

- Ulijarevic, M., Carrington, S., Leekam, S., 2016. Brief report: effects of sensory sensitivity and intolerance of uncertainty on anxiety in mothers of children with Autism Spectrum Disorder. *J. Autism Dev. Disord.* 46 (1), 315–319.
- Ung, D., Wood, J.J., Ehrenreich-May, J., Arnold, E.B., Fujii, C., Renno, P., et al., 2013. Clinical characteristics of high-functioning youth with autism spectrum disorder and anxiety. *Neuropsychiatry* 3 (2), 147–157.
- Ung, D., Selles, R., Small, B.J., Storch, E.A., 2015. A systematic review and meta-analysis of Cognitive-Behavioral Therapy for anxiety in youth with High-Functioning Autism Spectrum Disorders. *Child Psychiatry Human Dev.* 46 (4), 533–547.
- van Steensel, F.J., Bogels, S.M., Perrin, S., 2011. Anxiety disorders in children and adolescents with autistic spectrum disorders: a meta-analysis. *Clin. Child Family Psychol. Rev.* 14 (3), 302–317.
- van Steensel, F.J.A., Dirksen, C.D., Bogels, S.M., 2014. Cost-effectiveness of cognitive-behavioral therapy versus treatment as usual for anxiety disorders in children with autism spectrum disorder. *Res. Autism Spectrum Disord.* 8 (2), 127–137.
- Vasa, R.A., Mazurek, M.O., 2015. An update on anxiety in youth with autism spectrum disorders. *Curr. Opin. Psychiatry* 28 (2), 83–90.
- Vasa, R.A., Carroll, L.M., Nozollillo, A.A., Mahajan, R., Mazurek, M.O., Bennett, A.E., et al., 2014. A systematic review of treatments for anxiety in youth with autism spectrum disorders. *J. Autism Dev. Disord.* 44 (12), 3215–3229.
- Vasa, R.A., Mazurek, M.O., Mahajan, R., Bennett, A.E., Bernal, M.P., Nozollillo, A.A., et al., 2016. Assessment and treatment of anxiety in youth with Autism Spectrum Disorders. *Pediatrics* 137 (2).
- White, S.W., Oswald, D., Ollendick, T., Schill, L., 2009. Anxiety in children and adolescents with autism spectrum disorders. *Clin. Psychol. Rev.* 29 (3), 216–229.
- White, S.W., Bray, B.C., Ollendick, T.H., 2012. Examining shared and unique aspects of Social Anxiety Disorder and Autism Spectrum Disorder using factor analysis. *J. Autism Dev. Disord.* 42 (5), 874–884.
- White, S.W., Lerner, M.D., McLeod, B.D., Wood, J.J., Ginsburg, G.S., Kerns, C., et al., 2015. Anxiety in youth with and without Autism Spectrum Disorder: examination of factorial equivalence. *Behav. Therapy* 46 (1), 40–53.
- Wood, J.J., Gadow, K.D., 2010. Exploring the nature and function of anxiety in youth with Autism Spectrum Disorders. *Clin. Psychol. Sci. Practice* 17 (4), 281–292.
- Zainal, H., Magrati, I., Tan, J.W.L., Sung, M., Fung, D.S.S., Howlin, P., 2014. A preliminary investigation of the Spence Children's Anxiety Parent Scale as a screening tool for anxiety in young people with Autism Spectrum Disorders. *J. Autism Dev. Disord.* 44 (8), 1982–1994.

CHAPTER 4

Neurobiological Mechanisms of Anxiety in ASD

John D. Herrington^{1,2}, Valentina Parma³ and Judith S. Miller^{1,2}

¹The Children's Hospital of Philadelphia, Philadelphia, PA, United States

²University of Pennsylvania, Philadelphia, PA, United States

³Scuola Internazionale Superiore di Studi Avanzati, Trieste, Italy

INTRODUCTION

The majority of the existing literature on the cognitive neuroscience of ASD has focused on key diagnostic features—namely, social function, communication, and the systems that support these (e.g., visual perception and language). As a result, one might presume that we know very little about emotion systems in ASD, as affective processes do not figure prominently in the ASD diagnostic criteria. Then again, a strong case could be made that much of the existing literature on social information processing in ASD directly informs our understanding of affective processes in ASD, should we choose to view it that way. Indeed, the line between what is “social” and what is “emotional” is thin at best. This is clearly evident when one examines the kind of methodologies in widespread use to examine putative social information processes in ASD. For example, numerous experiments have been conducted in which an individual with ASD is asked to identify the emotion portrayed by an image of a face. What does it mean that most of these studies have interpreted the results in terms of social information processing and Theory of Mind, rather than affective processes? This choice of interpretation will often come as a surprise to an affective neuroscientist studying anxiety, who may conduct the identical experiment in a sample without ASD, without making any reference to Theory of Mind or social information processes. The bottom line is that most meaningful social interaction is also emotional. It is therefore probable that findings of different patterns of “social brain” activity in ASD represent important clues as to why difficulties with emotion processes are so common. This chapter will review evidence from central and peripheral nervous system research on why

anxiety may occur so frequently in the context of ASD. First, we will start with a review of amygdala involvement in anxiety and social information processes in ASD, covering prominent theories of function, structure, and integration between the two. In addition to being perhaps the most widely studied brain region in the cognitive neuroscience of both ASD and anxiety disorders, emerging evidence indicates that it may be the most important place to look when trying to understand their comorbidity. Second, we will review amygdala within the context of other brain structures – namely prefrontal cortex – as evidence implicates differences in amygdala/prefrontal cortex connectivity in both ASD and anxiety. Lastly, we will review the relationship between amygdala function and autonomic nervous system findings (electrodermal and cardiac autonomic activity). For a variety of reasons, the autonomic nervous system may prove more amenable to the large-scale research required to address the co-occurrence of anxiety in ASD.

THE RELATIONSHIP BETWEEN AMYGDALA, ANXIETY, AND ASD

Amygdala function. The most salient neurobiological account of the high rates of anxiety in ASD has to do with amygdala. This brain structure is among the most studied in all of human cognitive neuroscience; most of the content that follows is elaborated upon in much more detail in one of many review books and chapters (for example, see Whalen and Phelps, 2009). Amygdala has been implicated in a variety of processes related to emotional and social function. It is of great interest to our understanding of the human brain because it appears to function across a wide swath of human life—from the engagement of pervasive instincts and reactions (“fight or flight”) to the interpretation of the most subtle of social cues.

Ongoing, advanced theoretical work continues to shape our understanding of the role of amygdala in humans and non-humans (for reviews see Amaral et al., 2003a; Davidson, 2002; Phelps and LeDoux, 2005; Whalen, 1998). We find, however, that the literature on human amygdala function can be coarsely divided into two overlapping but somewhat distinct perspectives. The first perspective focuses on the role of amygdala in the “surveillance” of the environment for emotional and socially relevant

focuses on the role of amygdala in the experience and encoding of emotion (henceforth, called the emotion learning perspective).

The surveillance perspective. Amygdala is viewed as critical in coordinating biological systems to orient toward, process, and respond to significant external events. Much of the evidence in favor of this perspective comes from studies indicating that amygdala can be especially sensitive to degraded or peripheral socio-emotional information (e.g., detecting a “snake in the grass”; Whalen, 2007). Although controversial (see Pessoa and Adolphs, 2010), multiple lines of research indicate that amygdala receives some input from the external environment ahead of low-level perceptual structures (particularly those in visual cortex), suggesting that it has a privileged role in processing social and affective information (Morris et al., 1999; Pasley et al., 2004; Troiani et al., 2012). The surveillance perspective on amygdala function readily encompasses emotional as well as social information processes.

Most of the existing research on amygdala function in ASD is rooted in the surveillance perspective. These studies typically share a unidirectional perspective on amygdala activity in ASD—that it is underactive in situations where individuals rely on it to process social and emotional information (for reviews see Baron-Cohen et al., 2000; Herrington and Schultz, 2010; Pelpfrey et al., 2011). The underactive amygdala narrative is probably the most ubiquitous of all narratives in the cognitive neuroscience literature on ASD.

The emotion learning perspective. A robust and longstanding literature suggests that amygdala plays a critical role in fear learning across species (Davis, 1992; Kalin et al., 2004; Wilensky et al., 2006). Amygdala has been shown to coordinate with prefrontal cortex during the encoding and experience of emotion-based learning, and to coordinate with memory structures (i.e. hippocampus) in the consolidation and retention of that learning (for example Shaw et al., 2005). Although most of this literature focuses on negatively valenced emotions (i.e., fear), the human amygdala also appears to play a role in positive emotions as well (Hamann et al., 2002; Herrington et al., 2010). Not surprisingly, increased amygdala activity is one of the most widely agreed upon theories of the neurobiology of anxiety disorders—whereby the increased amygdala activity leads to elevated levels of worry and fear, and diminished habituation to feared stimuli and events (in the absence of sufficient coordination with other regulatory structures; Davis, 1992; Pine et al.,

Of course, individuals with ASD have higher rates of anxiety than the general population (Gadow, Devincent et al., 2005; Lecavalier, 2006; Sukhodolsky et al., 2008). Thus, we are faced with reconciling the fact that ASD is associated with *underactivity* of the amygdala, but that anxiety is associated with *overactivity*. We contend that the single most pressing issue in the cognitive neuroscience of anxiety in ASD concerns how we integrate these two empirical findings on amygdala function, and the two broader perspectives from which they are drawn.

To illustrate from our own data: in a large child sample ($N = 81$ ASD and 67 typically developing controls, or TDC; mean age = 12.5 years for both groups), symptoms of anxiety and ASD are correlated with amygdala function in opposite directions (Herrington, Miller, Pandey, and Schultz, 2016). Participants in this study completed a face identity task that elicits robust amygdala activity in both ASD and non-ASD samples. The parents of all participants completed the Social Communication Questionnaire (SCQ, a measure of core ASD symptoms; Chandler et al., 2007) and the parent-report version of the Screen for Child Anxiety Related Disorders (SCARED; Birmaher et al., 1997). The right panel of Fig. 4.1 shows the correlations between each of these two questionnaire measures

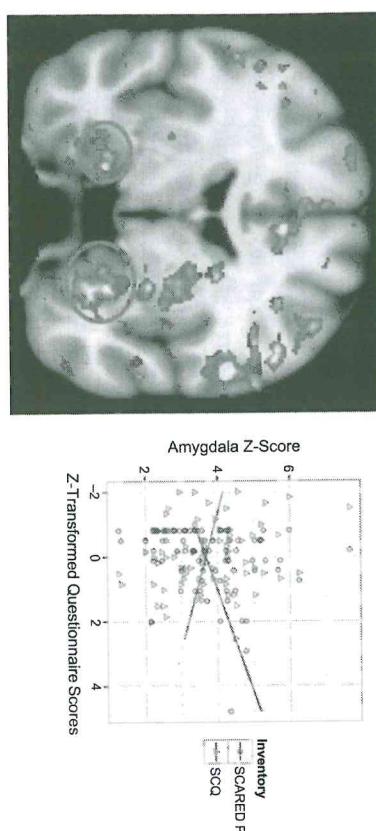


Figure 4.1 Anxiety and core ASD symptoms correlate with amygdala activity in opposite directions. Left panel: increased bilateral amygdala activity (circled) in control children ($N = 67$) compared to children with ASD matched for anxiety symptoms (i.e., low anxiety levels; $N = 24$). Right panel: correlations between right amygdala activity, the Panic/Somatic subscale of the Screen for Child Anxiety Related Emotional Disorders (SCARED; Birmaher et al., 1997), and the Social Communication Questionnaire (SCQ; Chandler et al., 2007). Questionnaire scores were z-transformed for illustration because the two inventories have very different ranges.

and peak activity within left amygdala in the ASD group (the questionnaires have different ranges and are therefore plotted as z-scores, using the ASD group mean and standard deviation for each). Although data from the Panic/Somatic subdomain of the SCARED are depicted in Fig. 4.1, the findings are nearly identical for the Separation Anxiety subscale and overall scale as well (relationships between amygdala activation and both social and generalized anxiety were less strong). Fig. 4.1 left panel illustrates significantly increased amygdala activity in the TDC group relative to individuals in the bottom quartile of SCARED scores (i.e., matched with the TDC group in anxiety). When comparing the TDC group to the other ASD quartiles, no significant difference was found within bilateral amygdala. These data point to two conclusions. First, social deficits and anxiety symptoms are associated with amygdala activity in opposite directions. Second, the longstanding findings of decreased amygdala activity only hold for those individuals with ASD and low levels of anxiety.

These data raise the question: how do we reconcile the association between *decreased* amygdala activation and social deficits in ASD with findings of *increased* amygdala activation and anxiety in ASD? And, by extension, what do the seminal findings of decreased amygdala activity as a putative biomarker of ASD have to say to the alarmingly high rates of anxiety symptoms in this population? Sadly, there is a striking lack of data available to address these questions. At the time this chapter was written, we knew of two functional imaging studies other than our own that formally integrated data on anxiety symptoms, with mixed findings.

Whereas Weng et al. (2011) reported no relationship between amygdala activity and anxiety scores on the Spence Child Anxiety Scale (Spence, 1998), Kleinhans et al. (2010) reported a significant correlation between social anxiety and amygdala activity (measured via the Social Avoidance and Distress Scale). Clearly these results are in need of replication, ideally using a variety of dimensional and categorical measures of anxiety in ASD in large samples of children with ASD.

Amygdala structure. In addition to studies of amygdala function, research on amygdala structure in ASD is also likely to play a role in our understanding of the co-occurrence of anxiety in ASD. This brief review focuses primarily on lesion studies in non-human primates and neuroimaging studies of morphometry (for a review of cytoarchitectural and post-mortem studies, see Schumann and Nordahl, 2011).

Lesion studies relate to brain structure and function; we have

The non-human primate lesion work of David Amaral, Margaret Baumann, and Cindy Schumann provides a vivid representation of the relationship between amygdala structure and anxiety in ASD. Although nearly a century of amygdala lesion studies in non-human primates have been cited as evidence of amygdala-mediated social deficits in ASD, a series of experiments conducted by Amaral et al. cast these studies in a new light (Amaral, 2002; Amaral et al., 2003b; Bauman et al., 2004a,b). Their experiments involved rhesus monkeys that were monitored in a seminaturalistic environment (a field cage permitting communication between conspecifics). They found that highly selective amygdala lesions did not appear to diminish the nature or quality of social interaction in adult monkeys. In fact, they reported that social behavior actually increased (due to the absence of typical social inhibition processes implemented by amygdala). In subsequent studies, they examined the effect of amygdala lesions earlier in development; they found that monkeys with amygdala lesions who were reared by their mothers demonstrated typical primate social behavior across a variety of metrics (i.e., postures, vocalizations, facial expression, etc.). Furthermore, their interest in conspecifics did not appear diminished (i.e., intact social motivation; see Chevallier et al., 2012).

Conversely, monkeys with amygdala lesions demonstrated behaviors consistent with abnormal fear processes. These behaviors include the inconsistent and inaccurate evaluation of threats in the environment, and hyperresponsivity to these threats. These and other results led the authors to the striking conclusion that the relationship between amygdala and social deficits in ASD may in fact be mediated by anxiety (Amaral et al., 2003b). The relationship between these findings and human imaging studies of amygdala structure are presently unclear. Findings on amygdala structure in ASD have been notoriously ambiguous, with multiple studies showing decreased volume (Aylward et al., 1999; Nacewicz et al., 2006; Pierce et al., 2001), increased volume (Howard et al., 2000; Mosconi et al., 2009; Munson et al., 2006), and null results (Haznedar et al., 2000). As pointed out by Schumann et al. (2010, 2011), discrepancies between findings are driven by multiple factors, among which two appear critical. First, it is likely that differences in amygdala structure are not specific to ASD, and may therefore relate to only part of the ASD phenotype (for instance, they have been associated with mood and anxiety disorders; Milham et al., 2005; J. C. Pfeifer et al., 2008). Second, amygdala growth

than cross-sectional differences in volume. In one of the only longitudinal studies of amygdala volume in ASD, Schumann et al. reported that individuals with ASD showed amygdala overgrowth earlier in development, but a later deceleration in this growth, yielding a null volume difference between ASD and control participants at adolescence (Schumann et al., 2004). The implication is that the observation of amygdala volume differences in ASD may turn critically on the age of the cohort under consideration.

Given the compelling findings from the non-human primate research connecting anxiety to amygdala function, it is surprising that there are no published studies formally testing the relationship between amygdala volume and anxiety disorders in ASD (though Juraneck et al., 2006, identified a positive correlation between amygdala volume and the anxious/depressed subscale of the Child Behavior Checklist). We recently completed data collection among 63 children with ASD and 37 non-anxious TDC children (mean age = 12 years) who underwent extensive psychodiagnostic assessment for anxiety disorders (including the Anxiety Disorder Interview Schedule developed by Silverman et al., 2001, and the ASD-specific addendum developed by Kerns et al., 2014). As predicted by Schumann et al., we did not see a significant difference between ASD and TDC groups in amygdala volume as a whole at this age. However, volume differences emerged when the co-occurrence of anxiety disorders were factored into the analysis. When breaking down the ASD group into children with and without a co-occurring anxiety disorder, we found a significant decrease in right amygdala volume for ASD children with anxiety relative to the TDC group. This result raises the intriguing possibility that previous studies examining the relationship between amygdala volume and the core symptoms of ASD missed out on an extremely important piece of information—the presence or absence of co-occurring anxiety.

Amygdala: Integration and future research. It is often difficult to integrate findings on brain function and structure, as they do not always show a reciprocal relationship. Structural differences may manifest clearly in signals from functional imaging, and differences in functional activation magnitude may be closely related or entirely unrelated to structural differences (at least at the scale where we can observe them with modern neuroimaging). Furthermore, functional and structural imaging do not always have analogous relationships with psychological variables. Nevertheless, the available data relating differences in amygdala function and structure

In reviewing these data, we are drawn to two lines of inquiry as future directions. The first concerns a deeper understanding of possible differential roles of amygdala subregions. Although frequently overlooked in human imaging research, a compelling case can be made that amygdala is not actually a single structure, but rather, a constellation of nuclei with putatively diverse functions (Swanson and Petrovich, 1998). Functional heterogeneity within amygdala has long been a focus of preclinical work; but, due to multiple limitations of MRI technology and methodology, it is extremely difficult to examine non-invasively in humans. There are nevertheless emerging theories regarding functional heterogeneity that could have a major impact on how we understand the neurobiology of anxiety in ASD. For example, recent data and methodological work by Bickart et al. (Bickart et al., 2014; Bickart et al., Hollenbeck et al., 2012) indicate that amygdala may be functionally subdivided into regions implementing socio-emotional perception, social approach/affiliation, and aversion/fear. Data such as these point to the hypothesis that the etiology of ASD involves differences in amygdala structure and function, and whether those differences relate to symptoms of anxiety as well as social deficits turns critically on the portions of amygdala affected in any given individual (and/or the connections between distinct portions of amygdala and other cortical structures).

A second important line of inquiry is more theoretical in nature. There is a pressing need to arrive at a clear understanding of precisely how amygdala simultaneously supports what might be categorized as approach (prosocial, affiliative) and avoidance (fear, aversion) tendencies in humans. There is a reason that the review above refers to two “perspectives” on amygdala function and not, say, two “theories.” It is improbable that these surveillance and emotion learning perspectives are genuinely distinct (to take one straightforward example, humans clearly leverage fear learning in the context of social information processing). But, when examining why anxiety occurs so often in ASD, how we resolve this distinction matters. To frame the debate in constructs that will be familiar to both ASD and anxiety disorder researchers: we need a better understanding of how this structure plays a simultaneous role in social motivation, social understanding, and in the experience of emotion (particularly negative emotion).

In reviewing these data, we are drawn to two lines of inquiry as future directions. The first concerns a deeper understanding of possible differential roles of amygdala subregions. Although frequently overlooked in human imaging research, a compelling case can be made that amygdala is not actually a single structure, but rather, a constellation of nuclei with putatively diverse functions (Swanson and Petrovich, 1998). Functional heterogeneity within amygdala has long been a focus of preclinical work; but, due to multiple limitations of MRI technology and methodology, it is extremely difficult to examine non-invasively in humans. There are nevertheless emerging theories regarding functional heterogeneity that could have a major impact on how we understand the neurobiology of anxiety in ASD. For example, recent data and methodological work by Bickart et al. (Bickart et al., 2014; Bickart et al., Hollenbeck et al., 2012) indicate that amygdala may be functionally subdivided into regions implementing socio-emotional perception, social approach/affiliation, and aversion/fear. Data such as these point to the hypothesis that the etiology of ASD involves differences in amygdala structure and function, and whether those differences relate to symptoms of anxiety as well as social deficits turns critically on the portions of amygdala affected in any given individual (and/or the connections between distinct portions of amygdala and other cortical structures).

A second important line of inquiry is more theoretical in nature. There is a pressing need to arrive at a clear understanding of precisely how amygdala simultaneously supports what might be categorized as approach (prosocial, affiliative) and avoidance (fear, aversion) tendencies in humans. There is a reason that the review above refers to two “perspectives” on amygdala function and not, say, two “theories.” It is improbable that these surveillance and emotion learning perspectives are genuinely distinct (to take one straightforward example, humans clearly leverage fear learning in the context of social information processing). But, when examining why anxiety occurs so often in ASD, how we resolve this distinction matters. To frame the debate in constructs that will be familiar to both ASD and anxiety disorder researchers: we need a better understanding of how this structure plays a simultaneous role in social motivation, social understanding, and in the experience of emotion (particularly negative emotion).

systems that may prove very important in our understanding of anxiety in ASD. These areas include portions of prefrontal cortex (PFC) that have long been implicated in affect and the regulation of affect. The identification of different roles for these structures is an active area of research (Blackford and Pine, 2012; Engel et al., 2007; Mohanty et al., 2007). Here we highlight some of these structures and how they relate to the etiology of ASD, and of anxiety within ASD.

Ventral PFC (i.e., the bottom portion of the frontal lobes) shares (primarily inhibitory) connections with amygdala and adjacent medial temporal cortex. This area can be further subdivided into ventrolateral and ventromedial PFC (vIPFC and vmPFC), with the latter corresponding to subgenual anterior cingulate cortex. vmPFC has been shown to coordinate with amygdala during fear learning, and appears to play a particular role in fear extinction (Delgado et al., 2008; Milad et al., 2007). Aberrant vmPFC activation has been observed in studies of multiple mood and anxiety disorders (for review see Davidson, 2002; Myers-Schulz and Koenigs, 2012).

Existing evidence indicates that vIPFC has a somewhat distinct role in emotion processes vis-à-vis vmPFC. vIPFC has been implicated in attentional biases toward emotional information (Britton et al., 2012; Waters et al., 2010). Anxiety disorders have long been associated with increased attentional capture by affective stimuli; this process is in fact interpreted as part of the etiology of anxiety (Bar-Haim et al., 2007; Heeren et al., 2011).

Support is accumulating for differences in attentional bias patterns toward and away from social and emotional information in ASD (Chawarska et al., 2010; Chevallier et al., in press; Isomura, Ogawa, Yamada et al., 2014). We nevertheless have very little data to speak to whether these effects are moderated by anxiety, as would be predicted by the anxiety disorder attentional bias literature. This is unfortunate, because attentional bias paradigms hold promise as potential non-verbal assessments of anxiety in ASD. However, there are now two studies suggesting that measures of attentional bias may *not* in fact be sensitive to anxiety symptoms in ASD (Hollocks et al., 2013; May et al., 2015). The question of whether anxiety-related attentional bias mechanisms are indeed different in ASD and non-ASD populations (and not a result of experimental confounds) is a pressing one, as it may help us determine the extent to which anxiety in ASD is a distinct phenomenon (Isomura et al., 2014).

AMYGDALA, PREFRONTAL CORTEX, AND BRAIN CONNECTIVITY IN ASD

in ASD, and are these related to anxiety? At present, the available data speaking to either question are sparse. More dorsal portions of PFC have been much more carefully studied in ASD, as these are implicated in Theory of Mind processes (see Herrington and Schultz, 2010). Watanabe et al. (2012) reported decreased vmPFC activity in ASD during the perception of social stimuli in ASD. Conversely, Dalton et al. (2005) reported increased ventral PFC activation in ASD during a face processing task.

Examinations of ventral PFC/amygdala connectivity may prove more valuable in understanding anxiety in ASD than studies of ventral PFC alone. A recurring theme in the developmental neuroimaging literature is the role of these areas in regulating amygdala function in the context of social and emotional processes. For example, it appears likely that the development of enhanced cognitive control and emotion regulation in adolescence is accompanied by enhanced regulation of amygdala by these structures (Giedd and Rapoport, 2010; though see J.H. Pfeifer and Allen, 2012, for counterarguments to this perspective). In this context, emotion regulation emerges from a balance between amygdala activity (which may in fact be “bottom up”) and PFC regulatory processes (which would be more “top down”). We and others often refer to this informally as the “balance model” of amygdala/PFC connectivity. Abnormalities in the development of this reciprocal relationship may in fact represent trans-diagnostic biomarkers of psychopathology, encompassing ASD, mood and anxiety disorders, attention deficit-hyperactivity disorder, and others (Arnsten and Rubia, 2012; Heatherton and Wagner, 2011; Passarotti et al., 2010; Pine et al., 2008).

A case can be made that the balance model has particular explanatory power in ASD, and in the context of co-occurring anxiety in ASD. This case is based on emerging findings of differences in both short and long-range brain connectivity in ASD (for review see Minshew and Williams, 2007). Although these findings have been reported among neuronal cell bodies and their connecting fibers (i.e., gray and white matter), some evidence exists to indicate that white matter may be particularly vulnerable (Barnea-Goraly et al., 2004; Cheon et al., 2011; Ke et al., 2009). These findings point to the largely untested hypothesis that individuals with ASD and co-occurring anxiety in ASD have differences in fibers that connect amygdala and adjacent temporal lobe areas to prefrontal cortex (white matter tracts of particular interest include the arcuate and uncinate fasciculus and the cingulum). Again, we may be presented with a situation where a

THE BRAIN IN CONTEXT: THE AUTONOMIC NERVOUS SYSTEM IN ANXIETY AND ASD

Brain imaging research promises to play an important role in our emerging understanding of the etiology of anxiety in ASD. However, there are limitations to what we can learn from brain imaging. The separation of the inherently overlapping symptom profiles of anxiety and ASD may require rather large numbers of well-characterized research participants to accomplish—a problem, given the difficulty and expense of both characterization and imaging. Furthermore, brain imaging studies are generally skewed toward more mildly affected individuals due to the inherent difficulty of the recording procedures (staying very still for an extended period of time inside an MRI machine). As a result, brain imaging research often runs the risk of yielding results that do not generalize well across the entire autism spectrum.

There are of course many ways to examine the neurobiology of anxiety in ASD that do not rely on imaging. Here we discuss the growing field of research on the autonomic nervous system (ANS) in ASD, for several reasons. ANS is considered a primary behavioral regulation system (Porges, 2001), controlling multiple organs and glands throughout the body via complex afferent–efferent pathways. There are multiple ANS manifestations of anxiety, some of which relate to amygdala function (Porges, 2001; Thayer et al., 2012). Given the complexity of the afferent/efferent interactions, dysfunction of the ANS is hypothesized as a possible etiological factor in social information processing differences in ASD. Fortunately, ANS measurement is tractable across the whole spectrum, irrespective of developmental and functional levels, given the relative ease of recording procedures (for this and other reasons, it also has significant appeal as a marker of treatment outcome). Last, but not least, recent technological advancements permit the recording

Although there are now a fair number of studies on ANS function in ASD (reviewed briefly here), only a handful of them formally consider the co-occurrence of anxiety.

The ANS can be divided in two main branches: the sympathetic nervous system (SNS, mediating “fight or flight” behavior, resulting in increased heart rate and hyperarousal), and the parasympathetic nervous system (PNS, responsible for “rest-and-digest” responses, characterized by bradycardia and lower arousal). Recent studies support ANS dysregulation in the majority of individuals with ASD, as evidenced by patterns of sympathetic hyperarousal and blunted parasympathetic vagal tone (Klusek et al., 2015; Patriquin et al., 2011). However, findings are still mixed, as revealed by the analysis of SNS and PNS indices such as electrodermal activity (EDA) and cardiac activity, respectively.

Electrodermal activity, anxiety, and ASD

Research on EDA (sometimes referred to as skin conductance, or the older term galvanic skin response) in ASD has increased in recent years. Greater EDA has long been associated with attentional orienting as well as increased anxiety (Dawson et al., 2007). Indeed, hyperarousal—an elevated state of physiological activation—is a defining feature of anxiety.

There is considerable variability in reports of tonic baseline measures of EDA among children with ASD (Hirstein et al., 2001; Joseph et al., 2008; McCormick et al., 2014; Schoen, Miller et al., 2009; van Engeland, 1984). Similarly mixed results are also found when analyzing phasic EDA to different sensory stimuli (Schoen et al., 2009; van Engeland, 1984). There is general agreement that this variability is driven in no small part by variability across samples (e.g., age, symptom severity), experimental parameters, and recording procedures (Hirstein et al., 2001; McCormick et al., 2014). However, this variability is also very likely related to clinically relevant variables with known relationships to EDA—namely, anxiety and arousal.

We recently tested this hypothesis among a group of 75 children with and without ASD and a comorbid anxiety diagnosis (i.e., a 2 X 2 design with presence/absence of ASD and anxiety diagnoses as factors; Parma et al., in preparation). We found that ASD and anxiety contributed uniquely to tonic EDA; whereas anxiety is associated with hyperarousal (increased EDA) in the typically developing group, the opposite pattern was evident when studying participants with ASD and anxiety (however, it should be noted that the difference in tonic EDA did not differ

about ANS function in ASD and anxiety, phasic, time-locked changes in EDA provide additional information regarding how individuals respond to social and emotional stimuli in the environment. The small literature on phasic EDA in response to emotion and social processes do not yet provide a clear pattern. In a study of direct vs. averted gaze identification, ASD showed larger EDA responses while looking at faces (Joseph et al., 2008), suggesting that emotional hyperarousal in ASD is present and may interfere with face processing (also see Dalton et al., 2005). However, opposite results (reduced EDA responses) have been reported when adults with ASD were asked to judge facial emotions (Hubert et al., 2009). Again, the relative role of confounding and clinical relevant variables has yet to be fully explored.

Cardiac activity, anxiety, and ASD

Classical conditioning paradigms measuring EDA provide valuable insight into amygdala-dependent socio-emotional arousal. In the conditioning context, EDA reflects the dynamics of learning to discriminate between threatening and safe stimuli (the greater the EDA, the greater the learning). South et al. (2011) demonstrated the increment in learning by those participants in the ASD group who showed the greater the SCR during conditioning. Interestingly, greater EDA corresponded also to greater social deficits and the most reduced social functioning.

A reliable assessment of PNS function can be obtained from the analysis of cardiac activity. The application of spectral analysis to heart rate yields a metric called heart rate variability (HRV), which refers to the variation in the duration of time intervals between heartbeats. Heart rate and HRV are governed by many components of physiology. One of these is the vagus nerve, a key component of the PNS that interfaces with the brain and the sinoatrial node of the heart (alongside multiple other organs; Berntson et al., 2007). Vagal control over the heart has the effect of increasing HRV (particularly in higher frequency bands, or respiratory sinus arrhythmia [RSA], when the high-frequency component occurs in conjunction with respiratory patterns). Increase HRV indicates a state of physiological calm (Berntson et al., 2007). Conversely, vagal control decreases under conditions of stress, yielding a decrease in HRV (Allen et al., 2014).

Decreased HRV has long been associated with disorders of social and emotional function, and has been shown to track changes in these symptoms in non-ASD samples (Bär et al., 2004; Porges et al., 1975; Tucker

relationship between HRV and individual differences in social communication and emotion regulation (for review see Porges, 2001). There is now a large body of evidence indicating that increased HRV is associated with increased social engagement, and that decreased HRV is associated with decreased quality and magnitude of social contact (Bal et al., 2010; Guy et al., 2014; Hallett et al., 2013; Neuhaus et al., 2010; Neuhaus et al., 2013). HRV may in fact provide a much-needed index of social functioning in ASD that is not verbally mediated.

Conversely, increased HRV is viewed as directly and indirectly associated with prosocial behavior. The dominant theories within biopsychosocial research on HRV rely on the premise that it is positively associated with an increased propensity for social engagement, an increase in the quality of that engagement, and better emotion regulation skills (see Porges's Polyvagal Theory [Porges, 2001; Porges et al., 2012], Thayer and Lane's Neurovisceral Integration Model [Thayer and Lane, 2000], and Bertson's Model of Autonomic Space [Bertson et al., 2008]). Most recently, it has been proposed that the Autonomic Space Model and Neurovisceral Integration Model together hold explanatory potential in the proposed socio-emotional deficits in ASD and its cortical, subcortical, autonomic and behavioral correlates (Benevides and Lane, 2015).

While HRV is associated with prosocial engagement behaviors among typically developing individuals, until recently there have been few studies of HRV in ASD. A growing number of recent studies consistently report decreased HRV in ASD (Bal et al., 2010; Guy et al., 2014; Hollocks et al., 2014; Neuhaus et al., 2013; Porges et al., 2012). We recently showed a significant decrease in HRV for a modest sample of youth with ASD compared to age- and IQ-matched controls (Guy et al., 2014), and have now replicated and extended this finding in a larger sample (43 ASD, 26 controls; in preparation). These data indicate a large and replicable effect between ASD and controls, regardless of medication status in ASD participants.

In principle, vagal control of HRV represents a compelling account for the co-occurrence of anxiety in ASD. Specifically, differences in PNS function may simultaneously account for the core social symptoms of ASD and symptoms of anxiety that often accompany them. Data related to HRV, anxiety, and ASD are only beginning to emerge. Neuhaus et al. (Neuhaus et al., 2013) reported a relationship between resting state HRV and internalizing symptoms (measured via the Child Behavior Checklist)

relationship between higher levels of anxiety in an ASD sample and decreased HRV subsequent to a psychosocial stressor (the Trier Social Stress Test). With the dramatic increase in availability of heart rate measures, we can expect to see much more research in this area in the future, ideally with large samples that permit the parsing of heterogeneity across multiple symptom domains (i.e., anxiety and social functioning).

CONCLUSIONS AND FUTURE DIRECTIONS

When looking back on the remarkable quantity of ASD cognitive neuroscience research from recent decades, it is hard not to get the sense that there has been a missed opportunity to deepen our understanding of why anxiety is so common in ASD. The missed opportunity stems largely from one cause—anxiety has seldom been considered a relevant dimension in ASD neurobiology research, and has therefore not been measured. In a sense this is quite understandable—the valid and reliable measurement of core ASD symptoms is challenging enough by itself. But we have entered an era where ASD is viewed as multidimensional, and translational research is increasingly prioritized. We seem poised to develop a much more mature understanding of the neurobiology of anxiety in ASD—one that requires the use of multi-method approaches.

Studies examining amygdala, ventral PFC, and their connectivity will likely be at the forefront of research on the neurobiology in ASD. Large-sample studies of well-characterized participants will be critical. But perhaps more important will be the willingness of cognitive neuroscientists to expand their research perspective beyond traditional ASD constructs (such as Theory of Mind), to encompass the rich literature on the neurobiology of affect.

Another critical area for future research relates to developmental progression. Researchers of anxiety in ASD face an ongoing conundrum: to what extent is anxiety part of the etiology of ASD versus a consequence of the symptoms of ASD? Neurobiology could provide critical insights on this question, especially if more research were to be conducted using younger populations followed longitudinally. Brain imaging is challenging in young children, whereas ANS measures are less so. More data are needed on the progression of peripheral biomarkers of anxiety and arousal in toddlers and preschoolers with ASD.

The proliferation of psychophysiological measures will open up new

by allowing for a more nuanced clinical characterization, describing treatment targets, and predicting and measuring responses to intervention. Furthermore, the accessibility and affordability of new technologies allows for the collection of data outside of laboratory settings on an unprecedented scale, thereby increasing our ability to isolate unique and overlapping relationships between psychological dimensions and the neurobiology of ASD. Indeed, biosensors are now often wireless and can stream to devices that are commonplace in many households (like smartphones). However, it is worth noting that many commercially available biosensors do not provide the type, quality, and reliability of data needed to devise anything but the coarsest indices (e.g., beats per minute). Whereas mobile tools for electrocardiography (ECG) may suffer less from movement artifacts, analogous tools for measuring EDA are especially problematic, because the noise introduced by movement is extremely hard to disentangle from the electrodermal signal itself. It is unclear how this particular problem will best be solved. Relatedly, the near absence of empirical, peer-reviewed studies validating mobile heart rate and electrodermal measures against gold-standard equipment is something that needs to be rectified.

And finally, more data are needed involving state manipulations of anxiety in ASD. The development of affective challenge paradigms such as the Laboratory Temperament Assessment Battery (Lab-TAB; Gagne et al., 2011) may provide critical information about the flexibility of affective systems—information that may not be available at rest or during traditional experimental tasks. It will require creativity on the part of cognitive neuroscience to implement these types of paradigms. But the payoff they provide might be very large, allowing us to obtain potentially critical information on the temporal dynamics of anxiety (and emotion regulation more generally) in ASD.

REFERENCES

- Allen, A.P., Kennedy, P.J., Cryan, J.F., Dinan, T.G., Clarke, G., 2014. Biological and psychological markers of stress in humans: focus on the Trier Social Stress Test. *Neurosci Biobehav. Rev.* 38, 94–124, <http://doi.org/10.1016/j.neubiorev.2013.11.005>.
- Amaral, D.G., 2002. The primate amygdala and the neurobiology of social behavior: implications for understanding social anxiety. *Biol. Psychiatry* 51 (1), 11–17.
- Amaral, D.G., Bauman, M., Capitanio, J., Lavenex, P., Mason, W., Mauldin-Jourdain, M., et al., 2003a. The amygdala: Is it an essential component of the neural network for

- Amaral, D.G., Bauman, M.D., Schumann, C.M., 2003b. The amygdala and autism: implications from non-human primate studies. *Genes Brain Behav.* 2 (5), 295–302.
- Arnsten, A.F.T., Rubia, K., 2012. Neurobiological circuits regulating attention, cognitive control, motivation, and emotion: disruptions in neurodevelopmental psychiatric disorders. *J. Am. Acad. Child Adolesc. Psychiatry* 51 (4), 356–367, <http://doi.org/10.1016/j.jaac.2012.01.008>.
- Aylward, E.H., Minshew, N.J., Goldstein, G., Honeycutt, N.A., Augustine, A.M., Yates, K.O., et al., 1999. MRI volumes of amygdala and hippocampus in non-mentrally retarded autistic adolescents and adults. *Neurology* 53 (9), 2145–2150.
- Bachevalier, J., Loveland, K.A., 2006. The orbitofrontal–amygdala circuit and self-regulation of social-emotional behavior in autism. *Neurosci. Biobehav. Rev.* 30 (1), 97–117, <http://doi.org/10.1016/j.neubiorev.2005.07.002>.
- Bal, E., Harden, E., Lamb, D., Van Hecke, A.V., Denver, J.W., Porges, S.W., 2010. Emotion recognition in children with autism spectrum disorders: relations to eye gaze and autonomic state. *J. Autism Dev. Disord.* 40 (3), 358–370, <http://doi.org/10.1007/s10803-009-0884-3>.
- Bär, K.-J., Greiner, W., Jochum, T., Friedrich, M., Wagner, G., Sauer, H., 2004. The influence of major depression and its treatment on heart rate variability and pupillary light reflex parameters. *J. Affect. Disord.* 82 (2), 245–252, <http://doi.org/10.1016/j.jad.2003.12.016>.
- Bar-Haim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, M.J., van IJzendoorn, M.H., 2007. Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study. *Psychol. Bull.* 133 (1), 1–24, <http://doi.org/10.1037/0033-2959.133.1.1>.

- Barnea-Goraly, N., Kwon, H., Menon, V., Eliez, S., Lortspeich, L., Reiss, A.L., 2004. White matter structure in autism: preliminary evidence from diffusion tensor imaging. *Biol. Psychiatry* 55 (3), 323–326.
- Baron-Cohen, S., Ring, H., Bullmore, E., Wheelwright, S., Ashwin, C., Williams, S., 2000. The amygdala theory of autism. *Neurosci. Biobehav. Rev.* 24 (3), 355–364.
- Bauman, M.D., Lavenex, P., Mason, W.A., Capitanio, J.P., Amaral, D.G., 2004a. The development of mother-infant interactions after neonatal amygdala lesions in rhesus monkeys. *J. Neurosci.* 24 (3), 711–721, <http://doi.org/10.1523/JNEUROSCI.3263-03.2004>.
- Bauman, M.D., Lavenex, P., Mason, W.A., Capitanio, J.P., Amaral, D.G., 2004b. The development of social behavior following neonatal amygdala lesions in rhesus monkeys. *J. Cogn. Neurosci.* 16 (8), 1388–1411, <http://doi.org/10.1162/08989290420441>.
- Benevides, T.W., Lane, S.J., 2015. A review of cardiac autonomic measures: considerations for examination of physiological response in children with autism spectrum disorder. *J. Autism Dev. Disord.* 45 (2), 560–575, <http://doi.org/10.1007/s10803-013-1971-z>.
- Bernston, G.G., Quigley, K.S., Lozano, Da, 2007. Cardiovascular Psychophysiology. In: Cacioppo, J.T., Tassinary, L.G., Bernston, G.G. (Eds.), *Handbook of Psychophysiology*, 3rd ed Cambridge University Press, New York, NY, USA, p. 182, -120.
- Bernston, G.G., Norman, G.J., Hawley, L.C., Cacioppo, J.T., 2008. Cardiac autonomic balance versus cardiac regulatory capacity. *Psychophysiology* 45 (4), 643–652, <http://doi.org/10.1111/j.1469-8986.2008.00652.x>.
- Bickart, K.C., Hollenbeck, M.C., Barrett, L.F., Dickerson, B.C., 2012. Intrinsic amygdala cortical functional connectivity predicts social network size in humans. *J. Neurosci.* 32 (42), 14729–14741, <http://doi.org/10.1523/JNEUROSCI.1599-12.2012>.
- Bickart, K.C., Dickerson, B.C., Barrett, L.F., 2014. The amygdala as a hub in brain networks that support social life. *Neuropsychologia* 63, 235–248, <http://doi.org/10.1016/j.neuropsychologia.2014.08.013>.

- Birmaher, B., Khetarpal, S., Brent, D., Cully, M., Balach, L., Kaufman, J., et al., 1997. The Screen for Child Anxiety Related Emotional Disorders (SCARED): scale construction and psychometric characteristics. *J. Am. Acad. Child Adolesc. Psychiatry* 36 (4), 545–553, <http://doi.org/10.1097/0004583-199704000-00018>.
- Blackford, J.U., Pine, D.S., 2012. Neural substrates of childhood anxiety disorders: a review of neuroimaging findings. *Child Adolesc. Psychiatric Clin. N. Am.* 21 (3), 501–525, <http://doi.org/10.1016/j.chc.2012.05.002>.
- Britton, J.C., Bar-Haim, Y., Carver, F.W., Holroyd, T., Norcross, M.A., Detloff, A., et al., 2012. Isolating neural components of threat bias in pediatric anxiety. *J. Child Psychol. Psychiatry Allied Dis.* 53 (6), 678–686, <http://doi.org/10.1111/j.1469-7610.2011.02503.x>.
- Chandler, S., Charman, T., Baird, G., Simonoff, E., Loucas, T., Meldrum, D., et al., 2007. Validation of the Social Communication Questionnaire in a Population Cohort of Children With Autism Spectrum Disorders. *J. Am. Acad. Child Adolesc. Psychiatry* 46 (10), 1324–1332, <http://doi.org/10.1097/chi.0b013e318127d81>.
- Chawarska, K., Volkmar, F., Klin, A., 2010. Limited attentional bias for faces in toddlers with autism spectrum disorders. *Arch. Gen. Psychiatry* 67 (2), 178–185, <http://doi.org/10.1001/archgenpsychiatry.2009.194>.
- Cheon, K.-A., Kim, Y.-S., Oh, S.-H., Park, S.-Y., Yoon, H.-W., Herrington, J., et al., 2011. Involvement of the anterior thalamic radiation in boys with high functioning autism spectrum disorders: a Diffusion Tensor Imaging study. *Brain Res.* 1417, 77–86, <http://doi.org/10.1016/j.brainres.2011.08.020>.
- Chevallier, C., Kohls, G., Troiani, V., Brodkin, E.S., Schultz, R.T., 2012. The social motivation theory of autism. *Trends Cogn. Sci.* 16 (4), 231–239, <http://doi.org/10.1016/j.tics.2012.02.007>.
- Chevallier, C., Parish-Morris, J., McVey, A., Rump, K., Sasson, N.J., Herrington, J.D., et al. (In press). Measuring social attention and Motivation in Autism Spectrum Disorder using eye-tracking: Stimulus type matters. *Autism Research*.
- Dalton, K.M., Nacewicz, B.M., Johnstone, T., Schaefer, H.S., Gernsbacher, M.A., Goldsmith, H.H., et al., 2005. Gaze fixation and the neural circuitry of face processing in autism. *Nat. Neurosci.* 8 (4), 519–526.
- Davidson, R., 2002. Anxiety and affective style: Role of prefrontal cortex and amygdala. *Biol. Psychiatry* 51 (1 diss), 68–80.
- Davis, M., 1992. The role of the amygdala in fear and anxiety. *Annu. Rev. Neurosci.* 15, 353–375, <http://doi.org/10.1146/annurev.ne.15.030192.002033>.
- Dawson, M.E., Schell, A.M., Filion, D.L., 2007. The electrodermal system. In: Cacioppo, John T., Tassinary, Louis G., Bernstein, Gary G. (Eds.), *Handbook of Psychophysiology*, 3rd ed. Cambridge University Press, New York, NY, pp. 159–181, x.
- Delgado, M.R., Nearing, K.I., Ledoux, J.E., Phelps, E.A., 2008. Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. *Neuron* 59 (5), 829–838, <http://doi.org/10.1016/j.neuron.2008.06.029>.
- Engels, A.S., Heller, W., Mohanty, A., Herrington, J.D., Banich, M.T., Webb, A.G., et al., 2007. Specificity of regional brain activity in anxiety types during emotion processing. *Psychophysiology* 44 (3), 352–363, <http://doi.org/10.1111/j.1469-8986.2007.00518.x>.
- Gadow, K.D., Devine, C.J., Pomeroy, J., Azizian, A., 2005. Comparison of DSM-IV symptoms in elementary school-age children with PDD versus clinic and community samples. *Autism* 9 (4), 392–415, <http://doi.org/10.1177/1362361305056079>.
- Gagne, J.R., Van Hulle, C.A., Aksan, N., Essex, M.J., Goldsmith, H.H., 2011. Deriving childhood temperament measures from emotion-eliciting behavioral episodes: Scale construction and initial validation. *Psychol. Assess.* 23 (2), 337–353, <http://doi.org/10.1037/a0021746>.

- Giedd, J.N., Rapoport, J.L., 2010. Structural MRI of pediatric brain development: what have we learned and where are we going? *Neuron* 67 (5), 728–734, <http://doi.org/10.1016/j.neuron.2010.08.040>.
- Guy, L., Souders, M.C., Bradstreet, L.E., DeLussey, C., Herrington, J.D., 2014. Emotion Regulation and Respiratory Sinus Arrhythmia in Autism Spectrum Disorder. *J. Autism Dev. Disord.* 44 (10), 2614–2620, <http://doi.org/10.1007/s10803-014-2124-8>.
- Hallett, V., LeCavalier, L., Sukhodolsky, D.G., Cipriano, N., Aman, M.G., McCracken, J.T., et al., 2013. Exploring the Manifestations of Anxiety in Children with Autism Spectrum Disorders. *J. Autism Dev. Disord.* 43 (10), 2341–2352, <http://doi.org/10.1007/s10803-013-1775-1>.
- Hamann, S., Ely, T., Hoffman, J., Kiltz, C., 2002. Ecstasy and agony: Activation of human amygdala in positive and negative emotion. *Psychol. Sci.* 3 (2), 135–141.
- Haznedar, M.M., Buchsbaum, M.S., Wei, T.C., Hof, P.R., Cartwright, C., Bienstock, C.A., et al., 2000. Limbic circuitry in patients with autism spectrum disorders studied with positron emission tomography and magnetic resonance imaging. *Am. J. Psychiatry* 157 (12), 1994–2001, <http://doi.org/10.1176/appi.ajp.157.12.1994>.
- Heatherton, T.F., Wagner, D.D., 2011. Cognitive neuroscience of self-regulation failure. *Trends Cogn. Sci.* 15 (3), 132–139, <http://doi.org/10.1016/j.tics.2010.12.005>.
- Heeren, A., Peschard, V., Philippot, P., 2011. The causal role of attentional bias for threat cues in social anxiety: a test on a cyber-ostracism task. *Cogn. Therapy Res.* <http://doi.org/10.1007/s10608-011-9394-7>.
- Herrington, J.D., Schultz, R.T., 2010. Neuroimaging of developmental disorders. In: Shenton, M., Turetsky, B.I. (Eds.), *Understanding Neuropsychiatric Disorders: Insights From Neuroimaging*. Cambridge University Press, Cambridge.
- Herrington, J.D., Heller, W., Mohanty, A., Engels, A.S., Banich, M.T., Webb, A.G., et al., 2010. Localization of asymmetric brain function in emotion and depression. *Psychophysiology* 47 (3), 442–454, <http://doi.org/10.1111/j.1469-8986.2009.00958.x>.
- Herrington, J.D., Miller, J., Pandey, J., Schultz, R.T., 2016. Anxiety and social deficits have distinct relationships with amygdala function in autism spectrum disorder. *Soc. Cogn. Affect. Neurosci.* 11 (6), 907–914, <http://doi.org/10.1093/scn/nsw015>.
- Hirshstein, W., Iversen, P., Ramachandran, V.S., 2001. Autonomic responses of autistic children to people and objects. *Proc. Biol. Sci.* 268 (1479), 1883–1888, <http://doi.org/10.1098/rspb.2001.1724>.
- Hollocks, M.J., Ozsvádjan, A., Matthews, C.E., Howlin, P., Simonoff, E., 2013. The relationship between attentional bias and anxiety in children and adolescents with autism spectrum disorders: attentional bias and anxiety in ASD. *Autism Res.* 6 (4), 237–247, <http://doi.org/10.1002/aur.1285>.
- Hollocks, M.J., Howlin, P., Papadopoulos, A.S., Khondoker, M., Simonoff, E., 2014. Differences in HPA-axis and heart rate responsiveness to psychosocial stress in children with autism spectrum disorders with and without co-morbid anxiety. *Psychoneuroendocrinology* 46, 32–45, <http://doi.org/10.1016/j.psyneuen.2014.04.004>.
- Howard, M.A., Cowell, P.E., Boucher, J., Broks, P., Mayes, A., Farrant, A., et al., 2000. Convergent neuroanatomical and behavioural evidence of an amygdala hypothesis of autism. *NeuroReport* 11 (13), 2931–2935.
- Hubert, B.E., Wicker, B., Monfardini, E., Deruelle, C., 2009. Electrodermal reactivity to emotion processing in adults with autistic spectrum disorders. *Autism* 13 (1), 9–19, <http://doi.org/10.1177/1362361308091649>.
- Isono, T., Ogawa, S., Yamada, S., Shibasaki, M., Masataka, N., 2014. Preliminary evidence that different mechanisms underlie the anger superiority effect in children with and without Autism Spectrum Disorders. *Front. Psychol.* 5, <http://doi.org/10.3389/fpsyg.2014.00441>.

- Joseph, R.M., Elhman, K., McNally, R., Keehn, B., 2008. Affective response to eye contact and face recognition ability in children with ASD. *J. Int. Neuropsychol. Soc.* 14 (6), 947–955, <http://doi.org/10.1017/S1355617708081344>.
- Juranek, J., Filipek, P.A., Berenji, G.R., Modahl, C., Osann, K., Spence, M.A., 2006. Association between amygdala volume and anxiety level: magnetic resonance imaging (MRI) study in autistic children. *J. Child Neurol.* 21 (12), 1051–1058.
- Kalin, N.H., Shelton, S.E., Davidson, R.J., 2004. The role of the central nucleus of the amygdala in mediating fear and anxiety in the primate. *J. Neurosci.* 24 (24), 5506–5515, <http://doi.org/10.1523/JNEUROSCI.0292-04.2004>.
- Kerns, C.M., Kendall, P.C., Berry, L., Sonders, M.C., Franklin, M.E., Schultz, R.T., et al., 2014. Traditional and atypical presentations of anxiety in youth with autism spectrum disorder. *J. Autism Dev. Disorders* 44 (11), 2851–2861, <http://doi.org/10.1007/s10803-014-2141-7>.
- Ke, X., Tang, T., Hong, S., Hang, Y., Zou, B., Li, H., et al., 2009. White matter impairments in autism, evidence from voxel-based morphometry and diffusion tensor imaging. *Brain Res.* 1265, 171–177, <http://doi.org/10.1016/j.brainres.2009.02.013>.
- Kleinmans, N.M., Richards, T., Weaver, K., Johnson, L.C., Greenison, J., Dawson, G., et al., 2010. Association between amygdala response to emotional faces and social anxiety in autism spectrum disorders. *Neuropsychologia* 48 (12), 3665–3670, <http://doi.org/10.1016/j.neuropsychologia.2010.07.022>.
- Klusek, J., Roberts, J.E., Losh, M., 2015. Cardiac autonomic regulation in autism and Fragile X syndrome: a review. *Psychol. Bull.* 141 (1), 141–175, <http://doi.org/10.1037/a0038237>.
- Lecavalier, L., 2006. Behavioral and emotional problems in young people with pervasive developmental disorders: relative prevalence, effects of subject characteristics, and empirical classification. *J. Autism Dev. Disord.* 36 (8), 1101–1114.
- May, T., Cornish, K., Rinehart, N.J., 2015. Mechanisms of anxiety related attentional biases in children with autism spectrum disorder. *J. Autism Dev. Disord.* 45 (10), 3339–3350, <http://doi.org/10.1007/s10803-015-2500-z>.
- McCormick, C., Hessl, D., Macari, S.L., Ozonoff, S., Green, C., Rogers, S.J., 2014. Electrodermal and behavioral responses of children with autism spectrum disorders to sensory and repetitive stimuli. *Autism Res.* 7 (4), 468–480, <http://doi.org/10.1002/aur.1382>.
- Milad, M.R., Wright, C.I., Orr, S.P., Pitman, R.K., Quirk, G.J., Rauch, S.L., 2007. Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biol. Psychiatry* 62 (5), 446–454, <http://doi.org/10.1016/j.biopsych.2006.10.011>.
- Milham, M.P., Nugent, A.C., Dreverts, W.C., Dickstein, D.P., Leibenluft, E., Ernst, M., et al., 2005. Selective reduction in amygdala volume in pediatric anxiety disorders: a voxel-based morphometry investigation. *Biol. Psychiatry* 57 (9), 961–966, <http://doi.org/10.1016/j.biopsych.2005.01.038>.
- Minschew, N.J., Williams, D.L., 2007. The new neurobiology of autism: cortex, connectivity, and neuronal organization. *Arch. Neurol.* 64 (7), 945–950, <http://doi.org/64/7/945>.
- Mohanty, A., Engels, A.S., Herrington, J.D., Heller, W., Ho, M.-H.R., Banich, M.T., et al., 2007. Differential engagement of anterior cingulate cortex subdivisions for cognitive and emotional function. *Psychophysiology* 44 (3), 343–351.
- Morris, J.S., Ohman, A., Dolan, R.J., 1999. A subcortical pathway to the right amygdala mediating “unseen” fear. *Proc. Natl. Acad. Sci. USA* 96 (4), 1680–1685.
- Mosconi, M.W., Cody-Hazlett, H., Poe, M.D., Gerig, G., Gimpel-Smith, R., Piven, J., 2009. Longitudinal Study of Amygdala Volume and Joint Attention in 2- to 4-Year-Old Children With Autism. *Arch Gen Psychiatry* 66 (5), 509–516, <http://doi.org/10.1001/archneuropsychiatr.2009.19>.
- Munson, J., Dawson, G., Abbott, R., Faja, S., Webb, S.J., Friedman, S.D., et al., 2006. Amygdala volume and behavioral development in autism. *Arch Gen Psychiatry* 63 (6), 686–693, <http://doi.org/10.1001/archpsyc.63.6.686>.
- Murphy, E.R., Foss-Feig, J., Kenworthy, L., Gaillard, W.D., Vaidya, C.J., 2012. Atypical Functional Connectivity of the Amygdala in Childhood Autism Spectrum Disorders during Spontaneous Attention to Eye-Gaze. *Autism Res. Treat.* 2012, 652408, <http://doi.org/10.1155/2012/652408>.
- Myers-Schulz, B., Koenig, M., 2012. Functional anatomy of ventromedial prefrontal cortex: implications for mood and anxiety disorders. *Mol. Psychiatry* 17 (2), 132–141, <http://doi.org/10.1038/mp.2011.88>.
- Nacewicz, B.M., Dalton, K.M., Johnstone, T., Long, M.T., McAuliff, E.M., Oakes, T.R., et al., 2006. Amygdala volume and nonverbal social impairment in adolescent and adult males with autism. *Arch. Gen. Psychiatry* 63 (12), 1417–1428, <http://doi.org/63/12/1417>.
- Neuhaus, E., Beauchaine, T.P., Bernier, R., 2010. Neurobiological correlates of social functioning in autism. *Clinical Psychol. Rev.* 30 (6), 733–748, <http://doi.org/10.1016/j.cpr.2010.05.007>.
- Neuhaus, E., Bernier, R., Beauchaine, T.P., 2013. Brief report: social skills, internalizing and externalizing symptoms, and respiratory sinus arrhythmia in autism. *J. Autism Dev. Disord.* <http://doi.org/10.1007/s10803-013-1923-7>.
- Pasley, B., Mayes, L., Schultz, R., 2004. Subcortical discrimination of unperceived objects during binocular rivalry. *Neuron* 42 (1), 163–172.
- Passarotti, A.M., Sweeney, J.A., Pavuluri, M.N., 2010. Neural correlates of response inhibition in pediatric bipolar disorder and attention deficit hyperactivity disorder. *Psychiatry Res.* 181 (1), 36–43, <http://doi.org/10.1016/j.psychresns.2009.07.002>.
- Patriquin, M.A., Scarpa, A., Friedman, B.H., Porges, S.W., 2011. Respiratory sinus arrhythmia: A marker for positive social functioning and receptive language skills in children with autism spectrum disorders. *Dev. Psychobiol.* 55 (2), 101–112, <http://doi.org/10.1002/dev.21002>.
- Pelphrey, K.A., Shultz, S., Hudac, C.M., Vander Wyk, B.C., 2011. Research Review: Constraining heterogeneity: the social brain and its development in autism spectrum disorder. *J. Child Psychol. Psychiatry* 52 (6), 631–644, <http://doi.org/10.1111/j.1469-7610.2010.02349.x>.
- Pessoa, L., Adolphs, R., 2010. Emotion processing and the amygdala: from a “low road” to “many roads” of evaluating biological significance. *Nat. Rev. Neurosci.* 11 (11), 773–783, <http://doi.org/10.1038/nrn2920>.
- Pfeifer, J.C., Welge, J., Strakowski, S.M., Adler, C.M., DellBello, M.P., 2008. Meta-analysis of amygdala volumes in children and adolescents with bipolar disorder. *J. Am. Acad. Child Adolesc. Psychiatry* 47 (11), 1289–1298, <http://doi.org/10.1097/CHI.0b03e31818185d299>.
- Pfeifer, J.H., Allen, N.B., 2012. Arrested development? Reconsidering dual-systems models of brain function in adolescence and disorders. *Trends Cogn. Sci.* 16 (6), 322–329, <http://doi.org/10.1016/j.tics.2012.04.011>.
- Phelps, E.A., LeDoux, J.E., 2005. Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron* 48 (2), 175–187, <http://doi.org/10.1016/j.neuron.2005.09.025>.
- Pierce, K., Müller, R.A., Ambrose, J., Allen, G., Courchesne, E., 2001. Face processing occurs outside the fusiform “face area” in autism: evidence from functional MRI. *Brain* 124 (10), 2059–2073.
- Pine, D.S., Guyer, A.E., Leibenluft, E., 2008. Functional magnetic resonance imaging and pediatric anxiety. *J. Am. Acad. Child Adolesc. Psychiatry* 47 (11), 1217–1221, <http://doi.org/10.1176/1545-1210-47-11-1217>.

- Porges, S.W., 2001. The polyvagal theory: phylogenetic substrates of a social nervous system. *Int. J. Psychophysiol.* 42 (2), 123–146.
- Porges, S.W., Walter, G.E., Korb, R.J., Sprague, R.L., 1975. The influences of methylphenidate on heart rate and behavioral measures of attention in hyperactive children. *Child Dev.* 46 (3), 725–733.
- Porges, S.W., Macellai, M., Stanfill, S.D., McCutie, K., Lewis, G.F., Harden, E.R., et al., 2012. Respiratory sinus arrhythmia and auditory processing in autism: Modifiable deficits of an integrated social engagement system? *Int. J. Psychophysiol.* <http://doi.org/10.1016/j.jpsycho.2012.11.009>.
- Rauch, S.L., Shin, L.M., Phelps, E.A., 2006. Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research—past, present, and future. *Biol. Psychiatry* 60 (4), 376–382, <http://doi.org/10.1016/j.biopsych.2006.06.004>.
- Schoen, S.A., Miller, L.J., Brett-Green, B.A., Nielsen, D.M., 2009. Physiological and behavioral differences in sensory processing: a comparison of children with autism spectrum disorder and sensory modulation disorder. *Front. Integrative Neurosci.* 3, 29, <http://doi.org/10.3389/neuro.07.029.2009>.
- Schumann, C.M., Nordahl, C.W., 2011. Bridging the gap between MRI and postmortem research in autism. *Brain Res.* 1380, 175–186, <http://doi.org/10.1016/j.brainres.2010.09.061>.
- Schumann, C.M., Hamstra, J., Goodlin-Jones, B.L., Lotspeich, L.J., Kwon, H., Buonocore, M.H., et al., 2004. The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. *J. Neurosci.* 24 (28), 6392–6401, <http://doi.org/10.1523/JNEUROSCI.1297-04.2004>.
- Schumann, C.M., Bloss, C.S., Barnes, C.C., Wideman, G.M., Carper, R.A., Akshoomoff, N., et al., 2010. Longitudinal magnetic resonance imaging study of cortical development through early childhood in autism. *J. Neurosci.* 30 (12), 4419–4427, <http://doi.org/10.1523/JNEUROSCI.5714-09.2010>.
- Schumann, C.M., Bauman, M.D., Amaral, D.G., 2011. Abnormal structure or function of the amygdala is a common component of neurodevelopmental disorders. *Neuropsychologia* 49 (4), 745–759, <http://doi.org/10.1016/j.neuropsychologia.2010.09.028>.
- Shaw, P., Brierley, B., David, A.S., 2005. A critical period for the impact of amygdala damage on the emotional enhancement of memory? *Neurology* 65 (2), 326–328, <http://doi.org/10.1212/01.wnl.0000168867.40688.90>.
- Silverman, W.K., Saavedra, L.M., Pina, A.A., 2001. Test-retest reliability of anxiety symptoms and diagnoses with the Anxiety Disorders Interview Schedule for DSM-IV: child and parent versions. *J. Am. Acad. Child Adolesc. Psychiatry* 40 (8), 937–944.
- South, M., Larson, M.J., White, S.E., Dana, J., Crowley, M.J., 2011. Better fear conditioning is associated with reduced symptom severity in autism spectrum disorders. *Autism Res.* 4 (6), 412–421, <http://doi.org/10.1002/aur>.
- Spence, S., 1998. A measure of anxiety symptoms among children. *Behav. Res. Therapy* 36 (5), 545–566, [http://doi.org/10.1016/S0006-7967\(98\)00034-5](http://doi.org/10.1016/S0006-7967(98)00034-5).
- Sukhadolsky, D.G., Scabill, L., Gadow, K.D., Arnold, L.E., Aman, M.G., McDougle, C.J., et al., 2008. Parent-rated anxiety symptoms in children with pervasive developmental disorders: frequency and association with core autism symptoms and cognitive functioning. *J. Abnormal Child Psychol.* 36 (1), 117–128.
- Swanson, L.W., Petrovich, G.D., 1998. What is the amygdala? *Trends Neurosci.* 21 (8), 323–331.
- Swartz, J.R., Wiggins, J.L., Carrasco, M., Lord, C., Monk, C.S., 2013. Amygdala habituation and prefrontal functional connectivity in youth with autism spectrum disorders. *J. Am. Acad. Child Adolesc. Psychiatry* 52 (1), 84–93, <http://doi.org/10.1016/j.jaac.2012.10.012>.
- Thayer, J.F., Lane, R.D., 2000. A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disorders* 61 (3), 201–216.
- Thayer, J.F., Ahn, F., Fredriksson, M., Sollers 3rd, J.J., Wager, T.D., 2012. A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36 (2), 747–756, <http://doi.org/10.1016/j.neubiorev.2011.11.009>.
- Troiani, V., Price, E.T., Schultz, R.T., 2012. Unseen fearful faces promote amygdala guidance of attention. *Soc. Cogn. Affect. Neurosci.* <http://doi.org/10.1093/scan/nss116>.
- Tucker, P., Adamson, P., Miranda, R., Scarborough, A., Williams, D., Groff, J., et al., 1997. Paroxetine increases heart rate variability in panic disorder. *J. Clin. Psychopharmacol.* 17 (5), 370–376.
- van Engeland, H., 1984. The electrodermal orienting response to auditory stimuli in autistic children, normal children, mentally retarded children, and child psychiatric patients. *J. Autism Dev. Disorders* 14 (3), 261–279.
- Watanabe, T., Yihata, N., Abe, O., Kuwabara, H., Inoue, H., Takano, Y., et al., 2012. Diminished medial prefrontal activity behind autistic social judgments of incongruent information. *PLoS One* 7 (6), e39561, <http://doi.org/10.1371/journal.pone.0039561>.
- Waters, A.M., Henry, J., Moggs, K., Bradley, B.P., Pine, D.S., 2010. Attentional bias towards angry faces in childhood anxiety disorders. *J. Behav. Therapy Exp. Psychiatry* 41 (2), 158–164, <http://doi.org/10.1016/j.jbtep.2009.12.001>.
- Weng, S.-J., Carrasco, M., Swartz, J.R., Wiggins, J.L., Kurapati, N., Liberon, I., et al., 2011. Neural activation to emotional faces in adolescents with autism spectrum disorders. *J. Child Psychol. Psychiatry Allied Discip.* 52 (3), 296–305, <http://doi.org/10.1111/j.1469-7610.2010.02317.x>.
- Whalen, P.J., 1998. Fear, vigilance, and ambiguity: Initial neuroimaging studies of the human amygdala. *Curr. Directions Psychol. Sci.* 7 (6), 177–188.
- Whalen, P.J., 2007. The uncertainty of it all. *Trends Cogn. Sci.* 11 (12), 499–500, <http://doi.org/10.1016/j.tics.2007.08.016>.
- Whalen, P.J., Phelps, E.A. (Eds.), 2009. The Human Amygdala. Guilford Press, New York.
- Wilensky, A.E., Schafe, G.E., Kristensen, M.P., LeDoux, J.E., 2006. Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. *J. Neurosci.* 26 (48), 12387–12396, <http://doi.org/10.1523/JNEUROSCI.4316-06.2006>.
- Veragani, V.K.Y., Pohl, R., Balon, R., Ramesh, C., Glitz, D., Weinberg, P., et al., 1992. Effect of imipramine treatment on heart rate variability measures. *Neuropsychobiology* 26 (1-2), 27–32, <http://doi.org/10.1159/000118892>.