies 1 and 2

Studies 1 and 2 add to our understanding of the different neurocognitive processes involved in anxious apprehension and anxious arousal in adolescence, as well as how these forms of anxiety develop. Study 1 provides preliminary support for the value in translating findings on anxious arousal gleaned from recent advances in studying rodents to the study of structural connectivity in humans. Specifically, it was found that the human homolog of an anxiogenic circuit in rodents was positively correlated with anxious arousal in youth (whereas the anxiolytic pathway did not). This provided evidence that risk for anxiety in early adolescence may involve disruptions in anxiogenic circuits (too much amplification of anxiety) relative to weakened anxiolytic circuits (too little regulation of initial anxiety responses). Secondly, Study 1 showed preliminary, albeit tentative, evidence that a circuit involved in inhibiting and avoiding bottom-up threat signals (right iFG average connectivity) was associated with anxious apprehension in adolescence. Notably, the two predictions that failed were (1) that an anxiolytic pathway thought to be related to anxious arousal was uncorrelated with anxious arousal and (2) that left iFG connectivity, thought to instantiate the simulation of future threats, was uncorrelated with anxious apprehension. Given the number of tests conducted and small sample size, study 1 should be thought of as a preliminary exploration of the structural correlates of types of anxiety in adolescence that require replication. Moreover, because informative results from Study 2 were found in data-driven analyses, future work should seek to replicate these preliminary findings. As such, given that this is the first exploration of transdiagnostic dimensions of anxiety in

adolescence, the present dissertation should be thought of more as a discovery-oriented project to establish more informed hypotheses about the development of anxiety in adolescence.

It should be noted, however, that the strength of inferences is stronger for the anxious arousal case than it is for anxious apprehension finding in Study 1. Findings for anxious arousal were motivated by precise causal manipulations of homologous neural circuits in rodents (e.g., Adhikari et al., 2015), whereas for anxious apprehension, left iFG connectivity hypotheses were based on correlational work in humans (e.g., Heller et al., 1997). Although lesion studies demonstrate that left iFG is involved in speech production (Blank et al., 2002), it remains to be demonstrated precisely if, to what degree, and how left iFG is involved in mental simulation involved in worry (e.g., Sharp, Miller & Heller, 2015). As such, present findings could merely indicate that the weighted structural degree of left iFG is not the appropriate biological correlate to measure internal mental rehearsal of negative future events. The same can be said for the tentative finding and theory supporting right iFG connectivity and anxious apprehension. Future work must investigate the temporal cascade of information processing presumably carried out by those high in anxious apprehension regarding orienting to threats and quickly disengaging from processing these threats in fear circuitry via inhibitory functions.

Study 2 built on findings in Study 1 by (1) using functional brain measures, (2) using a larger sample, (3) measuring the development of brain-anxiety relations longitudinally, and (4) implementing data-driven methods that can provide hypotheses for future studies. Results from Study 2 suggest primarily via data-driven approaches that are exploratory in nature that structural markers of types of anxiety found in Study 1 are not reflected in functional connectivity patterns comprising those same specific connections. This may be due to structure only partly constraining function (Bargmann & Marder, 2013). Indeed, it has been demonstrated that

functional connectivity of a given regional pair (e.g., amygdala-frontal cortex connectivity) is influenced by multiple indirect connections between two brain regions, as well as other phenomena such as volume transmission that can create global patterns of connectivity without necessarily needing axonal connections between regions (Anderson, 2014). As such, this distinction between what structural and functional connectivity can yield in terms of predicting anxiety is meaningfully different, and may lead to more precise mechanistic hypotheses regarding what aspects of information processing involved in types of anxiety might be more closely linked to structural or functional connectivity measures and their underlying biological causes.

Study 2 also demonstrated that functional connectivity may best predict levels of anxiety when integrating several connectivity metrics that include a common brain region. A data-driven procedure that requires further replication revealed that integrating the connectivity of all left amygdala connections predicted anxious arousal. The correlation between left amygdala and anxious arousal was marginally significant at wave 1 and statistically significant at wave 2. However, a follow-up test revealed the difference in these two correlation values were not significantly different from each other. Therefore, findings in Study 2 suggest that amygdala intrinsic connectivity may be an early marker of anxious arousal, which coheres with a large body of work showing that this marker is predictive of anxiety in childhood (e.g., Qin et al., 2014). When comparing this finding to Study 1 there is evidence that left amygdala connectivity may be especially relevant for the implementation of anxious arousal. Indeed, in Study 1, left but not right amygdala to dorsomedial prefrontal cortex positively correlated with anxious arousal after controlling for concurrent levels of depression and anxious apprehension. Moreover, the importance of left amygdala in predicting anxious arousal is supported by a previous study using

a similar design except on younger children showing the role left amygdala plays in predicting trait anxiety broadly defined (Qin et al., 2014).

Studies 1 and 2 also advanced an understanding of how structural and functional connectivity metrics involving iFG are differentially related to anxious apprehension and anxious arousal. In Study 2, left iFG connectivity was predictive of anxious apprehension at wave 2 and not at wave 1. This finding could indicate that as adolescents get older, the functional correlates of mentally simulating the future are more strongly predictive of anxious apprehension. More evidence, however, is needed to investigate the role that left iFG plays in aspects of information processing involved in chronic worry. Although theorists have suggested that chronic worry involves altered simulations of the future (i.e., prospection, Bulley, Henry, & Suddendorf, 2017), such hypotheses await experimental verification. One area that can make headway on this hypothesis is through leveraging computational explanations of planning (e.g., Keramati et al., 2016), and how that might be disrupted when planning to avoid uncertain negative events in one's future (e.g., Sharp & Eldar, 2019).

Present results across both studies suggest that right iFG structural connectivity may be better suited to measure the avoidance of immediate threat characteristic of anxious apprehension (e.g., Spielberg et al., 2013), and left iFG functional connectivity may track simulating future events in anxious apprehension in adolescence. Relationships found here could differ if functional connectivity is measured during certain tasks, in which intrinsic connectivity might differ from certain connectivity patterns only arising during the engaging of certain cognitive functions. As such, future studies should compare intrinsic connectivity to particular task-induced connectivity that is germane to the phenomena of interest. Although correlational evidence exists for associations between connectivity (Sharp, Miller & Heller, 2015), future

work should include experimental manipulations of these cognitive functions, and measure functional and structural connectivity within the same sample. Indeed, a major limitation of synthesizing studies 1 and 2 is that I assumed findings in study 1 held in study 2. This can only be verified on a sample where structural and functional connectivity is estimated from the same sample.

The future of studying the development of types of anxiety

The developmental neuroscience of anxiety is limited by immature theory

The inferences from the present study are limited by the inchoate theories by which the hypotheses were informed. First, I theorized that neural correlates found in adults and non-human animals of transdiagnostic types of anxiety could distinguish anxious apprehension and anxious arousal in early adolescence. Second, I predicted that these brain-behavior relationships would be present in both functional and structural connectivity. Note, these theories are not very detailed; they do not describe how, for instance, amygdala-prefrontal cortex functional or structural connectivity implement the information processing in e.g., anxious apprehension. Because of this, I did not have clear theoretical reasons to predict that certain structural connectivity measures might be important in predicting variation in one psychological function (simulating future negative consequences) involved in a type of anxiety over another psychological function (inhibiting bottom-up threat responding). Such predictions require a mechanistic model of how psychological functions are implemented in neurobiology (Bechtel, 2008; Picinnini & Craver, 2011; Thomas & Sharp, 2019). One method suited towards this end is called “effective connectivity” analyses, which requires specifying how different brain regions causally interact with each other across time, given an information processing theory of a given psychological phenomenon (Park & Friston, 2013).

The state of current theories of anxiety and its development is not yet ready for such work (e.g., Sharp & Eldar, 2019). A lack of strong theory about how psychological processes are implemented in neurobiological circuits is perhaps best reflected in more fine-grained work on anxiety in rodent studies, in which scientists mapping psychological functions to circuit activity claim the following:

> "An additional challenge for future studies will be to go beyond a functional and anatomical analysis of these circuits and address the computations they carry out. To tackle this challenge, future research needs to address how stimulus representations, associations and behavioural output programmes are encoded." (Tovote, Luthi and Fadok, 2015, p. 328)

The message here is that, although we understand how behavioral or cognitive states might covary with fine-grained changes in neurobiology by correlating behavior with neural interventions in rats (as noted above) or mapping self-reported mood states to changing MRI metrics in humans, we do not know have fine-grained explanations of how information processing is changing along with these neural changes. Doing this work requires further specifying the temporal dynamics of information processing in episodes of anxiety, such as how stimulus properties are encoded and transformed when attention is allocated to threat. As such, future work on the developmental neuroscience of anxiety is limited by theoretical progress in further specifying the psychological processes involved in types of anxiety.

Acquiring developmental neuroimaging data is necessary to spur progress

Inferences in the present set of studies are limited by the fact that only one year of development was measured. That said, the hope is that such inferences can inform hypotheses tested in more complex longitudinal designs. I found partial support for the first broad prediction that adult and non-human correlates of anxious apprehension and anxious arousal would present in adolescence: anxious arousal and anxious apprehension were distinguishable from self-

reported measures in youth, and a subset of the neural correlates found in adults and non-humans were uniquely associated with each dimension of anxiety. I then extended previous work by applying more cutting-edge data-driven techniques, and found that the set of left iFG connections could predict anxious apprehension, and that amygdala connections could predict anxious arousal. To my knowledge, this is the first demonstration that left iFG connections are predictive of anxious apprehension this early in life, which opens the path towards better understanding the development of chronic worry. Demonstrating that left iFG is predictive of chronic worry in both adulthood and early adolescence lends support to the idea that the internal processing of chronic worry in adolescence may not be much different from how it is instantiated in early adulthood.

I then found preliminary evidence to suggest that between-subject trends (e.g., between iFG connections and anxious apprehension at wave 2, or between amygdala and anxious arousal) are not reflected in within-subject changes (i.e., the longitudinal change-score analyses). To ensure this is a well-supported inference, further work must sample more time-points and along different scales. Study 2 examined longitudinal effects via a fixed effects analysis, in which slopes describing the relationship between change in anxiety and change in functional brain measures were held constant across subjects. Future studies should seek to determine how the dynamics of functional connectivity over time differentially relates to changes in anxiety across subjects by conducting random-effects analyses.

Integrating models from childhood through adulthood

Additional questions exist regarding how the biology and psychology of these types of anxiety are related to early signs of risk in childhood and infancy. As an example, it may be that neural correlates of inhibiting bottom-up threat signaling shown to be associated with anxious apprehension here may be present early on even if those implementing internal mental

simulation are not. Indeed, recent theories of a precursor of anxiety, behavioral inhibition, have recently been theorized to have subtypes that may map onto differences in anxious arousal and anxious apprehension (e.g., Perez-Edgar & Fox, 2018). For instance, two inhibited personality types that exist in childhood are those that are avoidant versus those that are sociable. The former is marked by an avoidance of social situations, whereas the latter is marked by the desire to be sociable with elevated fear responding when attempting to interact with peers (Poole, Tang & Schmidt, 2018). It may be that avoidance of processing immediate threats is an early marker of anxious apprehension, in which avoidance behavior is the result of an early precursor to chronic worry, whereas those with anxious arousal simply have an exaggerated bottom-up fear response when approaching potentially negative situations.

Combining computational approaches with developmental neuroimaging

Ultimately, the goal of such work is to inform more specific theories regarding the internal processing involved in anxious apprehension and anxious arousal, how that processing is carried out in the human brain, and what happens to individuals across their lives that leads to these phenomena becoming maladaptive. Findings here suggest that mental simulation of possible future negative events is part of anxious apprehension at older (12-14) compared to younger (11-13) ages. Moreover, evidence suggests that proximal threat detection, thought to be instantiated in amygdala, is primarily involved in anxious arousal.

More rigorous tests of hypotheses regarding the information processing involved in anxious apprehension and anxious arousal will require collaboration with computational cognitive science (e.g., Hauser et al., 2018). The mission of computational cognitive science is to formalize (specify mathematically) how information is processed. Once formalized, computational models enable predictions of how internal processing is modulated by an

organism interacting with various environments (e.g., Eldar et al., 2018), which can predict trial-level modulations in behavior and neural processing (Cohen et al., 2017). This has begun the difficult effort to specify impaired decision making and the implementing structures realizing these process in the brain for negative symptoms of schizophrenia (Maia & Frank, 2017) and mood instability (Eldar & Niv, 2015), but awaits application to anxiety (Sharp & Eldar, 2019).

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