**Anxiety in Autism is Linked to Aberrant Intrinsic Functional Connectivity Between Salience and Default Mode Networks**

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# Abstract

Anxiety is prevalent among 40%-50% of children with autism spectrum disorder (ASD) and exacerbates socio-emotional challenges. Increasing anxiety morbidity is marked by difficulties in emotion regulation, which are developmentally associated with a child’s early relationships. Default mode network (DMN) activity is associated with repetitive negative thinking and worrying. Salience network (SN) activity is associated with cognitive inflexibility and poor emotional interoception. Anxiety is associated with disruptions in SN-DMN connectivity. However, less is known regarding disruptions within and between these networks in clinical samples of youth with ASD and co-occurring anxiety relative to typically developing youths. This study used resting-state seed-based functional connectivity analyses to examine SN (insula and anterior cingulate) and DMN (medial prefrontal and posterior cingulate) connectivity within 74 ASD children with anxiety and 29 healthy controls. Underconnectivity between the SN and DMN was associated with increased anxiety in the ASD group relative to controls. Insular connectivity with the temporo-parietal junction was associated with anxiety severity and social deficits in ASD. We did not find evidence supporting a mediation model of social responsivity between SN-DMN functional connectivity and anxiety. These findings suggest that disruptions in SN-DMN connectivity may be a neural marker of anxiety in ASD, potentially impacting circuitry involved in cognitive flexibility (e.g., repetitive negative thought) and emotional awareness. Therefore, clinical interventions that engage salience and cognitive control circuitry (e.g., teaching social skills and emotion regulation strategies for navigating anxiety-inducing situations) may be beneficial in developing personalized treatments for autistic children with comorbid anxiety.

# Declaration of Student Involvement

The data for this thesis was collected from the mentor’s completed study assessing cognitive behavioral therapy for anxiety in ASD (R01HD083881, PI: Sukhodolsky) in the Sukhodolsky Lab at Yale University before the student’s training at the lab. The author generated the research questions, hypotheses, and aims with the collaboration and guidance of Dr. Karim Ibrahim and Dr. Denis Sukhodolsky. The author completed the neuroimaging and behavioral analyses based on training provided by Dr. Karim Ibrahim. The interpretation of study results was by the author, under the guidance of lab mentors.

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# Introduction

## Autism and anxiety

Autism spectrum disorder (ASD) is a neurodevelopmental condition diagnosed based on impairments in socialization and social communication and restricted and repetitive behaviors. In addition to the core ASD symptoms, functional impairments associated with other co-occurring disorders, particularly anxiety, can significantly impact social and emotional functioning. Research shows that approximately 50% of children and adolescents with ASD report clinically significant levels of anxiety (Kuusikko et al., 2008; Hollocks et al., 2019). Anxiety can be accurately evaluated in autistic children between the ages of 6 and 17 (Vasa et al., 2013) and is often higher than typically developing children.

Autism spectrum disorder and anxiety disorders contribute to and are associated with difficulties with emotion regulation. In ASD, studies report that youth on the spectrum have poorly differentiated emotional responses and less effectively monitor and modulate the valence, intensity, and expression of emotion (Losh & Capps, 2006; Samson et al., 2012). Emotion regulation challenges in ASD are typically associated with an increased prevalence of anxiety. Accordingly, ER deficits associated with elevated anxiety in children with ASD contribute to the increased use of psychiatric services within the population (Croen et al., 2006). Therefore, anxiety comorbidity in ASD can be investigated by understanding how children with autism regulate their distressing, anxious emotions.

The main assertions of this thesis work use psychoanalytic and neuroscience perspectives to conceptualize anxiety in ASD. Firstly, the experience and effects of anxiety in ASD are influenced by a child’s relationships with others. It is proposed that there is a distinct relational component associated with the increased levels of anxiety, and emotion regulation impairments, experienced by children with ASD. A psychoanalytic perspective will be used to explore how social deficits in ASD, through the parent-child relationship, impact the development of emotion regulation skills, resulting in increased susceptibility to anxiety disorders.

Secondly, the experience and effects of anxiety in ASD are influenced by brain networks that contribute to attentional and social functioning. This thesis will use a neuroscience perspective to focus on the influence of two critical networks in the brain, the default mode network (DMN) and the salience network (SN), on anxiety in ASD. The function of these brain networks has been reported as central to successful emotion regulation and is involved in the formation and development of a child’s social relations (Satpute & Lindquist, 2019; Seeley, 2019). Much research has therefore investigated and explored these networks in isolation. However, their interactions are not well characterized in the extant ASD literature, particularly in describing how difficulties with emotion regulation are associated with increased anxiety prevalence (Pfeifer & Peake, 2012; Chevallier et al., 2012; Nayar et al., 2022).

Thirdly, a neuroscience and psychoanalytic perspective can be used in conjunction to understand why anxiety prevalence is higher in ASD. This represents an attempt to integrate the neuroscience of networks associated with attentional and social processing and object-relational psychoanalysis. A child’s interactions with their parental figures – the processes of social responsivity and reciprocity used to regulate anxious emotions – utilize the same neural networks that engender social attention and perception (Pannekoek et al., 2014). The activity of the default mode and salience network provides the foundation for the relational processes underpinning emotional regulation of anxiety in ASD (Grabell et al., 2019; Mazefsky et al 2014). Abnormalities in the functioning of the salience and default mode networks associated with social deficits in autism, impact a child’s social relationships, and how they are used by the child to regulate anxious emotions.

Within this integrated approach, a Bayesian and free energy neuroscience framework will be used as a platform to explore the integration of a neuroscience and psychoanalytic perspective and how the neural correlates associated with disruptions in a child’s relationship with their parents affect the regulation of emotions associated with anxieties in ASD.

## Psychoanalytic perspectives on the neural correlates of anxiety in autism

ASD is a psychiatric disorder that affects social functioning. The psychoanalytic perspective asserts that the experience of emotions (including anxiety) is fundamental to social cognition. Emotions, including anxieties, prompt the structuralizing of the mind, being the impetus for the formation of the Id, Ego, and Superego (Freud, 1961). Early relationships, such as the anxieties associated with a child separated from their mother, are developmentally formative (Anna Freud, 1963). Through a developmental lines theory, Anna Freud proposed that emotions provide the impetus for and shape the caregiver-child relationship and subsequent learning processes (Anna Freud, 1963).

Our relationships with our parents are foundational to the management of anxiety. Psychoanalytic theorists frame a child's relationship with their caregiver in terms of 'object relations.' Winnicott (1971) proposed that a child learns to regulate anxious emotions through their relationship with their caregivers.

Emotion regulation in development is a dyadic process, as parents provide a dynamic scaffold via which children learn to regulate their emotions via parent modeling and co-regulation (Morris et al., 2017). Through sensitivity to socio-emotional reciprocity and responsivity and modeling via active co-regulation, a foundation for a child's self-regulation is established through their interactions with parents (Zhou & Yi, 2014; Gulsrud et al., 2010).

Object Relations theorists propose that within autism, a child's relationship with a parental security figure (the 'object') is disrupted, affecting the ego's management of anxieties produced in the id. It is proposed that within autism, a child's ability to interpret and understand the state of mind of the mother during emotional co-regulation is disrupted. This assertion is supported by clinical anecdotes within psychoanalytic literature (e.g., Durban, 2019; Durban, 2020).

Disruptions in development can be measured as deficits in social responsivity. Children on the spectrum display a range of social deficits unique to and symptomatic of the disorder. These social responsivity deficits include challenges with social cognition and facial processing, which interfere with the learning of emotion regulation in early childhood (e.g., Davies et al., 2011; Todorova et al., 2019; Nomi & Uddin, 2015; Eack et al., 2013; Kuusikko et al., 2009). These deficits have been linked to delays in the development of an ASD child's capacity to understand others' intentions by ascribing mental states to others, to mentalize, and construct a theory of mind of their caregivers.

A child's ability to mentalize the state of mind of their caregivers is essential. This is because, through the experience of anxiety being mentalized by the mother, a child typically learns how to tolerate and withstand anxieties, inheriting a working model for emotion regulation from their parent (Internal Working Models; Bowlby, 1973). Disruptions in social responsivity affect how an ASD child copes with and handles anxiety. Bion's theory of thinking (1967) provides a psychoanalytic mechanism for how disruptions in mentalizing a mother's state of mind during the learning of emotion regulation may make anxieties more prevalent.

## Bion’s theory of thinking and mentalizing anxiety in autism

In his work, Bion (1967) describes that the mother provides an 'alpha function' for development and growth. In typical development, the mother, through her interpretation of the mental state of her child, interprets 'beta elements' representing sensory-affective anxieties into 'alpha elements' representing metabolized thoughts that can symbolize anxiety and dissuade threat to the ego. A child's emotional experience is 'held' by the careful attention of the caregiver. By 'holding' anxiety and reflecting the emotions as an interpretable experience that can be tolerated, the child experiences reverie through the 'thinking breast' of the mother. The mother therein acts as a 'mirror turned lamp' reflecting and illuminating the child's own thinking processes (Wadell, 2002). Therein, the mother functions as a 'container' for the child's anxieties.

In ASD, object relations theorists propose that anxieties are perceived by the child as not being sufficiently 'held' by the mind of the caregiver (Winnicott, 1953). Challenges in social responsivity are therefore linked to challenges relating to the maternal object. Recent clinical studies suggest that social responsivity deficits are driven by mentalizing deficits in ASD, which affects the 'holding' comfort of the child-mother relationship (Moran et al., 2011; Fishman et al., 2014). Within a framework of Bion's theory of thinking, anxiety in ASD is experienced as incompletely 'contained' by the mother's mind in the child's mind (Hobson, 2011). Social responsivity deficits, therefore, interfere with the development of balanced representations of the mother's mind in the child, resulting in an incomplete containment of anxiety, leading to its increased prevalence in ASD.

## Neuroscience perspectives of anxiety in autism

A psychoanalytic perspective asserts that relational challenges between the child and parent contribute to anxiety in ASD. However, autism is also inherently a neurodevelopmental disorder with a genetic basis (Volkmar, 2012; Khundrakpam et al., 2020). Therefore, social responsivity deficits (mentalizing) which impact the development of anxiety from a psychoanalytic perspective, can also have a basis in the connectivity of neural networks in the brain (Luyten & Fonagy, 2015).

Network neuroscience is an emerging discipline that uses neuroimaging data to model and analyze functional and structural brain networks as collections of interacting neural ensembles (Krendl & Betzel, 2022). An understanding of the large-scale networks associated with psychopathologies can be derived from studies investigating the functional synchrony of the brain. Resting-state functional magnetic resonance imaging (rs-fMRI) provides a tool to characterize the brain’s intrinsic architecture from spontaneous fluctuations of blood oxygen level-dependent (BOLD) signals. Analyses of fMRI data using independent component analyses have identified Intrinsic Connectivity Networks (ICNs) that reflect strong couplings of blood oxygen level-dependent fluctuations representing coordinated neural activities (Lv and Wang et al., 2018). Imaging studies using resting-state fMRI have delineated several functional networks in the human brain, including the default mode, salience, and frontal executive networks. These canonical networks can be derived using various parcellations, including Yeo, Gordon, Powers, and Shen atlases (Yeo et al., 2011; Gordon et al., 2016; Shen et al., 2013). Contemporary fMRI research methods have led to a paradigm shift in our understanding of the neural correlates of anxiety and ASD. The dynamic interactions between these networks regulate shifts in attention, and the ongoing functioning of these networks imposes strong biases on information processing in the brain. The emerging view is that the etiology of psychopathological and cognitive disturbances is characterized by interactions among these networks (Menon, 2011). Two networks most frequently associated with anxiety in ASD are the default mode and salience networks (Padmanabhan et al., 2017; Toyomaki et al., 2017).

## The default mode network

The default mode network (DMN) is a key area associated with anxiety and social responsivity deficits. The default mode network is a collection of brain regions consisting of the posterior cingulate cortex (PCC), precuneus, medial prefrontal cortex (mPFC), temporoparietal junction (TPJ), and hippocampus (Padmanabhan, 2017). Default mode network activity in these connected regions supports a range of self-related mental processes, including self-referential thought and rumination. (Raichle et al., 2001). The default mode network is activated when mentalizing the thoughts of others and thus is involved in the social-cognitive processing of a 'theory of mind.'

Activity in the default mode network activity has been suggested to show atypical connectivity patterns in ASD relative to healthy controls during both task and rest. Atypical resting state connectivity in the default mode network is associated with social responsivity and mentalizing challenges (Kana et al., 2015; Chen et al., 2015). Children with ASD experience difficulties interpreting and understanding other people's mental states. Task-based studies in children with autism that manipulate theory of mind show that when viewing images, animations, or stories that test participants' understanding of other's mental states, activity in the TPJ and dorsal mPFC is attenuated (Schülte-Ruther et al., 2013). Studies of DMN activity also show reduced activation and connectivity between the PCC (Vander et al., 2014) and mPFC (Kana et al., 2015; White et al., 2014).

### The default mode network and childhood anxiety

Default mode network activity is linked to mood and anxiety disorders. Much research has found that default mode network activity is associated with ruminative thought patterns in depression (Kerestes et al., 2014; Mayberg et al., 2005) and worrying thoughts in anxiety (Anteraper et al., 2014; Xiong et al.; 2020). Rumination involves dwelling on negative events and the possible causes and implications of negative mood (Nolen-Hoeksema et al., 2004). Worry involves focusing thoughts on uncertain and future negative events (Borkovec et al., 1983). Emerging evidence suggests that the constructs of rumination and worry are similar in anxiety and depression and can be conceptualized as a transdiagnostic, dimensional construct of repetitive negative thinking (Topper et al., 2014).

Rumination in depression is characterized by hyperconnectivity of the default mode network and difficulties disengaging default mode network nodes during cognitively demanding tasks (Kerestes et al., 2013; Mayberg et al., 2005). Default mode network activity is also linked to worrying thoughts in anxiety. Worry is associated with less resting-state functional connectivity between the PCC and other default mode network regions in undergraduates (Burdwood et al., 2016) and in individuals with a generalized anxiety disorder (Feurer et al., 2021). Worries about performance anxiety on a math test are associated with weaker deactivation of the default mode network (Pletzer et al., 2015). Aberrant connectivity between the anterior cingulate and dorsal medial prefrontal cortex is associated with difficulties regulating default mode network activity, resulting in the increased salience and persistence of worrying thoughts in generalized anxiety (Paleusu et al., 2010). Therefore, patterns of repetitive negative thought are associated with default mode network hyper-connectivity, and it is proposed that because of this hyper-connectivity, individuals with anxiety find it challenging to focus their thinking away from self-focused negative patterns of thought.

### The default mode network in autism and anxiety

Abnormal default mode network connectivity that results in difficulties with mentalizing in autism may also result in children with autism being more susceptible to anxiety disorders. Children with ASD also demonstrate strong, persistent patterns of rigid thought and behavior (Nomi & Uddin, 2015), such as restricted, repetitive behaviors that are associated with default mode network hyper-connectivity (Uddin et al., 2009; Cole et al., 2019).

Research investigating default mode network connectivity within autistic populations with anxiety aligns with findings from studies in neurotypical individuals. Default mode network hyperactivity also makes children with ASD more prone to anxiety and worries. A strong positive association is found between depression symptoms, rumination, and default mode network connectivity (Gotham et al., 2014; Mazefsky et al., 2014), which is also predictive of an ASD child’s use of adaptive emotion regulation strategies (Rieffe et al., 2014). Individuals with ASD and elevated anxiety and rumination have stronger and more sustained neural reactivity in the default mode network to sad stimuli than individuals with ASD without anxiety (a response similar to typically developing individuals without anxiety) (Gotham et al., 2018). In studies of depression in ASD, an interaction effect was found between repetitive negative thinking and social responsivity deficits in ASD, such that individuals with the highest level of social impairment also preserved the most on negative thoughts and had the highest rates of depression (Gotham et al., 2014). Studies of emotion regulation suggest that coordinated activity between the TPJ within the default mode network and between the default mode network and insula plays an important role during emotional reappraisal and supports mindfulness activities (Grecucci et al., 2013). Hyperconnectivity within this region is associated with social responsivity deficits (Chen et al., 2015) and may make emotional reappraisal more challenging in ASD children (Hao et al., 2022). Therefore, an abnormally hyper-connected default mode network may be responsible for increased anxiety levels in ASD, making it harder for children on the spectrum to stop thinking about negative content.

 However, the hyper-connectivity of the default mode network alone does not fully account for (or provide a sufficient model to describe) the increased prevalence of anxiety disorders in ASD populations. Structural evidence suggests that disruptions of the default mode network are associated with anxiety but emphasizes that the relationship between network functional connectivity and behavior is likely nonlinear. In patients who had suffered a subacute ischemic stroke, increased connectivity in the left inferior frontal gyrus (iFG) and right middle frontal gyrus (mFG) was associated with post-stroke levels of anxiety (Vicentini et al., 2017). Research has found that default mode network hypo-connectivity is associated with increased anxiety (Burdwood et al., 2016), increased self-focused thoughts (Lian & Northoff, 2011), increased RRB (Comparan-Meza et al., 2021), and increased social deficits in ASD (Moseley et al., 2015). It is likely that abnormal development of the default mode network, in the context of its relationship with other networks in the brain, results in the difficulties observed in ASD and comorbid anxiety. Current models of brain network activity, therefore, emphasize that other networks in the brain also influence DMN activity. A key network implicated in this is the salience network.

## The salience network

The salience network is involved in the interpretation of social and emotional information as well as interoception. Salience network activity is essential for bringing to attention information about potential threats in the environment and integrating information about our emotions, and relating that in the context of other people and ourselves (Seeley, 2019).

 Core nodes in the salience network include the anterior insula (aINS), dorsal anterior cingulate cortex (dACC), as well as limbic areas, including the amygdala, ventral striatum, dorsomedial thalamus, and hypothalamus (Seeley, 2019). The two core functions of the salience network are (1) to mark sensory-affective stimuli for cortical processing and (2) to initiate appropriate control signals between key brain networks (Seeley, 2019). Altered connectivity within the salience network and between the salience network and the default mode network are implicated in anxiety comorbidity with ASD.

### The salience network and childhood anxiety

Abnormal activity in the salience network is associated with anxiety. Studies suggest that the salience network plays a central role in detecting the salience of emotions and triggering cognitive control via its functional connectivity with other brain networks. The anterior insula (aINS) and dorsal anterior cingulate cortices (dACC) have been investigated as neural candidates underlying increased vulnerability to anxiety in children. Activity in the anterior insula is linked to emotional awareness and subjective feelings generated within the body (Craig, 2010). Activity in the dACC is correlated with threat appraisal (Etikin, 2011). Research suggests that stronger connections between these regions reflect increased sensitivity to salient events, which biases attentional and perceptual processing. Elevated levels of functional connectivity within the salience network are found in children with higher trait anxiety levels (Geng et al., 2015; Kim et al., 2011; Baur et al., 2013).

In anxiety disorders, connectivity deficits within the salience network and between the salience network and other brain regions impact interoceptive processes. Connectivity within key nodes of the salience network, particularly between the aINS, and dACC, precedes default mode network activation in the dlPFC, mPFCC and PCC (Sridharan et al., 2008). It is proposed that weaker cognitive control of activity in these regions by the aINS-dACC pathway of the salience network is implicated in difficulties regulating anxious emotions (Bishop, 2009).

### The salience network in autism and anxiety

Challenges of introspection associated with salience network dysfunction relate directly to elevated anxiety levels in ASD. The triple network model can be used as a framework to understand the importance of the salience network in relation to the default mode network (Menon, 2018). Menon’s model highlights that the salience network has a crucial role in initiating network switching in the engagement of cognitive control networks involved in broader executive functioning, and the disengagement of the default mode network.

Within the triple network model framework, it is proposed that ASD individuals may have difficulties performing a ‘network switch’ away from self-referential, ruminative processing driven by a strong SN-DMN connection (Menon, 2018; Burrows et al., 2018). Within this model, salience network activity is associated with cognitive flexibility. Cognitive flexibility is the ability to switch between mental processes to produce appropriate behavioral responses (Scott, 1962). Deficits in this linked to aberrant salience network connectivity have been observed in children with ASD and are associated with levels of restricted repetitive behaviors and cognitive inflexibility (Dajani & Uddin, 2015; Lopez et al., 2005; Panerai et al., 2014). The salience network can therefore contribute to social-cognitive and affective dysfunction through its interaction with the default mode network (Menon & Uddin, 2010).

## Salience to default mode network connectivity:

### Network connectivity and cognitive inflexibility in autism and anxiety

Burrows et al. (2018) argue that in ASD, salience network overconnectivity with regions associated with negative self-thought driven by default mode network activity underlies increased susceptibility to anxiety disorders. It is proposed that the inflexible cognitive styles that characterize ASD contribute to increased repetitive negative thinking, increasing the risk for co-occurring anxiety. Burrows’ (2018) model is supported by research connecting default mode network activity to repetitive negative thinking in children with ASD and depression. Rumination in ASD predicts depression and emotion regulation (Crane et al., 2013; Gotham et al., 2014; Mazefsky et al., 2014; Rieffe et al., 2011). Fewer studies have examined worries linked to anxiety in children with ASD. Those who have, suggest that children with ASD endorse higher levels of worry than their typically developing counterparts (Worley & Matson, 2011; Gillott et al., 2001; Russell & Sofronoff, 2005). Therefore, repetitive negative thinking in ASD is a proposed transdiagnostic factor of anxiety psychopathology.

Within the triple network model of salience network function in ASD, the anterior insula -dorsal anterior cortex circuit of the salience network is proposed to impact cognitive control of prefrontal regions in the default mode network, including the activity of key regions in the medial prefrontal cortex and posterior cingulate cortex (Mathews & Mackintosh, 1998; Bressler & Menon, 2010). Research has found that high behavioral inhibition has been associated with salience network hyperconnectivity with the salience network and the default mode network, and decreased connectivity within the salience network (Pannekoek et al., 2013; Etkin et al., 2009; Liao et al., 2010). Decreased anterior insula (AI) to default mode network connectivity, particularly between the AI and mPFC and PCC, is associated with higher trait anxiety in adolescents (Geng et al., 2016) and self-report anxiety in children with ASD (Dennis et al., 2011). Given the default mode network’s association with negative and self-referential information in anxiety and depression, SN-DMN connectivity may be important for allocating attention self- and other- related information and is therefore connected to deficiencies in cognitive control and interoceptive processes associated with recognizing and understanding anxiety (Schimmelpfennig et al., 2023).

Children with autism experience difficulties ‘switching’ between a self-referential “me-focused” pattern of thought and a deliberate “task-focused” pattern of thought (Dajani & Uddin, 2015). Cognitive inflexibility (Hollocks et al., 2014; Lawson et al., 2015), insistence on sameness (Rodgers et al., 2012), and mentalizing deficits (Balaban & Bilici, 2022) have been associated with elevated anxiety and worries in ASD children. Hyperconnectivity between the salience and default mode network regions is therefore associated with self-focused, repetitive behavior, as well as difficulty in ‘switching’ or transitioning out of self-focused frames of mind (Posner et al., 2016; Padmanabhan et al., 2017; Burrows et al., 2017). This produces challenges in interpreting the mental state of others, in particular, parental figures, during emotional co-regulation. Salience network abnormalities associated with default mode network regulation ASD may therefore result in an increased risk of anxiety in ASD.

### Network connectivity distorts salience of emotions during mentalizing

Disrupted connectivity within the salience network and between the salience network and the default mode network in ASD may contribute to elevated anxiety. Altered functional connectivity patterns in the posterior/anterior insula have been hypothesized to contribute to ‘distortions in an emotional salience landscape’ (Ebisch et al., 2011), contributing to emotion regulation difficulties and elevated anxiety in ASD. In contrast to models of salience network and default mode network hyper-connectivity mentioned above, salience network hypo-connectivity within the local circuit of core nodes that comprise the salience network has also been hypothesized to interfere with its ability to appropriately mark sensory-affective information for socio-cognitive processing in children with ASD (Uddin & Menon, 2009), resulting in social deficits and reduced social motivation (Francis et al., 2019). A hypo-connected anterior insula-dACC may impair emotional awareness (Gu et al., 2013; Chevallier et al., 2012), and be unable to regulate default mode network activity efficiently (Wang et al., 2019). Both these patterns perpetuate anxiety by making it more challenging to interpret the mental states of others, including parental figures, when learning adaptive emotion regulation strategies. Bion’s Theory of thinking, in conjunction with modern Bayesian and free energy neuroscience principles, can be used as a clinical metaphor to understand how salience network abnormalities may result in social deficits and increased anxiety in autism. Functional activity within networks responsible for sensory integration and perception may use processes of Bayesian inference when organizing mental structures and when differentiating between internal and external mental events (Friston, 2003; Yufik & Friston, 2016). Activity within the brain follows the principle of a ‘Markov blanket”. Relating the salience network’s function to Bion’s Theory of thinking, the salience network may be responsible for marking sensory-affective ‘beta elements’ arriving from subcortical-limbic inputs for further thought and operation by subsequent neocortical processes or the ‘alpha element’ (Cieri & Esposito, 2019). Salience network activity, in coordinating responses with the default mode network thereby constitutes a portion of the contact barrier between an individual’s internal and external reality (For further information, see Mellor, 2018 or Bruineberg et al., 2021).

Abnormal integration of information within and between networks connected to the salience network may result in abnormal social perceptions, increasing anxiety in ASD. Sensory-affective information (beta-elements) that cannot be perceived or understood as thoughts, according to Bion (1967), are inherently anxiety-inducing (Tustin, 1991; Durban, 2019). Object relations theory connects anxiety in ASD with a sense of dis-integration within bodily states or a discontinuity between the body and the outside world (Meltzer, 1992; Tustin, 1990). In Bayesian terms, this could be considered a breakdown or disintegration in an individual’s internal Markov blanket (Holmes & Nolte, 2019).

Disrupted signaling within the salience network may lead to an abnormally high level of ‘prediction error’ in the processing of internal and external state information or a lack of consistency in how sensations are perceived and assigned meaning (Schimmelpfennig et al., 2023). This may result in the feeling of anxiety as ‘un-contained’, or a lack of reliability in the ASD child’s ability to use the maternal object as a ‘container’ for anxieties.

This description is supported by neuroscientific evidence suggesting that aberrances in the connection between core nodes of the salience network correlate positively with both sensory sensitivities (Uddin et al., 2013) and alexithymia in autism (Bernhardt et al., 2014; Poquerusse et al., 2018). The mislabeling of sensations as anxiety-provoking and anxieties derived from the dis-integration of sensory-affective information may result in social deficits and increased anxiety prevalence in ASD.

Salience network hypo-connectivity may result in a lack of integration of information within the AI-dACC pathway within the salience network, affecting the integrity of its afferent signaling to the default mode network (Kandilarova et al., 2018). This would result in stimuli being consistently perceived as more anxiety-provoking, evidenced by the increased formation of specific phobias in ASD through classical conditioning (Sukhodolsky et al., 2008). Evidence supports that the unpredictability, uncontrollability, and frequency that anxieties arising from the lack of integration of other salient stimuli by the salience network of ASD individuals result in an elevated prevalence of generalized anxiety through context conditioning (Green & Ben-Sasson, 2010).

Local circuit abnormalities that affect the integrity of a child’s understanding of emotional states may have knock-on effects on higher-level processing of information facilitated by the salience network through its connection with other core networks in the brain. Disrupted signaling and representations of sensory-affective information in the salience emotional ‘map’ may impact a child’s ability to shift attention away from their own thoughts and into the frame of mind of another person during emotion regulation (Ebisch et al., 2011).

## Developmental considerations of salience and default mode network connectivity

There is no current consensus within the extant literature on whether anxiety, social deficits in ASD, or mentalizing function generally are associated with salience or default mode network hypo- or hyper- connectedness. Contrasting theories of the role of hyper- or hypo- connectivity of the salience network with other networks in the brain can be reconciled under a developmental framework.

Hyper- or hypo- connectivity patterns within functional networks are not mutually exclusive across development. Characterizing deficits in autism or anxiety to be the result of either may be too simplistic, as multiple variables, including anatomic specificity, analytic technique, and the point in time of an individual’s developmental trajectory, all influence observed levels of network connectivity (Menon, 2013). Local circuit abnormalities within key nodes of the salience network likely contribute to abnormal signaling between networks (Peters et al., 2016). For instance, small-scale alteration in the expression of excitatory and inhibitory neurotransmitters may play a major role in sculpting local circuit properties. Varying hyper- or hypo- connectivity patterns may therefore impact the development of connections between larger-scale functional circuits (Gatto & Broadie, 2010; Rubenstein and Merzenich, 2003). Consistent with this view, recent studies in other disorders show that global functional hyperconnectivity between regions is associated with high-amplitude, low-level fluctuations within other neural sub-networks (Chen et al., 2022). It is likely, therefore, that neuroanatomical irregularities in neuron growth between key nodes in the salience network influence signal propagation and functional connectivity with other regions, which result in deficits in social cognition and behavior over development (Testa-Silva et al., 2012). However, It is still unclear how complex patterns of intrinsic connectivity affect the processing of sensory inputs or how functional connectivity of the salience network correlates with anxiety specifically.

The interactions between core nodes in the salience network (i.e., the anterior insula & and dorsal anterior cingulate) and default mode network (i.e., the medial prefrontal and posterior cingulate) associated with these behaviors are not well characterized in current literature. Currently, no work has studied how the connectivity of the salience and default mode networks impact social responsivity or its association with anxiety prevalence in ASD. Additionally, while psychoanalytic frameworks have applied Bion’s theory of thinking to network neuroscience models of psychosis (Mellor, 2018) and depression (Rabeyron, 2021), no work has explored the contemporary neuro-psychoanalytic implications of Bion’s Theory of Thinking, and free energy neuroscience in either anxiety or autism.

Furthermore, within current literature, a large proportion of evidence used to justify the role of repetitive negative thinking is based on typically developing adults reporting symptoms of depression. A smaller proportion of studies cited within current models look at specifically anxiety in ASD children. Because of the lack of research investigating the salience of emotions in ASD populations with anxiety, Burrow’s (2017) model implicating repetitive negative thinking may be missing a consideration of altered emotional states in anxiety.

Despite this, clinical evidence from psychodynamic and object-relations theory suggests that disruptions within salience network connectivity influence the representation and processing of emotions within the salience network (Svrakic and Zorumski, 2021). A psychodynamic perspective asserts that the experience of anxiety is a fundamental component of the challenges of identification associated within autistic relationships and emotion regulation. Hogeveen’s (2018) study supports this assertion that an additional element is implicated in the connection between ASD and comorbid anxiety. They found that the difference between self- and parent-reported symptoms mediated the association between Salience to Default Mode connectivity in anxiety. Hogeveen (2018) suggests that “insight” into an individual’s own emotional states is just as important as cognitive flexibility in ASD children to know when and how to engage in appropriate processes of emotion regulation.

Therefore, increased connectivity between networks associated with self-referential processing, including between the salience and default mode networks, may perpetuate cycles of negative self-thought (as per Burrow’s 2017 Model) but also impair emotional awareness. Both patterns contribute to Social Deficits, worsening comorbid anxiety. Currently, few studies have looked at both connectivity within the salience network or connectivity between the salience network and the default mode network and how it relates to anxiety, particularly in developmental populations and populations of ASD who experience clinical levels of anxiety.

# Method

## The present study, aims and hypotheses

Neuroscience and psychoanalytic research suggest that symptoms of anxiety and autism are associated with both connectivity within the salience network, as well as connectivity between the salience network (SN) and the default mode network (DMN). Our research aims and hypotheses are:

1. *Aim 1:* To investigate if differences in SN and DMN connectivity distinguish ASD vs HC. We will use SN cores in the dorsal anterior cingulate (dACC) and anterior insula (AI) to conduct resting-state seed-based connectivity analyses. *Hypothesis 1:* We predict that ASD children will show hyper-connectivity compared to healthy controls (HC).
2. *Aim 2:* To test if continuous measures of anxiety are associated with disruptions with salience and default mode network connectivity in ASD and if this relationship is mediated by the severity of social deficits. Within the ASD group, we will conduct connectivity analyses among core nodes of the SN (AI, dACC) and DMN (PCC, mPFC) using the Multidimensional anxiety scale for children (MASC-2) total score as a measure of anxiety. *Hypothesis 2:* In dimensional analyses, hyper-connectivity within the SN will be associated with increased severity of anxiety in youths with ASD and mediated by elevated social impairment.
3. *Aim 3:* To assess if common co-occurring behaviors with anxiety (externalizing and attention-deficit-hyperactivity disorder symptoms) mediate the relationship between anxiety severity and disrupted connectivity between SN and DMN networks. We investigate SN connectivity with the DMN as this network is a locus for self-referential thought. *Hypothesis 3:* We expect that co-occurring behaviors (externalizing and attention-deficit-hyperactivity disorder,) will mediate the relationship between anxiety severity, and connectivity between core nodes of the SN and DMN.

## Participants

Data was derived from a completed treatment study of cognitive behavioral therapy for anxiety in autism (Sukhodolsky et al., 2008; Sukhodolsky et al., 2013), and reports fMRI and clinical characterization data that was collected prior to initiating treatment (R01HD083881, PI: Sukhodolsky). Our initial sample included *n=154* children.

Participants with missing questionnaire items, missing IQ, age, or gender information were excluded from our analysis. Participants with incomplete fMRI data were also excluded from our analysis.

6 Participants were removed from analysis due to excessive head motion. There were no differences in any demographic or behavioral characteristics between the 6 children in the ASD group removed from analysis due to excessive head motion (appendix A).

Exclusion criteria for healthy controls was any history of ADHD, anxiety, disruptive behavior disorder or other psychiatric, genetic, or neurological disorders. Healthy controls were included in our study if they had no current or previous history of psychiatric or neurological disorders and a parent-rated MASC-2 T-score of below 54.

Inclusion criteria for the ASD group was a DSM diagnosis of both autism spectrum disorder based on the Autism Diagnostic Interview-Revised (ADI-R; Couteur et al., 2008) and/or the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2; Lord et al., 2000) and anxiety based on the Anxiety Diagnostic Interview Schedule (ADIS; Silverman & Albano, 1988). ASD participants were excluded from our analysis if they (1) had a significant medical condition, such as a seizure disorder, (2) were unable to meet MRI safety requirements due to the presence of metal medical implants or claustrophobia, or (3) had a history of head trauma or loss of consciousness. ASD participants had to either not be any medication or be on a stable dose of psychiatric medication for at least the past 6 weeks before participating in the study. ASD participants also had to have a full-scale IQ of at least 70.

After accounting for our exclusion and inclusion criteria, motion analyses and missing variables, our final sample included 103 children; *n=74* children with ASD, and *n=29* matched healthy controls. Within our sample, age was matched between ASD (*Mage=12.08*) and HC (*Mage=12.25*), but IQ differed between ASD (*MIQ=97*) and HC (*MIQ=108*) groups. 32 ASD participants reported taking psychiatric medications.Table 1 shows demographic and clinical characteristics of our participants.

## Ethics

Each participant’s parents provided informed consent for their children’s participation in the study. Consent was given by both parents, and children, adhering to the criteria specified by the institutional review board at the Yale School of Medicine. All identifying information for participants was excluded after collection, and de-identification, personal health information privacy and confidentiality was explained to each participant in consent and assent forms. Participating parents and children were fully briefed on the study’s risks and benefits, including the risks associated with fMRI scans. Approval for the study was obtained by the Yale IRB in compliance with the Declaration of Helsinki.

## Study measures:

### Demographics and medical forms

A demographics and medical history report was completed by parents collecting information regarding their child’s age, sex, race, ethnicity, handedness, and medication history (appendix M).

**Table 1:** *Participant demographics and clinical characteristics.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **Total Sample**  *(n=103)* | **ASD + Anxiety**  *(n=74)* | **Healthy Control**  *(n=29)* | ***p* - Value**  (Group test statistic) |
| **Age, Years, Mean** (*SD*) | 11.91 (1.83) | 11.93 (1.89) | 11.90 (1.82) | .09 |
| **IQ, Mean** (*SD*) | 102 (19) | 99 (20) | 110 (15) | .007\* |
| **Gender,** *n(%)* |  |  |  |  |
| Male | 75 (72) | 57 (77) | 18 (62) | .125 *()* |
| Female | 29 (28) | 17 (23) | 11 (38) |  |
| **Medication,** *n(%)* | - | 54 (73) | 0 |  |
| Neuroleptics | - | 6 (8) | - |  |
| SSRIs | - | 13 (18) | - |  |
| Stimulants | - | 16 (22) | - |  |
| Alpha Agonists | - | 19 (26) | - |  |
| **MASC Baseline Tscore** *(SD)* | 61 (16) | 68 (13) | 44 (5) | <.001\* |
| **SRS Baseline T-score** *(SD)* | 67 (16) | 75 (9) | 45 (4) | <.001\* |
| **CBCL Externalizing Baseline** *(SD)* | 52 (12) | 57 (9) | 40 (6) | <.001\* |
| **Motion, mean framewise displacement** *(SD)* | .189 (.221) | .213 (.253) | .126 (.064) | .07 |

**Table 1:** *Participant demographics and clinical characteristics*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **Total Sample**  *(n=103)* | **ASD + Anxiety**  *(n=74)* | **Healthy Control**  *(n=29)* | ***p -* Value**  (Group test statistic) |
| **Race,** *n(%)* |  |  |  | *.170* |
| White | 19 (65.5) | 57 (77.0) | 76 (73.8) |  |
| Black | 5 (18.5) | 3 (37.5) | 8 (7.8) |  |
| Asian/Pacific Islander | 3 (11.1) | 9 (12.3) | 12 (11.7) |  |
| Other/More than one race | 2 (7.4) | 5 (6.8) | 7 (6.8) |  |
| **Ethnicity,** *n(%)* |  |  |  | *.240 ()* |
| Hispanic | 4 (14.8) | 16 (21.9) | 20 (19.4) |  |
| Non-Hispanic | 25 (86.2) | 53 (71.6) | 78 (75.7) |  |
| Unknown | 0 | 5 (6.8) | 9 (4.9) |  |

### Clinical assessments

Children received a comprehensive diagnostic evaluation that included a clinical assessment with the child to establish a diagnosis of ASD. This was done using the (ADOS-2) Autism Diagnostic Observation Schedule, Second Edition and/or the Autism Diagnostic Interview-Revised (ADI). Research has shown that the ADOS-2 demonstrates high inter-rater reliability (Lord et al., 2000; Kamp- Becker et al 2009) and strong criterion validity (Chawarska et al., 2007) for ASD. A diagnostic interview with the parent was also conducted using the Autism Diagnostic Interview Revised (ADI-R).The ADI-R, like the ADOS demonstrates strong inter-rater reliability (Lord et al., 2006), and high diagnostic and criterion validity (Lord et al., 2003). Their combined use is the current diagnostic ‘gold standard’. Both the ADI-R and ADOS-2 contribute additively to clinical judgements and are purported to comprise a sufficiently consistent and rigorous application of the diagnostic criteria for ASD (Le Couteur et al., 2008; Risi et al., 2006).

The Differential Ability scales (DAS-II) were used to assess a child’s IQ and cognitive performance. Research suggests that the DAS-II has good test-retest reliability, demonstrates strong convergent validity (Dumont et al., 2008), and correlates well with other measures of IQ functioning (Farmer et al., 2016).

Anxiety comorbidity in our clinical (ASD) sample was assessed using the Anxiety Disorders Interview Schedule Child (ADIS-C) and Parent Version (ADIS-P; Silverman & Albano, 1996).Studies show that the ADIS is an appropriate measure for assessing anxiety in ASD (Kerns et al., 2017), demonstrates strong convergent and divergent validity, and strong test-retest reliability (Renno & Wood, 2013; Silverman et al., 2001).

### Behavioral measures:

The Multidimensional Anxiety Scale for Children, Second Version (MASC -2; March et al 1997)is a 50-item parent report scale that was used as a continuous measure of anxiety in our study. The questionnaire assesses emotional, cognitive, physical, and behavioral symptoms. The MASC-2 produces a total score, and scores on four subscales: Physical Symptoms, Harm Avoidance, Social Anxiety, and Separation/Panic. Studies using the MASC-2 report high test-retest reliability, favorable divergent and convergent validity, and good internal reliability within its subscales of anxiety (Wei et al., 2014 Baldwin and Dadds. 2007; Rynn et al 2006). MASC-2 total score was used in analyses.

The parent report of theSocial Responsiveness Scale- Second edition (SRS-2)is a 65-item continuous measure that was used to characterize social deficit associated with autism within our study. The SRS-2 is broken up into 5 subscales (social awareness, social cognition, social communication social motivation, and restricted interests and repetitive behavior), and produces a total score. Data suggests that the SRS-2 has robust test-retest reliability, high inter-rater reliability, and good internal consistency within each subscale (Constantino and Gruber, 2005). We used the SRS-2 total score in analyses.

The parent-reported Child Behavior Checklist (CBCL; Achenbach, 1999) Externalizing Behavior Problems subscale was used in analyses as a measure of externalizing symptoms. In total, the questionnaire has 118 items, 33 of which represent externalizing behaviors. The externalizing subscale is broken up into two broad-band syndrome scales: Aggressive behavior (e.g., “Gets into many fights”) and Rule breaking behavior (e.g., “Lies and cheats”). Data suggest that the CBCL has robust test-retest reliability, high inter-rater reliability, and good internal consistency within each subscale (Achenbach & Rescorla, 2001).

## Analytic plan

### Behavioral analysis:

All behavioral analysis was performed in R version 4.2.2 and SPSS version 28.0.0. Group differences in each of the behavioral variables and covariates of interest in the study were investigated using independent samples t-tests and chi-square tests in R.

### Clinical characteristics and demographics

Demographic variables (age and IQ) were compared using an independent sample t-test between groups. No significant difference in age was found between groups, *t(101) =-0.84, SE=.397, p=.933*. IQ was lower in the ASD sample vs HC, *t(101)=-2.53, SE=3.60,* *p=.013*.

Our sample was primarily male (71.8%). A chi-square test was used to compare the distribution of gender between healthy and ASD groups. The ratio of Male-Female participants were matched between groups, 𝑥2*(1, n=103)=3.49, p=.062*.

Our sample was primarily white (77.0%) and non-Hispanic (71.6%). Ethnicity ratios (Hispanic/non-Hispanic) were matched between our healthy control and clinical groups, 𝑥2*(1, n=94)=2.89, p=.236*. There were no differences in the proportions of racial groups between clinical and healthy control samples, 𝑥2*(3, n=96)=1.48, p=.170.*

Independent samples t-tests were conducted between groups for MASC-2, SRS-2, CBCL Externalizing as well as mean scanner motion (framewise displacement, FD). Mean scanner motion was roughly equivalent between ASD and Healthy control groups, *t(101)=1.80, SE=.049,* *p=.08*. Our ASD group scored higher on all behavioral measures, on the MASC-2, SRS-2 and CBCL-Externalizing than the control group (*p<.001*, See table 1).

## Neuroimaging analysis

### Image acquisition

All participants were scanned on a Siemens MAGNETOM PRISMA upgraded for echoplanar images (EPI) at the Yale Magnetic Resonance Research Center. Each session began with a localizing scan, followed by the collection of a high resolution T1-weighted anatomical image: Repetition time (TR)=2530ms, echo time (TE)=3.31ms, flip angle=60°, resolution=1x1x1mm, and the collection of 4.5 mm thick axial-oblique T1-weighted slices aligned with the anterior-commissure-posterior commissure line.

Functional data was collected at the same slice locations as the T1-weighted anatomical data, with an axial plane parallel to the anteromesial (AC-PC) line, utilizing an EPI gradient echo sequence for T2\* images: TR=3s, TE=25ms, flip angle=90°, field of view=21cm, matrix size=64x64. Participants completed one resting state run consisting of 155 volumes within a total run time of 5 minutes, 30 seconds. Participants were instructed to rest with their eyes open while watching a grey screen and crosshair. The first 4 volumes were discarded to allow the signal to reach a steady state.

### Image pre-processing

Image preprocessing and analysis was done using the Functional MRI of the Brain (FMRIB) Software Library (FSL Version 5.0.10; FMRIB, Oxford, United Kingdom).

Regarding spatial smoothing, the MCFLIRT linear realignment tool within FSL was used for motion correction and image realignment. Spatial smoothing was done using a 5mm full width and half maximum isotropic Gaussian kernel, and then put through a nonlinear high pass filter (60-s cut-off).

Individual participant analyses were conducted using FSL FMRI Expert Analysis Tool (FEAT). Functional images were registered to coplanar images, which were then registered to high-resolution T1-weighted images and normalized to the Montreal Neurological Institute 152 Template.

Participants with average motion of greater than 3mm or degrees of translation and rotation, respectively, were excluded (which is a common motion criterion benchmark for pediatric populations) (Greene et al., 2018; Frew et al., 2022).

Given that neuroimaging data in pediatric populations is particularly susceptible to noise and artifacts, Independent Components Analysis for Automatic Removal of Motion Artifacts (ICA-AROMA) was employed as part of our preprocessing and denoising workflow. ICA-AROMA is a robust approach used to denoise and remove motion artifacts in pediatric fMRI data (Ciric et al., 2017; Pruim et al., 2015), intended to preserve temporal degrees of freedom and homoscedasticity within the data while eliminating distance dependent artifacts without introducing false anticorrelations within the data. Before temporal filtering, noise components representing participant movement or scanner artefacts were removed, and the cleaned data was temporally high-pass filtered. At a single subject level, we regressed out white matter time series and cerebrospinal fluid signals from our data. Before scanning, subjects were also trained to minimize motion using a mock scanner equipped with motion-tracking software.

### Region of interest definition

A seed-based connectivity analysis was conducted in FSL to investigate the strength of salience to default mode network connectivity with anxiety severity in children with ASD. Based on prior literature (Seeley et al., 2007; Menon & Uddin, 2010), seed regions representing the salience network were selected in the anterior insula (aINS) and anterior cingulate cortex (ACC). Regions representing the default mode network (Andrews-Hanna et al., 2010; Brolidakis et al., 2016) were also selected in the medial prefrontal cortex (mPFC) and posterior cingulate gyrus (PCC). For each seed, Freesurfer was used to generate Regions Of Interest (ROI) based on the Destrieux Atlas and each participant’s unique structural image, producing subject-specific parcellations. The timeseries for each ROI was computed by extracting the average of all voxels in both left and right hemispheres of each subject specific ROI mask after preprocessing and denoising. Timeseries of each ROI used as a regressor in FSL FEAT within a general linear model (GLM). Cerebrospinal fluid and white matter time courses were then extracted using the Freesurfer processing stream and included as regressors of no interest. Trained researchers, blinded to group assignment, then visually inspected all neuroimaging outputs to ensure accuracy of parcellations and segmentations.

### Hypothesis 1: Seed based analyses

Hypothesis 1 was tested through seed-based analyses using a general linear model (GLM). For each ROI seed in the salience (aINS, dACC) and in the default mode network (mPFC, PCC), a general linear model using FMRIB’s Local Analysis of Mixed Effects (FSL-FLAME) was created. First, a categorical analysis was conducted comparing Healthy Control (HC) and ASD groups, then a dimensional analysis was conducted using the SRS-2 Total Scores, and MASC-2 Total Scores as continuous variables.

### Categorical analysis

FMRIB’s Local Analysis of Mixed Effects (FSL FLAME 1+2) was used, estimating the inter-subject random-effects component of mixed effects variance at each voxel between HC and ASD groups. We did not have a-priori hemispheric hypotheses, therefore analyses were conducted for salience and default network nodes including both hemispheres. For each ROI, a general linear model was created to evaluate group differences, while adjusting for IQ, age, motion, medication status and gender as covariates. To test our model for sensitivity analyses, a second general linear model was then conducted including the group interaction term, as well as T-scores on the CDI, CBCL Externalizing and ADHD subscales. Following this, supplemental analyses were also conducted, including an investigation of gender-related differences between groups at each ROI (Group \* Gender interaction).

### Dimensional analysis

Next, dimensional analysis was conducted to assess whether the association between salience (aINS and ACC) and default mode network (PCC and mPFC) seed activity and were associated with the severity of social impairment and anxiety. Anxiety severity (MASC-2 Total score) and social impairment (SRS-2 Total score) were modelled as continuous variables to maximize statistical power, along with the interaction term between anxiety and social responsivity (MASC-2 \* SRS-2). Motion, IQ, age, gender and medication status were included as covariates in our model.

For both dimensional and categorical analyses, corrections for multiple comparisons were done at a whole brain, and voxel-wise level using a standard cluster formation threshold of *p<.001.* Clusters were reported if they surpassed a cluster-wise threshold of *p<.05.* Based on a family wise error correction rate derived from random field theory, a z-threshold of *z>3.1* was used to define clusters as groups of continuous voxels.

To visualize our results and interactions, beta coefficient values were averaged from clusters that surpassed the *p<.05* threshold and extracted for each subject using FSL’s Featquery. These coefficient values were compared across groups for our subsequent mediation and post-hoc analyses. FSLeyes version 5.0.10 was used to generate all neuroimaging figures.

## Hypothesis 2 and 3: Mediation analysis

Hypotheses 2 and 3 were tested using mediation analyses. Our independent variable was seed-based functional connectivity between the salience (SN) and default mode (DMN) networks. We had no a-priori hypothesis of which of our four seeds representing the salience (aINS and ACC) and default mode (mPFC and PCC) networks would be significantly associated with our behavioral variables, therefore, a series of mediation analyses were conducted on an exploratory basis to investigate the mediation effect of social responsivity between seed-based connectivity and anxiety. Our dependent variable was anxiety. We had two *a-priori* hypotheses: Hypothesis 2 proposes that social responsivity mediates the association between salience to default mode network seed connectivity and anxiety. Hypothesis 3 proposes that comorbidities (ADHD, and externalizing symptoms) mediate the association between salience to default mode network seed connectivity and anxiety. Therefore, the effects of social responsivity, ADHD, and externalizing symptoms were investigated as mediators in three separate mediation models (Figure 1). The indirect effect of social responsivity or comorbid symptoms is represented by the product of paths *a* and *b*. Path *c* and *c’* represent the total effect of SN-DMN seed connectivity on anxiety alone, and the direct effect of SN-DMN seed connectivity on our mediator variable.

Mediation analysis provides a mechanistic understanding of indirect effects, which portrays the influence of an independent variable on a dependent variable through a mediator variable. Following Hayes (2017), a significant effect of statistical mediation would indicate that social responsivity, ADHD symptoms or externalizing would mediate the association between seed connectivity from our regions of interest in the default mode and salience networks and anxiety. Mediation analyses were originally designed to establish causal inference from longitudinal data. However, our study uses cross sectional data, therefore, this thesis does not claim to establish causal directionality between these variables. Nevertheless, various statistical frameworks support the use of exploratory mediation models to investigate complex relationships, such as the association between network functional connectivity and behavioral measures (MacKinnon et al., 2007; Serang et al., 2017). Mediation analyses were conducted using the PROCESS macro in R (Hayes, 2017). To test for the statistical significance of the indirect effect, an alpha level of .05, and a bootstrapping procedure that computed unstandardized estimates from 5,000 bootstrap samples were used for each mediation model.

**Figure 1:** *Proposed Mediation model of Social responsivity (SRS-2 T-score) and psychopathological comorbidities (ADHD, Externalising CBCL Subscales) between salience (aINS, dACC) to default mode (mPFC, PCC) seed connectivity and anxiety (MASC-2 T-score).*

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## Regression assumptions and Descriptive statistics:

Our seed-based analyses in FSL and PROCESS mediation analyses both rely on regression models. Violations of these regression assumptions may result in incorrect statistical inferences in small samples. Shapiro-Wilk analyses were conducted on SPSS to test assumptions of normality for behavioral variables. Skewness and Kurtosis were also investigated. Behavioral data in clinical populations does not typically conform to a normal distribution (Counsell et al., 2011), thus, we did not expect this especially given that MASC-2 scores were an inclusion criterion for our ASD sample.

Shapiro-Wilk analyses showed that the MASC-2, SRS-2, and CBCL-Externalizing subscales within the total sample were not normally distributed (appendix B). Within the total sample, MASC-2 kurtosis was smaller than -1.0, indicating a platykurtic distribution. Kurtosis in SRS-2 and CBCL-Externalizing variables were also trending towards a platykurtic distribution. MASC-2, SRS-2 and CBCL Externalizing behavioral scores within our ASD sample were slightly negatively skewed (appendix C), which was expected given the clinical nature of our sample, and our inclusion criteria. The healthy control group displayed a positive skewness in MASC-2 and SRS-2 scores, indicating a lower level of anxiety and social impairment, which was expected in a typically developing population. A Shapiro-Wilk test was also conducted for all demographic variables. Age was not normally distributed *(W=.517, p=.037*). All other demographic variables displayed a normal distribution (appendix B).

Given that the size of our sample is relatively large for a neuroimaging study (*n=103*), violations of the assumption of normality should have a minimal impact on inferences made within our analyses (Knief and Forstmeier, 2021). For all variables, skewness and kurtosis ranged from -2.0 to +2.0. Given that our sample size is relatively large, we can assume that behavioral and demographic data still retain a normal univariate distribution (Cain, Zhang & Yuan, 2016).

Durban-Watson tests were used to calculate the first-degree autocorrelation within our three mediation models using functional connectivity and anxiety (MASC-2 T-score). No autocorrelations were found in our model with social responsivity (SRS-2 T-score), CBCL Externalizing or ADHD mediators (appendix D).

Each mediation model was then assessed for multicollinearity. All collinearity statistics for all three models were within acceptable ranges. Collinearity tolerance for all variables was greater than .25, VIF for all variables was less than 2 (appendix E).

Homoscedasticity was assessed by visual inspection of scatterplots of regression standardized residuals for each mediation model. All plots showed a random, rectangular shape, indicating homoscedasticity could be assumed (appendix F).

No heavy tails or outliers were found within our data, and no values were above or below absolute values that would be considered problematic for our statistical analysis.

### Correlations between psychometric measures

In the total sample,Pearson’s bivariate correlations were conducted between all behavioral variables, and mean scanner motion (framewise displacement, FD) within the scanner. MASC-2 total scores were significantly correlated with CBCL Externalizing Behavior subscale scores *(r=.482, p<.001, CI[0.314, 0.619]).* Mean scanner motion was significantly correlated with IQ *(r=-.219, p=.027, CI[-.400, -.025])* and with age *(r=-.344, p<.001, CI[-.505, -.159]).* SRS-2 did not significantly correlate with other behavioral variables. No other significant correlations were found. Correlations for the total sample are reported in appendix G.

In our ASD subsample, an identical Pearson bivariate correlation analysis was also conducted. Social responsivity scores (SRS-2) were correlated with CBCL Externalizing behavior subscale scores *(r=0.499, p<.001, CI[.304, .0654])* and negatively correlated with IQ *(r=-0.231, p=.005, CI[-0.437, -0.001])*. Mean scanner motion was also negatively correlated with age *(r=-0.396, p=.005, CI[-0.574, -0.183]).* Beyond these correlations, no other correlations were found. Correlations for the ASD subsample are reported in appendix H.

For both correlations in the total sample and ASD subsample, a 5000-iteration bootstrap was performed to control for type-1 error. These correlations were used to guide the selection of covariates selected during FEAT analyses.

### Potential covariates and psychotropic medication use:

Independent samples t-tests were used to compare all demographic variables between the ASD and HC groups. Age, gender distribution, and scanner motion were roughly equivalent between our ASD and Healthy control groups. Participants in the ASD group on average had a lower IQ than healthy controls *(p=.007*; Table 1). Given that differences in demographic variables were found between our ASD and HC groups, age, gender, IQ, and motion were included as covariates in all subsequent analyses.

73% of ASD children within our sample reported taking psychiatric medications. Group level comparisons were also conducted within our ASD sample comparing individuals who had medication prescriptions and those without. ASD participants on medication had higher SRS-2 T scores, *t(73)=3.09, p=.003, (95% CI[2.39,11.12]),* but had equivalent IQ, age, scanner motion, CBCL-externalizing subscale, and MASC-2 anxiety scores compared to those not on medication (appendix I). Post-hoc comparisons of neuroimaging findings show no differences in functional connectivity within the ASD sample associated with medication status. Regardless, medication status was also included as a covariate in subsequent analyses.

Finally, given that a DSM-5 diagnosis of autism and a clinically level of anxiety was an inclusion criterion for study participation in our study for ASD individuals, group-level differences in MASC-2 and SRS-2 T-scores were expected and observed. Given findings from prior literature (Neuhaus et al., 2021), a higher level of endorsement of the CBCL Externalizing behavior subscale was also expected and observed between sample groups. Full descriptive findings, comparisons between demographics, and clinical characteristics can be found in table 1.

# Results

## Salience network: Anterior insula and anterior cingulate seed connectivity

Anterior insula seed functional connectivity analyses in the total sample (*n=103*), using a categorical model, contrasting our clinical and healthy control group, show that after adjusting for covariates of age, IQ, gender, motion and medication status, autistic children showed under-connectivity between the bilateral anterior insula, a region in the salience network, and the bilateral temporo-parietal junction, a region in the default mode network *(p=.023, zmax=4.01)* (Figure 2). Coordinates for clusters of activation are reported in table 2A.

Our finding of underconnectivity between the anterior insula and temporo-parietal junction characteristic of our autism and comorbid anxiety sample were supported by concurrent analyses within the total sample *(n=103)* using a dimensional model that included social responsivity (SRS-2 T-score), anxiety severity (MASC-2 T-score) and their interaction term (SRS\*MASC). Increasing anxiety severity was associated with decreasing bilateral anterior insula to temporo-parietal junction connectivity *(p=.012, zmax=5.05) (*Figure 3*).* All findings remained significant after adjusting for covariates including age, gender, IQ, motion and medication status. Coordinates for clusters of activation are reported in table 2B.

Regarding associations with SRS-2, in both the total sample *(n=103)*, and the ASD subsample *(n=74),* increasing social responsivity deficits was associated with hyper-connectivity of the anterior insula to ACC (*p=.037, zmax = 4.34*) and PFC *(p=.048, zmax=4.17) (*Figure 4*).* Using our anterior insula seed, social responsivity deficits were associated with functional connectivity of the anterior insula with itself *(p<.001, zmax=8.95).* This association likely represents within-network connectivity in the salience network. Allfindings remained significant after adjusting for age, gender, IQ, motion, and medication status. Coordinates for clusters of activation are reported in table 2C.

**Table 2:** *Anterior insula seed connectivity peak clusters in the total sample**(n=103)*.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Region** | **Hemisphere** | **BA** | **MNI Coordinates** | | | **z-score max** | ***p*** |
|  |  |  | **x** | **y** | **z** |  |  |
| **(A)** Categorical model adjusted for covariates\*, HC > ASD contrast. | | | | | | | |
| TPJ | Right | 39 | -56 | -50 | 36 | 4.29 | .002 |
| TPJ | Left | 40 | 52 | -42 | 30 | 4.05 | .004 |
| **(B)** Dimensional model adjusted for covariates\*, continuous association with anxiety (MASC-2) | | | | | | | |
| aINS | Left | - | 34 | 8 | -6 | 5.05 | .013 |
| aINS | Right | - | -30 | 12 | -10 | 5.12 | .033 |
| **(C)** Dimensional model adjusted for covariates**\*,** continuous association with social responsivity (SRS-2) | | | | | | | |
| aINS | Right | 13 | 34 | 14 | 6 | 8.95 | <.001 |
| aINS | Left | 13 | -34 | 6 | 6 | 5.90 | <.001 |
| ACC | Left | 32 | -8 | 10 | 34 | 4.34 | .038 |
| PFC | Left | 10 | -38 | 50 | 14 | 4.17 | .048 |

\**Note*: Covariates include Age, IQ, Gender, Medication Status and Motion.

|  |  |
| --- | --- |
| **(A)**  **A graph with red and blue dots  Description automatically generated with medium confidenceA picture containing brain  Description automatically generated**  **aINS – TPJ Functional Connectivity** | **A picture containing text, diagram, plot, screenshot  Description automatically generated(B)** |

**Figure 2:** *Decreased functional connectivity between the anterior insula (aINS) and temporo-parietal junction (TPJ) observed in children with ASD contrasted against Healthy Controls (HC).* **(A)** Significant clusters of group level differences in temporo-parietal junction functional connectivity in the total sample *(n=103)*. Contrasts Of Partial Estimate Means (COPE-means) Beta coefficients were averaged and extracted from the highlighted regions and displayed in panel B. **(B)** Violin Boxplots of COPE-mean Beta coefficients of anterior-insula to temporal-parietal junction connectivity in ASD and HC groups.

**A graph with red and blue dots

Description automatically generated with medium confidenceFigure 3:** *Increasing anxiety severity associated with decreasing bilateral anterior insula connectivity (aINS) with the temporo-parietal junction (TPJ).* **(A)** Bilateral insular seed functional connectivity regions associated with significant clusters of group level differences in Anxiety. Contrasts Of Partial Estimate Means (COPE-means) Beta coefficients were averaged and extracted from the highlighted regions and displayed in panel B. **(B)** Regression Plot of Anxiety (MASC-2 Total Score) and COPE-mean Beta coefficients of anterior-insula to temporal-parietal junction connectivity in the total sample *(n=103),* separated by group.

|  |  |
| --- | --- |
| **(A)**    **aINS Seed Functional Connectivity** | **(B)**  **A graph with red and blue dots  Description automatically generated with medium confidence**  **Anxiety (MASC-2 Total Score)** |

**A graph with red and blue dots

Description automatically generated with medium confidenceFigure 4:** *Increasing severity of social impairment associated with anterior insula (aINS) seed connectivity with the anterior cingulate (ACC), dorso-lateral prefrontal cortex (dlPFC) and bilateral insula*. **(A)** Significant clusters of group level differences in functional connectivity associated with Social Responsivity (SRS-2 Total Scores). Contrasts Of Partial Estimate Means (COPE-means) Beta coefficients were averaged and extracted from the highlighted regions and plotted in panel B. **(B)** Regression Plot of SRS-2 Social Responsivity and COPE-mean Beta coefficients of anterior-insula seed functional connectivity in the total sample *(n=103),* separated by group.

|  |  |
| --- | --- |
| **(A)**  **A picture containing brain  Description automatically generated** | **(B)**  **A graph with red and blue dots  Description automatically generated with low confidence** |

Functional connectivity analyses using an anterior cingulate cortex (ACC) seed showed no significant categorical group differences or dimensional associations with anxiety severity, or social responsivity in the total sample between ASD and Healthy controls, or within our ASD subsample.

## Default mode network: Posterior cingulate and medial pre-frontal cortex seed connectivity

Within the total sample *(n=103),* there were no group differences in default mode connectivity for mPFC and PCC seeds. There were also no associations found with social responsivity (SRS-2 T-score) or anxiety (MASC-2 T-score) using mPFC and PCC default mode network seeds in the total group or ASD subsample.

## Interim summary of results:

Our primary finding was that there was underconnectivity between the anterior insula and temporal parietal junction in our ASD participants compared to healthy controls. Underconnectivity between these regions was not anticipated in our hypotheses; instead, we expected that core nodes within the salience and default mode network would be hyper-connected. Dimensional analyses using the same anterior insula seed in the ASD subsample indicated social responsivity and anxiety were associated with anterior insula hyperconnectivity with the anterior cingulate and dorso-lateral prefrontal cortex, and hyperconnectivity in the anterior insula with itself. Therefore, to further understand our primary finding of underconnectivity between the anterior insula and temporo-parietal junction associated with anxiety symptoms in ASD, social responsivity was investigated as a mediator for this relationship in both the total sample (*n=103*) and ASD subsample *(n=74)*.

## Social responsivity mediation analyses

Mediation regression analysis was conducted to investigate social functioning as a mediator for the relationship between anxiety and decreased salience (anterior insula) to default mode (temporo-parietal junction) network connectivity.

Mediation analysis in the total sample *(n=103*) indicated that the association linking anxiety and functional connectivity between the default and salience network nodes was fully mediated by an indirect effect of social responsivity *(β=-0.16, SE=0.06, 95%CI[-0.30, -0.05])* (figure 5, panel A*).* Approximately 44% of the variance was accounted for by social functioning as a predictor *(R2=.44)* (appendix J, table 11). The statistical significance of this indirect mediation effect in the total and ASD sample were tested using a percentile bootstrap approach with 5000 samples. These findings however were not replicated in the ASD subsample *(n=74)*. Social functioning was not a significant mediator of anxiety severity (figure 5, panel B). The overall regression model was nonsignificant (*F(1,72)=0.019, p=.892*). (appendix J, table 12).

Follow up analyses were also conducted investigating aINS-TPJ connectivity as a mediator for the association between social responsivity and anxiety within the ASD subsample. No significant mediation effect was found.

**Figure 5:** *Mediation model of the indirect effect of anterior insula (aINS) to temporo-parietal junction (TPJ) functional connectivity on anxiety (MASC) through social responsivity (SRS).* **(A)** Mediation analysis using the total sample *(n=103)*. **(B)** Mediation analysis within the ASD subsample *(n=74)*.

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## Anxiety comorbidity mediation analyses:

Mediation regression analysis was also used to investigate common co-occurring behaviors with as mediators for anxiety’s relationship with decreased salience (anterior insula) to default mode (temporo-parietal junction) network connectivity.

A mediation analysis in the total sample *(n=103)* indicated that the CBCL Attention Deficit and Hyperactivity (ADHD) subscale behaviors mediated the connection between anxiety and aINS-TPJ functional connectivity *(β=-0.13, SE=0.05, 95%CI[-0.22, -0.05])* (figure 6, panel A) *.* Approximately 4.7% of the variance was accounted for ADHD as a predictor *(R2=.047)* (Appendix K, Table 13). The statistical significance of this indirect mediation effect in the total and ASD sample were both also tested using a percentile bootstrap approach with 5000 samples. These findings however were not replicated in the ASD subsample *(n=74)*. ADHD was not a significant mediator of anxiety severity (figure 6, panel B). The overall regression model was also nonsignificant (*F(1,72)=0.060, p=.807*)(Appendix K, Table 14).

The CBCL Externalizing behavior subscale did not mediate the relationship between anxiety and functional connectivity in the total sample *(B=-11.6, SE=-1.94, 95% CI[-23.4, 0.24], β=-0.19, p=.055).* Within the ASD subsample, this mediation regression was also nonsignificant. *(F(1,72)=0.06, p=.807)*

**Figure 6:** *Mediation model of the indirect effect of anterior insula (aINS) to temporo-parietal junction connectivity (TPJ) on anxiety (MASC) through CBCL Attention Deficit and Hyperactivity subscale behaviors (ADHD).* **(A)** Mediation analysis using the total sample, *(n=103)*. **(B)** Mediation analysis within the ASD subsample, *(n=74)*.

**(A)**

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# Discussion

The current study investigated the relationship between anxiety in ASD and the functional connectivity within and between the salience, and default mode networks. Seed-based analysis was conducted investigating the association between four key regions in the salience (anterior insula and anterior cingulate cortex) and default mode (medial prefrontal cortex and posterior cingulate cortex) networks with anxiety and social responsivity. We found partial support for our first hypothesis; functional hypo-connectivity between the anterior insula (aINS) and temporo-parietal junction (TPJ) was associated with group-level differences in anxiety between autism and healthy control populations. Our second and third hypotheses were not supported. The association between functional hypo-connectivity of the aINS–TPJ pathway and anxiety was not mediated by social responsivity scores in our ASD sample. Other co-occurring behaviors (externalizing and ADHD) also did not mediate the relationship between functional hypo-connectivity and anxiety in ASD. These findings will be discussed in the context of the study hypotheses and previous literature from neuroscientific and psychoanalytic fields.

## Discussion of significant results, findings and implications

### Hypothesis 1: Seed based functional connectivity analysis

Firstly, we aimed to investigate if differences in salience network connectivity distinguish ASD participants with anxiety and healthy controls. We hypothesized that autism would be characterized by functional hyper-connectivity within our ASD sample. Our hypothesis was partially supported: functional connectivity of the anterior insula in the salience network and temporo-parietal junction in the default mode network distinguished our clinical group, and this pattern of connectivity was predictive of anxiety in ASD. However, functional hypo-connectivity between the anterior insula to temporo-parietal junction was the distinguishing factor associated with anxiety between our ASD and HC groups.

### The anterior insula to temporo-parietal junction pathway and anxiety in ASD

Group-level differences in the functional connectivity of the anterior insula and temporo-parietal junction were found between the autism and HC groups. Anterior insula to temporo-parietal junction pathway hypoconnectivity was dimensionally associated with anxiety (MASC-2 T-score) in ASD. These results support studies suggesting that anterior insular dysfunction as a hub for integrating social and affective information is associated with increased anxiety levels in ASD. Studies report that decreased resting state connectivity of the anterior insula with the default mode network is associated with anxiety levels (Xia-an et al., 2018; Angeletti et al., 2021). Activity in the TPJ has been referenced by multiple other studies to be associated with theory of mind and mentalizing tasks (Ozdem et al., 2019; Jiang et al., 2021). Neuroimaging studies suggest that the role of the TPJ in the default mode network is specifically related to the embodied simulation of movement (Kilroy et al., 2021), which supports mentalizing activity through close communication with the anterior insula via the middle longitudinal fasciculus (Saur et al., 2008). Some studies have also likened the interface between the anterior insula and temporo-parietal junction to function as a 'switch' or 'circuit breaker' within the default mode network, which allow the subsequent activation of frontoparietal networks involved in preparing and applying goal-directed selection for stimuli and responses (Anticevic et al., 2010). Disrupted communication between the anterior insula and temporo-parietal junction may affect the anterior insula's role to 'switch' between brain states and disrupt the integration of self- and other- related information (Wu et al., 2015). Hypo-connectivity between these regions may increase difficulties transitioning out of repetitive negative thinking, increasing anxiety (Burrows et al., 2017). Therefore, disruptions to the anterior insula – temporo-parietal junction pathway may affect mentalizing and interoceptive processing. This aberrant processing may subsequently result in anxious emotions.

### Anterior insula to anterior cingulate connectivity and social responsivity

A dimensional association was also found between anterior insula and anterior cingulate cortex connectivity and social responsivity scores (SRS-2 T-scores). Increasing hyper-connectivity of the anterior insula (aINS) and anterior cingulate (ACC) pathway was associated with greater reported social deficit in our total sample. This consistent with prior research showing that the aINS-ACC pathway is associated with attentional control and conflict monitoring (Burrows et al., 2017; Menon, 2011), and supports the representation of emotional states during emotion regulation (Oschner and Gross, 2007). Hyperconnectivity of this pathway within the SN is associated with cognitive inflexibility and is proposed to be a mechanism contributing to levels of social deficit in ASD (Mesulam, 1990; Yeo et al., 2015). Our findings support this research, suggesting that disruptions in this salience network pathway characterize social responsivity deficits in individuals with autism and comorbid anxiety.

### Anterior insula to prefrontal cortex connectivity and social responsivity

Lastly, a dimensional association was found between the anterior insula and prefrontal cortex connectivity and social responsivity (SRS-2 T-scores). Increasing hyper-connectivity between the anterior insula and prefrontal cortex was associated with greater reported social deficit in our total sample. Our findings of hyper-connectivity between the anterior insula and prefrontal cortex associated with social responsivity contrast results showing hypo-connectivity of the anterior insula with the temporo-parietal junction associated with anxiety. Other studies have reported that anterior insula hyper-connectivity with the prefrontal cortex is associated with executive dysfunction in ASD, which may contribute to and increase social responsivity challenges that are observed within our sample (Courchesne & Pierce, 2005; Weller et al., 2009; Menon & Uddin, 2010). Hogeeveen’s (2018) study also reported overconnectivity between the anterior insula and fronto-parietal network associated with anxiety in ASD. Differing functional connectivity patterns between the anterior insula with regions in the default mode network may represent distinct mechanisms for anxiety pathology and social responsivity deficits. This effect may also be unique to ASD individuals with comorbid anxiety. Future research is needed to disentangle these contrasting findings of anterior insula connectivity.

### Developmental implications for salience and default mode network connectivity

Overall, findings from seed-based analyses emphasize that connectivity of the anterior insula is central to the psychopathology of anxiety in autism. Functional connectivity between the anterior insula, with the temporo-parietal junction (TPJ), the anterior cingulate cortex (ACC), and the prefrontal cortex (PFC), were associated with anxiety and social responsivity. In the context of Menon’s (2018) triple network model, our results support the importance of the salience network as a cognitive control center. Menon’s (2018) model highlights the crucial role of salience network activity, particularly the anterior insula, for initiating network switching leading to the engagement of the CEN and the disengagement of the DMN (Menon & Uddin, 2010). Our findings support Menon’s assertion that the anterior insula is an integral causal outflow hub (Sidharan et al., 2008), associated with the generation of appropriate behavioral responses to salient stimuli through its connections within the salience network, and with the default mode network (Menon, 2018). Our study also supports Burrow’s (2017) hypothesis that repetitive negative thinking is associated with hyperconnectivity within the salience network, resulting in maladaptive switching of attention and cognitive inflexibility. Hyperconnectivity between the anterior insula and anterior cingulate cortex at rest may represent a hyper-reactivity to social-emotional stimuli stemming from difficulties processing emotionally relevant information during social target detection (Skokauskas & Gallagher, 2010). Increased within-network connectivity in the salience network in children with ASD at rest could prevent flexibility switching between brain networks, particularly when processing social, face-related information (Safar et al., 2018). These contribute to an increased level of hostile attribution biases (Niditch et al., 2012) and exacerbate repetitive negative thinking patterns (Lydon-Staley et al., 2019), both of which are associated with increased anxiety in ASD.

Expanding on Burrow's (2017) model, our results add to the current literature by suggesting that functional hypo-connectivity, particularly with the temporo-parietal junction may be another important interface connecting the salience and default mode networks associated with anxiety in ASD. Activity within the TPJ is associated with embodied perspective-taking (Martin et al., 2020). Prior research has shown that aINS-TPJ connectivity mediates the relationship between neural correlates of interoception and social fear (Stern et al., 2017). Therefore, in ASD, aberrant integration of self-related signals due to hypo-connectivity along the aINS-TPJ pathway may contribute to increased levels of anxiety (Greucci et al., 2013). The atypical integration of information during embodied perspective taking associated with a hypo-connected aINS-TPJ pathway may make emotions harder to recognize and regulate, as a 'self-concept' of one's emotions provides an anchor to understand emotional regulation in others (Holzel et al., 2011; Chien et al., 2012; Hao et al., 2022). Within ASD, increasing alexithymia and decreasing self-awareness of internal affective states is associated with elevated levels of anxiety (Hogeveen et al., 2018; Poquerusse et al., 2018).

Given that we found varying patterns of hyper- and hypo- connectivity, our findings emphasize that increased repetitive negative thinking in ASD could be the result of the dynamic interaction of functional connectivity within the salience network (i.e., in the aINS-ACC pathway) and between the salience and default mode networks (i.e., in the aINS-TPJ pathway). Differing connectivity profiles of the anterior insula are likely moderated by developmental stage. Resting-state fMRI studies show age effects associated with anterior insula connectivity in ASD. Autistic adults and adolescents show social responsivity deficits are associated with aINS-ACC pathway hypo-connectivity (Uddin et al., 2013). In contrast, within network hyperconnectivity of the insula and salience network is found in children, but not in adolescents and adults with ASD (Nomi & Uddin, 2015). Anterior insular hyper-connectivity likely decreases with age, spurred by pubertal and behavioral challenges that individuals with ASD begin to experience in adolescence, such as the formation of friendships, or romantic relationships, subsequently transitioning into insular network hypo-connectivity during adolescence and adulthood (Nomi, et al., 2016; Nomi et al., 2019).

Developmental changes in anterior insula connectivity may result in time- and task-sensitive connectivity differences of the anterior insula within the salience network and between the anterior insula and the default mode networks. Studies investigating temporal data from brain states using measures of dynamic functional connectivity suggest that time-sensitive differences in the signaling and coordination of cognitive control networks result in patterns of hypo- and hyper-connectivity that are unique to autism development (Damiani et al., 2018; Roy & Uddin., 2021). These trends are observed in our resting-state study, as the anterior insula was simultaneously hypo-connected with the default mode, and hyper-connected to the salience and prefrontal cortical networks. As children age, these connectivity patterns likely shift (Nomi and Uddin, 2015). Therefore, developmental changes in the signaling within the SN, centered around the anterior insula, may result in a cascade of changes in the insular pathways associated with the emergence of anxiety pathology in ASD during middle to late childhood (Menon et al., 2020; Nomi et al., 2019).

Connectivity between the anterior insula and temporo-parietal junction may be a key pathway affected by changes in insular development, whose aberrant functioning promotes repetitive negative thinking associated with anxiety development (Hao et al., 2020; Hogeveen et al., 2018; Boeheme et al., 2015). A tendency to perseverate on negative self-thoughts in ASD may therefore arise from the conjunction of (1) aINS-ACC hyperconnectivity which affects cognitive switching capacities (Schimmelpfennig et al., 2023), with (2) aINS-TPJ hypo-connectivity which results in aberrances in self-representation (Lombardo et al., 2011; Donaldson et al., 2018). Difficulties inhibiting emotional information or disengaging from negative thoughts may be due to network inefficiencies that result in higher activity levels - more effort - required to switch away from upsetting thoughts (Burrows et al., 2017). Abnormal embodied self-representations may exacerbate the impact of repetitive negative thoughts linked to cognitive inflexibility in ASD (Lartseva et al., 2014). Patterns of repetitive negative thinking and challenges in emotional awareness may represent a vulnerability associated with aberrant salience network development (Burrows et al., 2017), as well as an opportunity for interventions to target anxiety in individuals with autism spectrum disorders through leveraging therapies that activate salience to default mode network pathways. This interpretation of our results is supported by research from psychoanalytic literature.

### Developmental implications for object relations psychoanalysis

Our evidence suggests that the ‘containment’ of anxiety within Bion’s theory of thinking is associated with mentalizing and salience of emotion in the anterior insula (aINS) to temporo-parietal junction (TPJ) pathway. Psychoanalytic work in object relations theory asserts that during ‘containment,’ or ‘holding,’ the maternal object represents the ‘embodiment’ of anxious emotions (Tustin, 2015). A child’s understanding of the maternal object’s attempts at comfort requires a process of introjection into the maternal object as a ‘container’ for anxiety (Hamilton et al., 1994; Klein, 1984 Bion, 1962). Experiencing maternal reverie requires that a child understands how a mother empathizes with and interprets a child’s projected anxiety. Therefore, a child’s self-concept of emotional awareness is an important component in how the maternal object ‘holds’ anxiety (Bowlby, 1958; Bollas, 2018). If processes of emotional awareness are disrupted, the experience of ‘holding’ felt as incomplete, and anxiety is experienced as ‘uncontained’ (Bion, 1962; Durban, 2019; Emanuel, 2015).

The anterior insula to temporo-parietal junction pathway supports the experience of maternal reverie through processes of embodiment (i.e., through automatic mimicry or imitation) (Molnar-Szakacs & Uddin, 2022; Oliver et al., 2018; Gu et al., 2013). The atypical integration of information due to a hypo-connected aINS-TPJ pathway may affect how a child experiences the embodiment of emotions by the maternal object (Pouillaude, 2018; Plank et al., 2022; Arzy et al., 2006). Our study suggests that aberrant hypo-connectivity associated with the aINS-TPJ pathway may affect emotional awareness by contributing to a sense of ‘dis-embodiment’ or sense of ‘dis-integration’ associated with anxious emotions in ASD (Brenner, 2022b; Durban, 2019). Therefore, this results in the perception that anxious experiences as being incompletely ‘mentalized’ by the maternal object. Under a contemporary Bayesian approach to object relations theory, disrupted signaling within the anterior insula and temporo-parietal junction may contribute to ‘prediction error’ in the processing of internal and external state information (Wen et al., 2021; Holmes & Nolte, 2019), resulting in difficulties in the child experiencing anxiety as sufficiently ‘held’ by the mind of the mother. This association implies that aberrant aINS-TPJ connectivity is associated with mentalizing, but also alexithymia deficits in ASD, which is observed in clinical case studies of autism (da Silva, Vasco & Watson, 2018), as well as demonstrated in functional neuroimaging studies (Lassalle et al., 2019).

However, there is contrasting evidence between neuroscience and psychoanalytic literature: some studies show that default mode network activity associated with empathic response is preserved in ASD (Schultz et al., 2015). Findings suggest that underconnectivity associated with theory of mind deficits might be specific to TPJ but not the adjacent supra-marginal gyrus. Studies using empathic judgment tasks and emotional egocentricity paradigms suggest that connectivity of regions adjacent to the TPJ support intact self-other distinctions during empathy (Hoffman et al., 2016). This implies that some pathways supporting the integration of social and emotional information between the SN-DMN networks (likely via the aINS-TPJ pathway) are preserved in ASD. Methodological differences could account for some level of variability in the neuroimaging results. This could be due to a difference between task-based and resting-state studies (Hasson et al., 2009). Functional connectivity differences observed at rest do not necessarily reflect functional connectivity differences during tasks (Mennes et al., 2013).

Alternatively, empathy and mentalizing could use the aINS-TPJ pathway for embodied simulation of mental states and socio-emotional cognition differently in participants with ASD. A Lacanian psychoanalytic perspective suggests that the construction of emotional understanding in ASD follows a different developmental trajectory, as children with ASD may relate to the maternal object differently. Brenner (2021) explains this through differences in the use of language in autism to encode and interpret emotions. Anxieties in ASD are not experienced as embodied by the object but are symbolized using a ‘signed language’, of repetitive or restrictive motions (Xie et al., 2023; Sterponi et al., 2014; Prizant, 1983). This is proposed to be a ‘private language’ which dissuades anxiety pre-linguistically, outside the operation of an alpha function (Brenner, 2022a). Therefore, Restricted, repetitive behaviors constitute a method of shielded interactions with the environment used to alleviate anxiety (Brenner, 2022b). Developmentally, a reliance on self-focused, restricted repetitive behaviors to regulate anxiety may result in or develop from patterns of hyper-connectivity within the SN (exemplified by the aINS-ACC pathway), which are associated with cognitive inflexibility (Lartseva et al., 2014). Therefore, within a Lacanian psychoanalytic framework, cognitive inflexibility associated with repetitive ruminative thinking may arise from the maladaptation of the salience network to the experience of anxiety (Uddin, 2019; Chen et al., 2016). Future research can expand on this from a psychoanalytic perspective through qualitative investigations of how restricted repetitive behaviors, or idiosyncratic speech, frame or regulates the experience of anxieties in autism. Future imaging research may also consider investigating the neural correlates of restricted-repetitive behaviors in ASD and emotion regulation.

## Discussion of non-significant results

### Hypothesis 1: Null findings from default mode network seed connectivity

In addition to our primary findings, seed-based ROI analyses were conducted using DMN seeds in the posterior cingulate cortex and medial prefrontal cortex. We found no significant differences in connectivity within the default mode network across groups. No significant relationships were also found in the connectivity of these regions within the default mode network with our behavioral variables of anxiety and social responsivity.Null findings from our seed-based analysis of default mode network regions associated with social responsivity contrast existing literature. Prior studies have demonstrated altered DMN activity in children with ASD (Padmanabhan et al., 2017). Meta-analyses of resting state abnormalities in functional connectivity of the default mode network emphasize group differences at rest in children with ASD when compared against healthy controls (Wang et al., 2021). Studies have reported decreased resting state functional connectivity in various nodes of the default mode network, such as mPFC (Kennedy et al. 2008), precuneus (Assaf et al. 2010; Doyle-Thomas et al., 2015) and angular gyrus (Murdaugh et al., 2012).

Fewer studies investigating resting state functional connectivity have been done specifically comparing functional connectivity in individuals with ASD and comorbid anxiety. Studies comparing samples of individuals with autism, autism and anxiety, and anxiety without autism contrasted with healthy controls report comorbid anxiety in autism is characterized by decreased functional connectivity between the DMN, amygdala and striatum (Bartolotti, Sweeney and Mosconi, 2020). Recent studies also suggest that decreased resting state connectivity of the DMN and bilateral insula characterize autism and anxiety in adult populations (Tung et al., 2021). Despite analyses comparing samples of children (e.g., Lin et al., 2020) and adults (e.g., Tung et al., 2021; Hull et al., 2017) including the mPFC, and PCC seeds as regions of interest, differences in resting state functional connectivity within DMN regions in ASD children are not reported. In this sense, our results are in line with findings from prior literature, indicating that functional connectivity differences within the default mode network may not be distinctive of autistic children with comorbid anxiety. Therefore, the neural correlates of ASD with comorbid anxiety may be distinct from autism without comorbidities.

Overall, our seed-based resting-state analyses suggest that anxiety in autism is associated with disruptions to the functional connectivity between the salience and default mode networks and within the salience network. However, as our experiment uses resting state fMRI data, the causal relationships between these functionally connected regions are not clear. Therefore, the mechanisms linking anxiety, and social responsivity, associated with functional connectivity between the anterior insula, anterior cingulate, and temporo-parietal junction, remains an avenue for future research. There is also a lack of neuroimaging research in autistic populations with comorbid anxiety and an absence of current psychoanalytic theory to inform our therapy and understanding of autism with comorbid anxiety. Burrows (2017) proposed that the functional connectivity between these regions mediates the association between repetitive negative thinking, cognitive flexibility, and anxiety. We attempted to investigate this by firstly including default mode network seed ROIs in our analyses, and secondly, by investigating social responsivity as a mediator for the relationship between SN-DMN connectivity patterns and anxiety.

### Hypothesis 2: Social responsivity did not mediate the relationship between SN-DMN connectivity and anxiety in autism.

In our second hypothesis, we aimed to test if continuous measures of anxiety and social responsivity were associated with disruptions in salience to default mode network connectivity in autism. We hypothesized that within our dimensional analysis, SN-DMN hyper-connectivity would be associated with increasing severity of anxiety in youths with autism, mediated by elevated social impairment. Our mediation hypothesis was not supported. A significant mediation effect was found of social responsivity between anxiety and salience (aINS) to default mode (TPJ) hypo-connectivity in the total sample (n=103). However, this effect was not replicated in our ASD subsample (n=74).

Currently, no other autism research has investigated social responsivity as a mediator for the neural correlates associated with anxiety. Social deficits in autistic children who report challenges with mentalizing also have challenges in emotion regulation and awareness (Poquéresse et al., 2018), which correlates with disrupted connectivity of the anterior insula with key salience network nodes, such as the anterior cingulate (e.g., Uddin, 2015) and with default mode network nodes, such as the temporo-parietal junction (e.g., Hao et al., 2022). Hogveeen’s (2018) prior work used a measure of parent-minus-child reports of anxiety to measure emotional awareness impairments associated with increased emotion regulation difficulties in children with ASD. Their measure of emotional insight was statistically dependent on their outcome variable (parent-report anxiety), which may have confounded their results. A strength of our study is that we attempted to replicate their mediation analysis using a mediator that was statistically independent of our anxiety outcome variable, and subsequently found no mediation effect. Therefore, based on our current results there is insufficient evidence to suggest that social responsivity deficits associated with SN-DMN dis-connectivity mediate the association between salience to default mode network dysfunction and anxiety in ASD. A failure to replicate this mediation model suggests that the relationship between the behavioral and neural correlates of anxiety and social deficit in ASD may not fit a mediation model.

The presence of a modulation effect of SN-DMN connectivity on the relationship between social responsivity and anxiety scores may explain why an effect was not found in our mediation model. In his triple network model, Menon (2017) proposes that the anterior insula, particularly through its connection with the anterior cingulate, mediates the connectivity of afferent pathways from the salience network to the default mode network. This theoretical assertion forms the basis for Hogeveen’s (2018) mediation analysis and the premise for the current study’s mediation analysis. However, the absence of a mediation effect of social responsivity from salience network connectivity to anxiety may indicate that the anterior insula functions as a modulator of connectivity between the default mode and salience networks instead of a mediator. This is supported by work showing that engagement or disengagement of the DMN is modulated by signals from the anterior insula (Ulrich et al., 2022). Furthermore, recovery from anxiety disorders via pharmacological and CBT-based therapies has also been associated with increased connectivity between the anterior insula and temporo-parietal junction (Santos et al., 2019). Future studies that evaluate modulators for salience and default mode functional connectivity, social responsivity, and anxiety may provide insight into how the cognitions associated with RNT and cognitive inflexibility contribute to anxiety comorbidities in ASD.

### Hypothesis 3: Comorbid disorders did not mediate the relationship between SN-DMN functional connectivity and anxiety.

Lastly, we aimed to test if social responsivity or other behaviors that frequently co-occurred with anxiety mediated the association between functional connectivity and anxiety (MASC-2 scores) in autism. Our hypothesis was not supported. A significant mediation effect was found between social responsivity symptoms and ADHD symptoms between anxiety and functional connectivity in our total sample (n=103). However, again, this effect was not replicated in our ASD subsample (n=74).

The mediation effects observed within the total sample that are absent in our ASD sample are likely the result of group-level differences between our healthy and clinical samples. Our sample and inclusion criteria limit our interpretation of our mediation analyses. Only ASD individuals with clinical anxiety were included in our study. Therefore, our categorical group variables (autism diagnosis) may have been colinear with social responsivity (SRS-2) or anxiety (MASC-2) variables. Because of this, a mediation effect of social responsivity on SN-DMN connectivity in our total sample may be confounded with ASD diagnosis, as individuals in our clinical group consistently endorse greater levels of social responsivity deficits and anxiety disorder problems than healthy controls.

Likewise, group-level differences introduced by the clinical inclusion criteria for our ASD and Healthy groups may explain why a mediation effect of ADHD symptoms was found in the total sample but was absent in our ASD sample. ADHD is frequently comorbid with autism and anxiety (Avni et al., 2018). Sub-clinically, individuals with ASD endorse higher ADHD symptoms (Leitner, 2014). Therefore, because individuals with ASD endorse higher levels of subclinical ADHD symptoms than typically developing children, group-level differences associated with ASD diagnoses may introduce a confounding effect into our mediation model in the total sample. Future research investigating samples of autism and comorbid anxiety should therefore include 1) an ASD-only sample and 2) an anxiety-only sample, in addition to a control and experimental clinical group.

## Methodological limitations

There are several methodological limitations to consider in our study. Firstly, we are limited by our use of the social responsiveness scale as a behavioral measure in our seed-based imaging analyses and mediation model. In our study, social responsivity was used as a proxy for mentalizing. Investigating a mediation model of anxiety via social responsivity is not the same as investigating mentalizing behavior itself. The Social Responsiveness Scale (SRS-2) is a general measure of autistic traits associated with social deficits, including social awareness, cognition, communication, restricted repetitive behaviors, and motivation (Constantino et al., 2005). These are subcomponents associated with mentalizing and theory of mind deficits in autism but are not equivalent to mentalization-specific assessments such as the Reflective Functioning Questionnaire (RFQ; Fonagy et al., 2016) or Mentalization Breakdown Interview (MBI; Ulvestad et al., 2023). Therefore, some components of mentalizing may not be captured by the SRS-2. Additionally, the SRS-2, a much broader measure of social impairment, may also capture components of behavior not affected by mentalizing. Furthermore, the SRS-2 is not equivalent to a task-based measure, or an objective rater’s evaluation of mentalizing behaviors. Future studies should include mentalizing questionnaires by both parent and child and independent ratings by clinicians.

Secondly, we are limited by our use of a seed-based neuroimaging approach when investigating functional connectivity between brain networks. Seed-based approaches require the a-priori selection of regions of interest. Given the unique clinical characteristics of our sample (ASD with comorbid anxiety), it may have been better not to have had a-priori assumptions based on prior literature, as doing so may have biased our findings towards regions associated with SN–DMN connectivity. Our seed ROIs in the aINS, ACC and MPFC, and PCC represent only four anatomical regions that are typically considered part of the salience and default mode networks (Raichle, 2015). Studies show that functional connectivity of the SN and DMN with the motor cortex (Masuda et al., 2019), thalamus (Pagni et al., 2023) and amygdala (Ibrahim et al., 2019; Ibrahim et al., 2022) are also associated with social responsivity deficits and anxiety in ASD. These regions of interest were not included in our analyses. However, they may influence anxiety and social responsivity via their interactions, indirectly affecting the connectivity of salience and default mode network pathways. To address functional connectivity differences associated with anxiety in ASD at a network level, future research can leverage graph-based analytic techniques in conjunction with seed-based connectivity to supplement results by identifying brain regions whose connectivity may be meaningfully related to anxiety and social responsivity differences. In these graph-based network analyses, relationships between brain regions are represented as node and edge topographical graphs that can be investigated at different levels of scale using specific measures to capture connectome attributes at local (region-specific) and global (network-wide) scales (Termenon et al., 2016). These measures can include the strength, efficiency, and centrality of connections that can reveal important theoretical implications for understanding brain networks.

Thirdly, we are limited by our sample demographics. An inclusion criterion for our autism sample included having a verbal IQ high enough (IQ>70) to participate in cognitive behavioral therapy for anxiety. Our findings, therefore, may not generalize to non-verbal participants with ASD. Additionally, our study's sample size was predominantly male, with a 3:1 male-to-female gender ratio. This ratio is consistent with meta-analytic reports of the male-to-female ratios of ASD (Loomes et al., 2017). However, it limits the generalizability and interpretability of our findings for females with ASD. Prior research shows that the presentation of ASD is distinct in males and females; however, the neural mechanisms underlying these differences have not been explored in detail (Alloy et al., 2016; Ordaz et al., 2016). Our posthoc analyses showed gender effects during a clinical group by gender analysis. Group differences in anterior insula connectivity associated with anxiety were only found within our male ASD participants. Furthermore, within the female ASD subgroup, anterior insula connectivity with the TPJ, ACC, and PFC was not associated with anxiety (appendix L). Therefore, based on our results, there is insufficient evidence to suggest that patterns of functional connectivity in the SN and DMN associated with anxiety and social responsivity are identical in both boys and girls with ASD. Due to our uneven male-to-female participant ratio, our study likely has insufficient power to detect differences between males and females with ASD after separation by both gender and clinical groups. Future studies should therefore aim to recruit equal ratios of males to females. Future studies should also aim to investigate anxiety severity and network connectivity in girls from underrepresented populations and minority groups. This can be done by pooling data over multiple sites using publicly available neuroimaging databases such as the Adolescent Brain Cognitive Development Study (Casey et al., 2018) or Autism Brain Imaging Data Exchange (Craddock et al., 2013). However, discussions on the implications of neuroscience research are conducted largely from scholarly expert opinion without directly involving minority communities. Therefore, a consideration for future research is to promote diversity, equity, and inclusion in neuroscience by actively involving minority populations of interest in research via active collaboration with community partners. This can be done by making participation in neuroimaging studies more accessible through initiatives such as Community-Based Participatory Research (Espinosa & Verney, 2021; La Scala et al., 2023). Simultaneously leveraging large data repositories and engaging local communities in research would greatly add to explorations of gender, socio-economic and ethnic effects in salience and default mode network connectivity associated with anxiety in ASD, allowing researchers to identify clinical implications most relevant to their local communities.

## Clinical implications

Our findings suggest that anterior insula and temporo-parietal junction functional connectivity may be a resting-state biomarker associated with anxiety in ASD children. Based on current findings, salience network abnormalities in the processing of self-relevant emotional information suggest that clinical interventions which increase emotional awareness through engaging salience and cognitive control circuity may be beneficial in developing personalized treatments for autistic children with comorbid anxiety. Current clinical interventions which do this include mentalization, and mindfulness-based therapies. Research suggests that improved emotion regulation through increased emotional awareness are primary treatment mechanisms in Mindfulness Based Interventions (MBI) and Mentalization Based Therapy (MBT) (Malberg, 2021; Roemer, Williston and Rollins, 2015). These approaches provide an alternative to CBT in autistic adolescents and adults, as the high-level cognitive strategies taught in CBT can be difficult to deploy during times of high stress or anxiety.

Improvements in functional connectivity in the aINS-TPJ pathway be linked to recovery from anxiety disorders and associated with either improvements in emotional awareness or reductions in cognitive inflexibility. Future studies could use longitudinal data sets comparing treatment effects on SN-DMN functional connectivity associated with cognitive behavioral, mentalizing, or mindfulness-based therapies at baseline and after treatment. Alternatively, Granger causality analyses may be conducted on resting state fMRI data to investigate how network connectivity between the anterior insula and temporo-parietal junction is causally related to anxiety or anxiety recovery (Seth et al., 2015). Salience and default mode network connectivity associated with anxiety may be a predictor of therapy response for ASD children that can be used in the future to support clinical decision-making when selecting treatment options.

From a diagnostic perspective, differences in functional connectivity between key salience (insular) and default mode (temporo-parietal) networks may be distinctive to the development of anxiety symptomology in ASD. Including an ASD-only and an anxiety-only participant, sample would enable future research to identify if anxiety in autism is a distinct nosological category from anxiety without comorbidities (Vasa & Masurek, 2015). Further investigations of the mechanisms underlying the association between SN-DMN functional connectivity, social responsivity, and anxiety challenges in ASD would present a strong justification for modifications of existing therapies for ASD populations experiencing anxiety to focus on building cognitive flexibility and emotional awareness skills through activities such as mindfulness, which engage salience and cognitive control circuity.

A psychoanalytic perspective supports modifications of these therapies for ASD populations. Promoting mindfulness through increasing emotional awareness facilitates the creation of representation-promoting and object-building interpretations within the therapeutic environment (Conner et al., 2019; Silani et al., 2008). By forming interpretations of the transference, the therapist, saturated with anxieties of being, “absorbs” the patient’s unconscious material, disentangling and transforming it. This constitutes the gradual construction of a good internal object through the therapist's presence (Klein, 1984). Translating psychoanalytic interpretations of autistic transference using modern frameworks of Bayesian neuroscience and salience network activity may offer clinicians to an opportunity to refine the therapeutically active components of mindfulness and mentalization-based therapies and adapt them for use in neurodivergent populations (Holmes & Nolte, 2019). Therefore, future studies that combine qualitative and quantitative methods to investigate how changes in the salience network connectivity are associated with the development and transformation of object relations in the therapeutic setting (i.e., through longitudinal treatment change studies) may also provide a valuable addition to both clinical neuroscience and psychoanalytic literature.

## Concluding statement

Salience and default mode network connectivity may play an essential role in the pathology of social and emotional awareness deficits experienced by individuals with anxiety and ASD. The current study highlights that the anterior insula is an essential hub in the salience network associated with cognitive inflexibility. Insular connections with key nodes in the default mode network may also be implicated in deficits in emotional awareness. Insular connectivity with the temporo-parietal junction may be an important biomarker associated with social-emotional challenges in autism with comorbid anxiety. Engaging salience and default mode networks through therapy may be key mechanisms of change that can be leveraged in therapy to help ASD children cope with anxiety by improving emotional awareness and developing cognitive flexibility skills. Future research may also consider using Bayesian neuroscience frameworks to bridge object relations psychoanalysis and contemporary imaging network neuroscience.

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# Appendicies

## Appendix A:

**Table****3.** *Comparsion of ASD cases excluded due to high motion in the final sample*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Low Motion** *(n = 68)* | **High Motion**  *(n =6)* | **T-test for equality of means** | | | **95% Confidence Interval** | |
| *Mean (SD)* | *Mean (SD)* | *t (df = 73)* | *p* | Standard error | Upper | Lower |
| **Age** | 11.3 (1.92) | 10.5 (1.53) | -1.10 | .274 | .800 | -2.46 | 0.71 |
| **IQ** | 98.8 (20.4) | 94.3 (16.4) | -0.43 | .665 | 10.4 | -25.1 | 16.1 |
| **SRS-2 T-score** | 75.0 (8.72) | 71.3 (8.14) | -1.08 | .314 | 3.66 | -11.0 | 4.00 |
| **MASC-2 T-score** | 67.8 (13.0) | 62.5 (19.2) | -0.94 | .535 | 5.61 | -16.4 | 5.86 |
| **CBCL - Externalizing** | 58.0 (9.32) | 54.0 (11.8) | -0.99 | .326 | 3.99 | -11.8 | 3.98 |

## Appendix B:

**Table 4.** *Shapiro-Wilk Tests of Normality for variables of interest*

|  |  |  |
| --- | --- | --- |
| Variable | Shapiro-Wilk Test | |
|  | *W* | *p Value* |
| Full Sample | *(df = 103)* | |
| MASC -2 | .938 | <.001 |
| SRS-2 | .928 | <.001 |
| CBCL-Externalizing | .963 | .005 |
|  |  |  |
| Age | .974 | .037 |
| Gender | .517 | <.001 |
| IQ | .928 | .650 |
| Race | - | - |
| Ethnicity | - | - |

## Appendix C:

**Table 5.** *Skewness and Kurtosis for variables of interest in total and ASD samples*

|  |  |  |
| --- | --- | --- |
| **Variable** | **Normality values** | |
|  | **Skewness (SE)** | **Kurtosis (SE)** |
| **Full Sample** *(n = 103)* |  |  |
| MASC-2 | .154 (.204) | -1.14 (.406) |
| SRS-2 | -.510 (.215) | -.896 (.427) |
| CBCL-Externalizing | -.078 (.209) | -.916 (.414) |
|  |  |  |
| Age | .027 (.199) | -.911 (.396) |
| IQ | .125 (.226) | -.275 (.447) |
| Race | . | . |
| Ethnicity | . | . |
| **ASD Sample** *(n = 74)* |  |  |
| MASC-2 | -.096 (.204) | -1.13 (.406) |
| SRS-2 | -.175 (.215) | -.896 (.427) |
| CBCL-Externalizing | -.078 (.209) | -.916 (.414) |
|  |  |  |
| **HC Sample** *(n = 29)* |  |  |
| MASC-2 | 1.12 (.398) | 1.04 (.778) |
| SRS-2 | 1.43 (.421) | 1.97 (.821) |
| CBCL-Externalizing | .635 (.403) | -.465 (.778) |

## Appendix D:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Model** | **R** | **R2** | **Adjusted R2** | **St. Error** | **Durbin-Watson** |
| **i** | .659**i** | .434 | .416 | 12.4 | 1.98 |
| **ii** | .503**ii** | .253 | .230 | 14.1 | 1.58 |
| **iii** | .473**iii** | .224 | .199 | 14.4 | 1.53 |

**Table 6.** *Durban Watson Tests and model summaries for Mediation models*

1. *Predictors*: Functional Connectivity Contrast of Partial Estimate Means (copemean), Social Responsivity (SRS-2) T-score, Copemean \* SRS-2 interaction. Dependent Variable: Anxiety (MASC-2) T-score
2. *Predictors*: Functional Connectivity Contrast of Partial Estimate Means (copemean), Externalising (EXT) CBCL Subscale, Copemean \* EXT interaction. Dependent Variable: Anxiety (MASC-2) T-score
3. *Predictors*: Functional Connectivity Contrast of Partial Estimate Means (copemean), Attention-Deficity Hyperactivity (ADHD) CBCL Subscale, Copemean\*ADHD interaction. Dependent Variable: Anxiety (MASC-2) T-score

## Appendix E:

**Table 7.** *Multicolinearity Statistics for mediation regression models*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Model** | **Unstandardized B** | **Coefficients St. Error** | **Standardized coefficient Beta** | ***t*** | ***p*** | **Colinearity Statistics** | |
| **Tolerance** | **VIF** |
| **i. Social Responsivity (SRS-2) Mediation Model** | | | | | | | |
| Constant | 18.3 | 5.52 | - | 3.32 | .001 | - | - |
| Contrast of Partial Estimates Mean | -5.76 | 8.18 | -.060 | -.704 | .483 | .810 | 1.24 |
| SRS-2 T-score | .656 | .081 | .636 | 8.10 | <.001 | .954 | 1.05 |
| SRS-2 interaction | -.052 | .101 | -.045 | -.518 | .605 | .783 | 1.28 |
| **ii. Externalising Symptom (EXT) CBCL Subscale Mediation Model** | | | | | | | |
| Constant | 27.9 | 6.66 | - | 4.18 | <.001 | - | - |
| Contrast of Partial Estimates Mean | -11.3 | 8.99 | -.123 | -1.26 | .212 | .809 | 1.24 |
| CBCL Externalizing | .646 | .125 | .465 | 5.17 | <.001 | .963 | 1.04 |
| CBCL Externalizing Interaction | -.065 | .150 | -.043 | -.430 | .668 | .783 | 1.28 |
| **iii. Attention Deficit Hyperactivity (ADHD) CBCL Subscale Mediation Model** | | | | | | | |
| (Constant) | 16.0 | 9.99 | - | 1.596 | .114 | - | - |
| Contrast of Partial Estimates Mean | -9.50 | 9.80 | -.100 | -.969 | .335 | .784 | 1.28 |
| CBCL ADHD | -.042 | .141 | -.032 | -.299 | .766 | .714 | 1.40 |
| CBCL ADHD Interaction | .762 | .166 | .440 | 4.599 | <.001 | .901 | 1.11 |

## Appendix F:

**Figure 7.** *Mediation**Regression Standardized Residual Plots*

**Panel 1**: SRS Mediation Model (Regression Standardized Residuals)

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**Panel 2:** Externalizing Mediation Model (Regression Standardized Residuals)

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**Panel 3:** ADHD Mediation Model (Regression Standardized Residuals)

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## Appendix G:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Table 8.** *Correlation table of Behavioral Variables* *in the Total sample*,*(n = 103)* | | | | | | |
| Characteristic | **1.** | **2.** | **3.** | **4.** | **5.** | **6.** |
| 1. **SRS-2 Total Score** | - |  |  |  |  |  |
| 1. **MASC-2 Total Score** | 0.161 | - |  |  |  |  |
| 1. **CBCL Externalizing Behavior Subscale** | 0.511\* | -0.032 | - |  |  |  |
| 1. **Age** | -0.003 | 0.096 | -0.181 | - |  |  |
| 1. **IQ** | -0.230 | -0.142 | 0.014 | 0.004 | - |  |
| 1. **Motion (Framewise Displacement, FD)** | 0.022 | -0.145 | 0.231 | -0.383\* | -0.258\* | - |
| *\* p < .05* |  |  |  |  |  |  |

## Appendix H:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Table 9.** *Correlation table of Behavioral Variables,* ASD subsample,*(n = 74)* | | | | | | |
| Characteristic | **1.** | **2.** | **3.** | **4.** | **5.** | **6.** |
| 1. **SRS-2 Total Score** | - |  |  |  |  |  |
| 1. **MASC-2 Total Score** | 0.149 | - |  |  |  |  |
| 1. **CBCL Externalizing Behavior Subscale** | 0.499\* | -0.043 | - |  |  |  |
| 1. **Age** | -0.231\* | -0.153 | 0.011 | - |  |  |
| 1. **IQ** | -0.033 | 0.08 | -0.161 | 0.005 | - |  |
| 1. **Motion (Framewise Displacement, FD)** | 0.021 | -0.138 | 0.217 | -0.195 | -0.396\* | - |
| *\* p < .05* |  |  |  |  |  |  |

## Appendix I:

Contrasts of Partial Estimate means for Anterior Insula Seed connectivity with the Temporo-Parietal Junction associated with behavioral variables (SRS and MASC) are presented below. No significant differences in anterior insula seed connectivity were found between medicated vs unmedicated ASD, *(p > .50).*

**Table 10.** *Descriptive statistics and t-test of ASD subsample separated by medication status.*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Medication**  (n = 32) | **No medication**  (n = 42) | **t test for equality of means** | | | **95% Confidence Interval** | |
| Mean(SD) | Mean(SD) | *t* (df = 73) | *p* | Standard error | Upper | Lower |
| **Age** | 12.1 (1.68) | 11.4 (1.82) | 1.50 | .139 | 0.442 | -0.22 | 1.54 |
| **IQ** | 95.9 (18.9) | 102.0 (20.5) | -1.21 | .232 | 5.05 | -16.2 | 4.00 |
| **SRS-2 T-score** | 78.1 (9.21) | 71.6 (8.33) | 3.09 | .003\* | 6.75 | 2.39 | 11.1 |
| **MASC-2 T-score** | 70.9 (14.1) | 67.5 (13.0) | 1.01 | .316 | 3.41 | -3.33 | 10.2 |
| **CBCL - Externalising** | 59.8 (9.80) | 55.4 (8.14) | 1.93 | .059 | 2.23 | -.179 | 8.93 |
| **Scanner Motion**  **(Mean Framewise displacement)** | .171 (.010) | .228 (.295) | -.880 | .382 | .064 | -.185 | .072 |

**Figure 8.** *Violin boxplot of COPE-mean Beta coefficients of anterior insula to temporo-partial junction connectivity associated with social responsivity (SRS-2) total scores.* A picture containing text, diagram, plot, line

Description automatically generated

**Figure 9.** *Violin boxplot of COPE-mean Beta coefficients of anterior insula to temporo-partial junction connectivity associated with anxiety (MASC-2) total scores.*

A picture containing text, diagram, plot, screenshot

Description automatically generated

## Appendix J:

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ***Path*** | ***Effect*** | ***B (SE)*** | ***Std. Beta*** | ***t*** | ***p value*** | ***95% Confidence Interval*** | |
|  |  |  |  |  |  | **Lower** | **Upper** |
| **a** | FC† 🡪 SRS | -21.2 (7.82) | -0.26 | -2.71 | .008\* | -36.7 | -5.67 |
| **b** | SRS 🡪 MASC | 0.66 (0.08) | 0.64 | 8.30 | < .001\* | -19.2 | 6.38 |
| **(Total) c** | FC† 🡪 MASC | -20.3 (8.05) | -0.24 | 2.53 | .001\* | -36.3 | -4.36 |
| **(Direct) c'** | FC† 🡪 MASC | -6.41 (6.44) | -0.08 | -0.99 | 0.32 | -19.2 | 6.38 |
| **(Indirect) ab** | FC† 🡪 SRS 🡪 MASC | -13.9 (5.37) | -0.17 | -2.59 | .005\* | -0.29 | -0.05 |
| †FC (Functional Connectivity)  Note: All coefficients and confidence intervals based on 5000 bootstrap sample | | | | | | | |

**Table 11:** *Regression path coefficients of social responsivity mediating anxiety and SN-DMN connectivity in the total sample.*Mediation model of anterior insula to temporo-parietal junction connectivity on anxiety (MASC) through social responsivity (SRS) within the total sample, *(n = 103).*

**Table 12:** *Regression path coefficients of social responsivity mediating anxiety and SN-DMN connectivity in the ASD subsample.*Mediation model of anterior insula to temporo-parietal junction connectivity on anxiety (MASC) through social responsivity (SRS) within the ASD sample, *(n = 74).*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ***Path*** | ***Effect*** | ***B (SE)*** | ***Std. Beta*** | ***t*** | ***p value*** | ***95% Confidence Interval*** | |
|  |  |  |  |  |  | **Lower** | **Upper** |
| **a** | FC† 🡪 SRS | 0.74 (5.40) | 0.02 | 0.14 | .892 | -10.0 | 11.5 |
| **b** | SRS 🡪 MASC | 0.24 (0.17) | 0.16 | 1.40 | .167 | -0.10 | 0.58 |
| **(Total) c** | FC† 🡪 MASC | -5.44 (7.89) | -0.08 | -0.69 | .490 | -21.2 | 10.3 |
| **(Direct) c'** | FC† 🡪 MASC | -5.27 (7.94) | -0.08 | -0.66 | .510 | -21.1 | 10.6 |
| **(Indirect) ab** | FC† 🡪 SRS 🡪 MASC | 0.18(1.60) | 0.02 | 0.13 | .450 | -0.04 | 0.05 |
| †FC (Functional Connectivity)  *Note*: All coefficients and confidence intervals based on 5000 bootstrap sample | | | | | | | |

## Appendix K:

**Table 13:** *Regression path coefficients of ADHD mediating anxiety and SN-DMN connectivity in the total sample.* Mediation model of anterior insula to temporo-parietal junction connectivity on anxiety (MASC) through CBCL Attention Deficit and Hyperactivity subscale behaviors (ADHD) within the total sample, *(n = 103).*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ***Path*** | ***Effect*** | ***B (SE)*** | ***Std. Beta*** | ***t*** | ***p value*** | ***95% Confidence Interval*** | |
|  |  |  |  |  |  | **Lower** | **Upper** |
| **a** | FC† 🡪 ADHD | -14.1 (4.67) | -0.29 | -3.02 | <.001\* | -23.4 | -4.82 |
| **b** | ADHD 🡪 MASC | 0.75 (0.16) | 0.43 | 4.55 | <.001\* | 0.44 | 1.06 |
| **(Total) c** | FC† 🡪 MASC | -18.0 (8.30) | -0.22 | -2.18 | .032\* | -34.4 | -1.58 |
| **(Direct) c'** | FC† 🡪 MASC | -7.44 (7.87) | -0.09 | -0.95 | .367 | -23.6 | 8.68 |
| **(Indirect) ab** | FC† 🡪 ADHD 🡪 MASC | -10.5 (3.90) | -0.13 | -2.69 | .004\* | -0.22 | -0.04 |
| †FC (Functional Connectivity)  *Note*: All coefficients and confidence intervals based on 5000 bootstrap sample | | | | | | | |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ***Path*** | ***Effect*** | ***B (SE)*** | ***Std. Beta*** | ***t*** | ***p value*** | ***95% Confidence Interval*** | |
|  |  |  |  |  |  | **Lower** | **Upper** |
| **a** | FC† 🡪 ADHD | -5.86 (5.02) | -0.14 | -1.17 | .247 | -15.9 | 4.15 |
| **b** | ADHD 🡪 MASC | 0.04 (0.20) | 0.02 | 0.20 | .849 | -0.35 | 0.43 |
| **(Total) c** | FC† 🡪 MASC | -1.99 (8.10) | -0.03 | -0.25 | .807 | -18.1 | 14.2 |
| **(Direct) c'** | FC† 🡪 MASC | -1.77 (8.23) | 8.23 | -0.21 | .831 | -18.2 | 14.2 |
| **(Indirect) ab** | FC† 🡪 ADHD 🡪 MASC | -0.218 (1.56) | -0.01 | 0.14 | .444 | -0.05 | 0.05 |
| †FC (Functional Connectivity)  *Note*: All coefficients and confidence intervals based on 5000 bootstrap sample | | | | | | | |

**Table 14:** *Regression path coefficients of ADHD mediating anxiety and SN-DMN connectivity in the ASD subsample.* Mediation model of anterior insula to temporo-paretial junction connectivity on anxiety (MASC) through CBCL Attention Deficit and Hyperactivity subscale behaviors (ADHD) within the ASD sample, *(n = 74).*

## **Appendix L:**

Gender effects associated withanterior insula seed functional connectivity.

**Figure 10:** *Decreased functional connectivity between anterior insula and temporo-parietal junction observed only in boys with ASD*. **(A)** Significant clusters of gender by group level differences in functional connectivity in the total sample *(Z = 4.24, p = .035).* Contrasts Of Partial Estimate Means (COPE-means) Beta coefficients were extracted from the highlighted regions. **(B)** Violin Plots of COPE-mean Beta coefficients of anterior-insula to temporal-parietal junction connectivity

|  |
| --- |
| **(A)**  A close-up of a brain  Description automatically generated with low confidence |
| **A picture containing text, diagram, plot, line  Description automatically generated(B)** |

**Table 15:** *Gender specific* *anterior insula seed connectivity peak clusters in total sample*,Categorical Gender \* Group Analyses, *(n = 103)*.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Region** | **Hemisphere** | **BA** | **MNI Coordinates** | | | **z-score max** | ***p*** |
|  |  |  | **x** | **y** | **z** |  |  |
| TPJ | Right | 39 | -58 | -50 | 38 | 4.24 | *.035* |
| TPJ | Right | 39 | -48 | -46 | 30 | 3.78 | *.035* |

**Appendix M**: *Demographics and Medical History Questionnaire*

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