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RESEARCH ARTICLE

An amygdala-centered hyper-connectivity signature of threatening face processing predicts anxiety in youths with autism spectrum conditions

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Abstract

Anxiety is exceedingly prevalent among individuals with an autism spectrum condition (ASC). While recent literature postulates anxiety as a mechanism encompassing an underlying amygdala-related elevated baseline level of arousal even to nonthreatening cues, whether this same mechanism contributes to anxiety in those with an ASC and supports the transdiagnostic nature of anxiety remains elusive. In this case–control study of 51 youths (26 ASC), we assessed autism and anxiety via the Autism-Spectrum Quotient and the State–Trait Anxiety Inventory, respectively. Hemodynamic responses, including amygdala reactivity, to explicit and implicit (backwardly masked) perception of threatening faces were acquired using functional Magnetic Resonance Imaging (fMRI). For explicit fear, ASC individuals showed significantly greater negative correlations between the amygdala and the attentional deployment-parietal network. For implicit fear, ASC individuals showed significantly stronger correlations of the amygdala with the prefrontal networks, temporal pole, and hippocampus. Additionally, an fMRI-based neurologic signature for anxiety in ASCs was identified via the LibSVM machine learning model using amygdala-centered functional connectivity during the emotional processing of explicit and implicit stimuli. Hypervigilance to implicit threat in ASCs comorbid with anxiety might exacerbate explicit threat reactivity; hence the use of attentional avoidance patterns to restrict affective hyperarousal for explicitly perceived socioemotional stimuli. Consequently, developing an attention-independent behavioral/neural marker identifying anxiety in ASCs is highly warranted.

Lay Summary

This study identifies a dissociation of amygdala reactivity dependent on explicit and implicit threat processing. Implicit anxiety in individuals with an autism spectrum condition (ASC) could outweigh explicitly induced threat. When explicitly perceiving socioemotional stimuli, ASC individuals with anxiety might use attentional avoidance patterns to restrict affective hyperarousal.

KEYWORDS
amygdala, autism spectrum condition (ASC), explicit, implicit, threat

Yu-Chun Chen and Chenyi Chen contributed equally to this study.

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INTRODUCTION

Anxiety is the most prevalent co-occurring mental illness in individuals with a diagnosis for an autism spectrum disorder (ASD) (Rodgers & Ofield, 2018). Recent studies have postulated an underlying universal mechanism of anxiety which involves a hyper-or-elevated level of arousal at baseline even to nonthreatening stimuli, and that is mediated by gene expression (Chen et al., 2020; Top Jr. et al., 2016). Previous conceptualizations concerning the neuropathological changes central to the impairments in social interaction incurred by ASD individuals have emphasized amygdala dysfunction as one of its causes (Amaral et al., 2003; Baron-Cohen et al., 2000; Schultz, 2005). Concurrently, the amygdala is equally central to the threat processing system involved in the experience of anxiety (Sah, 2017). The aberrant amygdala hyperactivation found in individuals with high anxiety might explain why they exhibited hypersensitivity when processing emotionally ambiguous stimuli, and how they experienced the unconstrained viewing of undefined stimuli as more aversive. However, the studies conducted to examine the mechanisms underlying the link between anxiety and amygdala dysfunction in ASD are sparse.

One functional Magnetic Resonance Imaging (fMRI) study using a paradigm requiring selective attention toward and away from social information found that individuals with both anxiety and ASD exhibited similarly increased levels of attentional directedness toward task-irrelevant, peripheral social information as was seen in individuals diagnosed with anxiety only, pointing at the transdiagnostic nature of the negative valence, as conceptualized in the RDoC (NIH's Research Domain Criteria) (Herrington, Maddox, McVey, et al., 2017). Furthermore, in order to delineate the neural biology of ASD with co-occurring anxiety, Herrington and colleagues concurrently considered two seeming contradictory neural models that suggest hyperactivation of amygdala in anxiety, but hypoactivation of amygdala in ASD (Herrington et al., 2016). Based on their findings, since amygdala activity represents a hybrid signal processing of emotion and social information that cannot be reduced to either alone, the distinct relationships between the anxiety and social deficits with amygdala function in ASD is not dissociable without looking into the amygdala function as the whole connectivity network, and not only as a singular amygdala reactivity. These separate mechanisms would be underpinned by varying neural networks despite of common fMRI activations observed at the gross anatomical level.

Whilst anxiety is a real and serious problem for many people on the autistic spectrum, relatively few neuroimaging studies have addressed this issue. One preliminary study with a small sample size ($N = 12$) reported that adults with ASD exhibited comparable amygdala engagement in response to implicit (backward masking) presentations of anxious (fearful) faces (Hall et al., 2010). While non-masked stimuli for the explicit processing consisted of fearful faces stimuli presented for 200-ms, backwardly masked stimuli for the implicit processing consisted of 17-ms of fearful faces subsequently followed by 183-ms of neutral faces; thus preventing the conscious processing of threatening stimuli. Another study ($N = 20$) using fear conditioning tasks, showed anxiety was related to an impairment in the ability of the amygdala to differentiate between threatening versus safe cues (Top Jr. et al., 2016). These findings suggest that anxiety in ASD might be linked to an abnormal amygdala activation, or different connectivity networks. One possibility is that the co-occurring altered amygdala-centered functional connectivity, even to "unseen" stimuli, may hamper the balance among the modulatory roles the amygdala plays in a wide array of networks, and in which it is highly possible to go beyond the function of emotion (Pessoa & Adolphs, 2010).

The amygdala, as a key brain structure associated to anxiety, is responsible for salience detection in the environment, including threats (Davis & Whalen, 2001). As such, the extent to which the amygdala reacts in response to threatening stimuli (anger and/or fear) has been linked to anxiety (Etkin et al., 2004; Etkin & Wager, 2007; Most et al., 2006). Given that ambiguous cues significantly increase the threat processing system's activity, it is no surprise that fearful faces elicit more reactivity in the amygdala than angry faces (Whalen et al., 2001), as fearful faces signal the possible existence of danger without providing information about its source. Consequently, amygdala reactivity as elicited by fearful faces has been attested to be a reliable way for examining the neural correlates underpinning anxiety (Bishop et al., 2004; Chen et al., 2017). Neuroimaging research has demonstrated that there is engagement of the amygdala during implicit processing, in addition to the explicit processing, of threats (Morris et al., 1998; Whalen et al., 1998). Implicit

processing is presumed to be subserved by a direct neural pathway to the amygdala, which permits threatening stimuli to be processed in a speedy, automatic, and unconscious manner (LeDoux, 1996). The paradigm using fMRI in combination with backwardly masked stimuli presentation provides a novel opportunity to examine behavioral and neural responses indicative of implicit threat processing (Dimberg et al., 2000; Morris et al., 2001; Whalen et al., 1998). One fMRI study adopting such paradigm, revealed that the mode of functional connectivity in amygdala pathways vary as a function of the awareness level for signals of fear, suggesting excitatory feedforward connections along the subcortical pathways to the amygdala during the implicitly masked condition (Williams et al., 2006). Relevant to this issue, another study found that negative amygdala-parietal connectivity contributed to the effect of reducing negative affect through the emotion regulation strategy of attentional deployment, indicating that this negative reentrant feedback of the amygdala could be necessary to afford such awareness (Ferri et al., 2016).

Atypical attention during social interactions is a well-demonstrated feature of ASD (Fan et al., 2014; Klin et al., 2002). Moreover, longer gaze fixation in those individuals with autistic traits has been associated with heightened hemodynamic activity in the amygdala (Dalton et al., 2005). When explicitly perceiving socioemotional/threatening stimuli, individuals with ASD might tend to use attentional avoidance patterns to restrict affective hyperarousal. Conversely, individuals with ASD might fail to inhibit the hyperarousal ascribed to anxiety when implicitly perceiving socioemotionally threatening stimuli, as implicit perception refers to a perceptual state in which individuals fail to report the presence of a stimulus, in spite of the fact that the stimulus has actually been processed (Tamietto & de Gelder, 2010).

Here, this fMRI study used the backwardly masked paradigm to elucidate how perceiving explicit and implicit threat would affect amygdala engagement and its related functional connectivity across two participant groups: ASD with anxiety, and controls. In regards to anxiety as indicated by amygdala reactivity to threat (Bishop et al., 2004; Etkin et al., 2004), the exceeded implicit anxiety in individuals with ASD might exacerbate the over-arousal level to the explicitly induced fear. Based on evidence from ASD comorbid with anxiety (Herrington et al., 2016; Herrington, Maddox, McVey, et al., 2017), it is reasonable to suppose that anxiety in ASD might arise from the aberrant amygdala-centered reactivity to both explicit and implicit threat processing, compromising preexisting hyperarousal triggered by implicit perception of threatening stimuli. Given the view that these excitatory feedforward connections along this pathway might be overflowed for automatic responses to "unseen" fear (Pessoa & Adolphs, 2010), a stronger subcortical pathway to the amygdala would be expected to be found in ASD individuals with high anxiety. We thus hypothesize that ASD individuals with high anxiety would exhibit a stronger negative amygdala-parietal connectivity during the explicit fear condition to compensate for their excessive processing of negative affect.

MATERIALS AND METHODS

Participants

Twenty-six youths with autism spectrum condition (ASC) and 25 matched controls (CTL) (aged 14–24 years) participated in this study. ASC individuals with anxiety were recruited from a community autism program and referred to clinical psychiatrists. The inclusion criteria was as follows: (1) must previously have been diagnosed with ASD by certificated psychiatrists, and according to the Diagnostic and Statistical Manual of Mental Disorders-5 (APA, 2013); (2) possess DSM-5 ASD severity grades from mild (Level 1) to moderate (Level 2) in both social communication and restricted interests and repetitive behaviors domains, with stability during the last 2 months. For the exclusion criteria, all participants with neurological abnormalities, a history of epilepsy or seizures, head trauma, and IQ < 75, were excluded. The participants in the age- and sex-matched control group were recruited from the local community, and screened for major psychiatric illnesses by conducting the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID-I), research version (First et al., 2002). The subjects did not participate in any intervention or drug programs during the experimental period. All participants had normal or corrected-to-normal visual acuity. All participants, or their respective legal guardians, provided written informed consent. All procedures were approved by the Ethics Committee of National Yang-Ming University (YM102035) and conducted in accordance with the Declaration of Helsinki. Due to the observational nature of the present study, we were not aware of the necessity for the prospective clinical trial registration; thus, this research was retrospectively registered in a publicly accessible database at ClinicalTrials.gov (Autism, Emotional Processing, and the Amygdala; NCT04549506), nevertheless, as an observational study type. We did, however, prospectively upload our full-length manuscript, in which further detailed methods and results can be found, on to the Research Square preprint server (https://doi.org/10.21203/rs.3.rs-15528/v1). The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Procedures

Before fMRI scanning, each participant underwent assessments with the State–Trait Anxiety Inventory

(STAI) to determine their self-reported anxiety levels (Spielberger et al., 1970), as well as the Autism-Spectrum Quotient (AQ) (Baron-Cohen, Wheelwright, Skinner, et al., 2001). The STAI is a commonly used measure of trait and state anxiety that has 20 items for assessing trait anxiety, and 20 for state anxiety. State anxiety (STAI-S) indicates anxiety in specific situations, and trait anxiety (STAI-T) determines anxiety as a general trait. A cut point of 39–40 of the STAI-S has been suggested to detect clinically significant symptoms for state anxiety (Julian, 2011). The Autism Quotient (AQ) is a 50-item self-report measure, which assesses autistic traits in individuals with no intellectual disability.

The paradigm for the fMRI scanning was derived from the work by Etkin et al. (2004). The visual stimuli consisted of black and white pictures of male and female faces with fearful and neutral facial expressions, which were chosen from the Pictures of Facial Affect (Ekman & Friesen, 1976). The faces were oriented to maximize interstimulus alignment of eyes and mouths, and then artificially colorized (red, yellow, or blue) and equalized for luminosity. During fMRI scanning, subjects performed the color identification task, in which they were asked to judge the color of each face (pseudo-colored in either red, yellow, or blue) and to indicate the answer by a keypad button press. Each stimulus presentation involved a 200-ms fixation cross to cue subjects to focus on the center of the screen, followed by a 400-ms blank screen and a 200-ms face presentation. Participants then had 1200-ms to respond with a key press, indicating the color of the face. Non-masked stimuli consisted of a 200-ms fearful- or neutral-expression face. Backwardly masked stimuli consisted of 17-ms of a fearful or neutral face, followed by a 183-ms neutral face mask belonging to a different individual, but of the same color and gender as the previous one. Each epoch (12-s) consisted of six trials of the same stimulus type (explicit fearful [EF], explicit neutral [EN], implicit fearful [IF], or implicit neutral [IN]), but were randomized with respect to color and gender. The presentation order of the total 12 epochs (two for each stimulus type) and 12 fixation blocks (with a 12-s fixation cross) were pseudo-randomized. To avoid stimulus order effects, we used two different counterbalanced run orders. The stimuli were presented using Matlab software (MathWorks, Inc., Sherborn, MA) and were triggered by the first radio frequency pulse for the functional run. The stimuli were displayed on VisuaStim XGA LCD screen goggles (Resonance Technology, Northridge, CA). The screen resolution was 800 x 600, with a refresh rate of 60 Hz. Behavioral responses were recorded by a fORP interface unit and saved in the Matlab program.

Immediately after fMRI scanning, participants underwent the detection task, during which they were shown all of the stimuli again and alerted of the presence of fearful faces. The subjects were administered a forced-choice test under the same presentation conditions as those during scanning and asked to indicate whether they observed a fearful face or not. The detection task was designed to assess possible awareness of the masked fearful faces. The chance level for correct answers was 50%. The performance was determined by the calculation of a detection sensitivity index (d') based on the percentage of trials in which a masked stimulus was detected when presented ["hits" (H)] and adjusted for the percentage of trials a masked stimulus was "detected" when not presented ["false alarms" (FA)]; [d' = z-score (percentage H) − z-score (percentage FA), with chance performance = 0 ± 1.74] (M. J. Kim et al., 2010; Whalen et al., 2004).

Functional MRI data acquisition, image processing, and analysis

Functional and structural MRI data were acquired on a 3 T MRI scanner (Siemens Magnetom Tim Trio, Erlanger, German) equipped with a high-resolution 32-channel head array coil. A gradient-echo, T2*-weighted echoplanar imaging (EPI) with a blood oxygen level-dependent (BOLD) contrast pulse sequence was used for functional data. To optimize the BOLD response in the amygdala (Morawetz et al., 2008), 29 interleaved slices were acquired along the AC-PC plane, with a 96 x 128 matrix, 19.2 x 25.6 cm^2 field of view (FOV) and 2 x 2 x 2 mm voxel size, resulting in a total of 144 volumes for the functional run (TR = 2-s, TE = 36-ms, flip angle = 70°, slice thickness 2 mm, no gap). Parallel imaging GRAPPA with factor 2 was used to increase the speed of acquisition. Structural data were acquired using a magnetization-prepared rapid gradient echo sequence (TR = 2.53-s, TE = 3.03-ms, FOV = 256 x 224 mm^2, flip angle = 7°, matrix = 224 × 256, voxel size = 1.0 x 1.0 x 1.0 mm^3, 192 sagittal slices/slab, slice thickness = 1 mm, no gap).

Image processing and analysis were performed using SPM8 (Wellcome Department of Imaging Neuroscience, London, UK) in MATLAB 7.0 (MathWorks Inc., Sherborn, MA). Structural scans were co-registered to the SPM8 T1 template, and a skull-stripped image was created from the segmented gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) images. These segmented images were combined to create a subject-specific brain template. EPI images were realigned and filtered (128-s cut-off), then co-registered to these brain templates, normalized to Montreal Neurological Institute (MNI) space, and smoothed (8 mm FWHM). The voxel size used in the functional analysis was 2 x 2 x 2 mm^3. All subjects who completed scanning had less than one voxel of in-plane motion.

Preprocessing for the T1-weighted images involved using the following DARTEL algorithm: new segment—generate roughly aligned GM and WM images of the subjects; create template—determine nonlinear

deformations for warping all the GM and WM images so that they match each other; and normalize to MNI space—images were normalized to the MNI template and were smoothed with an 8-mm full-width at half-maximum Gaussian filter. Then, the GM, WM, and CSF structures of each patient were obtained after processing. A two-level approach for block-design fMRI data was adopted using general linear model implemented in SPM8. Fixed effects analyses were performed at the single subject level to generate individual contrast maps, and random effects analyses were performed at the group level. At single subject level, contrast images were calculated comparing each explicitly and implicitly presented fearful face block with the neutral baseline. Shorthand (e.g., EF − EN) was used to indicate the contrasts of regressors (e.g., EF > EN). Movement parameters from the realignment output were included as regressors of no interest. Error bars signify SE. To isolate the effects of fear content of the stimuli from other aspects of the stimuli and the task, we subtracted neutral (EN) or masked neutral (IN) activity from fearful (EF) or masked fearful activity (IF), respectively. The explicit perception of fearful faces was denoted as non-masked fear (EF − EN), and the implicit perception of fearful faces as masked fear (IF−IN). These contrast images were then entered to the second-level group analysis. The resulting first-level contrast images were then entered into a full factorial analysis: 2 [group: ASC vs. control (CTL)] x 2 (attention: explicit [EF–EN] vs. implicit [IF−IN]). In addition, participant's age was included as a nuisance regressor. Whole-brain activations were corrected for multiple comparisons with family-wise error (FWE) rate at $p < 0.05$. Monte Carlo simulation implemented using 3dClustSim: https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html determined that a 10-voxel extent at height threshold of $p < 0.001$ uncorrected (cut-off, $t = 3.178$) yielded a FWE-corrected threshold of $p < 0.05$, accounting for spatial correlations in neighboring voxels (significance level: voxel $p = 0.001$, $\alpha = 0.05$ with 5000 Monte Carlo simulations after GM masking).

Based on evidence from ASD with co-occurring anxiety (Herrington, Maddox, Kerns, et al., 2017; Herrington, Maddox, McVey, et al., 2017), we used MarsBar (see http://marsbar.sourceforge.net/) to conduct the regions of interest (ROIs) analyses for the right and left amygdala. Signals across all voxels with a radius of 4 mm were averaged and evaluated for the masked and non-masked comparisons in the right and left amygdala. The size of amygdala ROI was determined according to a recent functional connectivity study in ASD (Shen et al., 2016). The individual mean parameter estimates (beta values) were then subject to a mixed analysis of variance (ANOVA), as to test for main effects of group (ASC vs. CTL) and attention (explicit [EF–EN] vs. implicit [IF−IN]), as well as group-by-attention interactions.

Functional connectivity analysis

The psychophysiological interaction (PPI) assesses the hypothesis that the activity in one brain region can be explained by an interaction between cognitive processes and hemodynamic activity in another brain region. The interaction between the first and second regressors represented the third regressor. The individual time series for the right amygdala was obtained by extracting the first principal component from all raw voxel time series in a sphere (4-mm radius) centered on the coordinates of the subject-specific amygdala activations. These time series were mean-corrected and high-pass filtered to remove low-frequency signal drifts. The physiological factor of the right amygdala activity was then multiplied by the psychological factor to constitute the interaction term. PPI analyses were then carried out for each subject, and involved the creation of a design matrix with the interaction term, the psychological factor, and the physiological factor as regressors. The psychological variable was used as a vector coding for the specific task (1 for fear, −1 for neutral) convolved with the hemodynamic response function to examine the whole-brain correlations of the physiological factor that change depending on the emotion perception, namely, to search for the brain regions that showed significant correlations with the right amygdala activity only in the fear condition, but not in the neutral condition. PPI analyses were separately conducted for each group (ASC vs. CTL) in order to identify brain regions showing significant changes in functional coupling with the amygdala during explicitly and implicitly perceived fear. Subject-specific contrast images were then entered into random effects analyses to compare the group effect. Due to the insufficient power in the PPI analysis with a total of 144 volumes, we applied a cluster correction procedure with a more liberal initial cluster-forming threshold of $p < 0.01$, but a larger cluster size for explorative purposes. To control for multiple comparisons at FWE rate of $p < 0.05$, clusters bigger than 54 were considered significant for PPI analyses according to the Monte Carlo simulation of 3dClustSim (significance level: voxel $p = 0.01$, $\alpha = 0.05$ with 5000 Monte Carlo simulations after GM masking).

Subsequently, a multiple regression model was run separately for each seed to estimate the regression coefficient between all voxels and the interaction time series (along with task, movement, and linear drift as nuisance regressors). The strength of association between all voxels and the interaction time series was measured with R2 values. These coefficients of determination were square-rooted then multiplied by the sign of their respective estimated beta weights to obtain directionality of association. The correlation coefficients of the interaction time series were then converted to z-scores using Fisher's transformation. The resulting statistical maps were then included in a second-level group analysis (ASC vs. CTL)

by running a voxel-based two-sample *t*-test on the z-scores of the interaction effect for each seed separately (contrast value 1 for ASC, −1 for CTL). Finally, in order to examine how the group effect in the PPI results was driven by the nature of the correlations, namely, positive or negative correlations, we reported the descriptive statistics for each group.

Classification using machine learning

After identifying the functional pathway of the amygdala in ASC individuals during both explicit and implicit processing of fear, the parameter estimates of functional connectivity were extracted for further diagnostic classification analyses. Parameter estimates across all voxels with a radius of 4 mm surrounding the coordinates that showed the peak *t* values were averaged and extracted accordingly. Data from two fMRI runs of each participant were averaged and then used for the training, and test data set were represented by a $d = 12$-dimension feature vector that includes the parameter estimates of the medial prefrontal cortex (mPFC), inferior frontal cortex, temporal pole, hippocampus, superior parietal cortex, and fusiform gyrus, from both explicit and implicit conditions. For a total of 51 participants, the feature vector was: ASC 26 × 12, CTL 25 × 12. The ASC tag was 1, the CTL tag was −1 and the training set was sent to a linear support vector classifier for classification (http://www.csie.ntu.edu.tw/∼cjlin/libsvm/) (Chang & Lin, 2011). The total of 51 × 12 feature matrix submitted to a fivefold cross-validation procedure that iteratively divided the feature matrix into six groups (in this example, 10 × 12 feature matrix/group), trained the classifier to discriminate conditions based on four of the feature matrix groups (in this example, a 40 × 12 feature matrix), and tested the accuracy of the obtained functional connectivity pattern for predicting diagnostic value of ASC on the test data set (in this example, a 10 × 12 feature matrix). This cross-validation procedure yields a percent accurate classification for each of the six tests (% of participants accurately decoded), which are then averaged to produce the overall prediction accuracy. Finally, to test whether the prediction validity of the proposed functional connectivity model is significantly superior against a chance level of 50%, we applied a Monte Carlo simulation approach in which the labels of participants (ASC vs. CTL) were randomly shuffled and the prediction accuracy distribution was built upon 10,000 classifying permutations. For all the reported results, a linear kernel Support Vector Machine (SVM) classifier was trained, and default parameters were used.

TABLE 1 Demographic and clinical variables of the participants in the study

	ASC ($N = 26$)	CTL ($N = 25$)	*p* value
Age, years	19.5 (1.07)	21.6 (0.61)	0.10
Sex			
Male	26 (100%)	23 (92%)	0.16
AQ	26.12 (1.62)	18.56 (1.38)	0.001
Social skill	5.38 (0.53)	3.52 (0.48)	0.012
Attention switch	6.12 (0.42)	5 (0.42)	0.065
Attention to detail	4.31 (0.42)	2.88 (0.41)	0.018
Communication	5.42 (0.5)	4.64 (0.32)	0.2
Imagination	4.88 (0.47)	252 (0.39)	<0.001
STAI-T	47.92 (2.2)	41.52 (1.69)	0.025
STAI-S	41.46 (2.49)	35 (1.96)	0.047

Note: Data are presented as mean (SE) or number of participants (%).
Abbreviations: ASC, autism spectrum condition; STAI, State–Trait Anxiety Inventory.

RESULTS

Demographics and dispositional measures

The demographic variables collected by the laboratory researchers in this study re-confirmed the clinical referral (Table 1). The ASC group, compared with the control group, scored higher on the AQ ($t_{49} = 3.53$, $p = 0.001$, Cohen's $d = 1.0$) in social skill, attention to detail, and imagination subscale, as well as the STAI-T ($t_{49} = 2.31$, $p = 0.025$, Cohen's $d = 0.6$) and STAI-S ($t_{49} = 2.03$, $p = 0.048$, Cohen's $d = 0.6$).

Behavioral performance

For the color identification task within the fMRI scanning, the two (group: ASC vs. CTL) x two (attention: explicit vs. implicit) x two (emotion: fear vs. neutral) ANOVA on the accuracy (±SEM) did not yield any effect (all $p > 0.1$). ASC individuals and controls appeared to have comparable performance (96.1 ± 1% vs. 96.8 ± 1%). As regarding to the reaction time (RT), the results showed a main effect of emotion ($F_{1,49} = 6.95$, $p = 0.011$, $\eta^2 = 0.124$) and group ($F_{1,49} = 10.1$, $p = 0.003$, $\eta^2 = 0.171$) as well as an interaction between attention and emotion ($F_{1,49} = 5.92$, $p = 0.019$, $\eta^2 = 0.108$). ASC took longer RTs in the color identification task than CTL (573 ± 35 vs. 413 ± 36 ms). While overall fear relative to neutral exerted longer RTs (503 ± 27 vs. 484 ± 24 ms), this negativity bias was unbalanced, and biased toward the implicit condition (explicit: 493 ± 27 vs. 484 ± 25; implicit: 512 ± 27 vs. 484 ± 24), suggesting the validity of the backwardly masked paradigm.

For the detection task outside the fMRI scanner, ASC and controls performed above chance level in the explicit condition (d', mean ± SD: 1.928 ± 0.186 and 2.174 ± 0.146), and at chance level in the implicit

condition (0.112 ± 0.045 and 0.078 ± 0.016). Between the groups, the overall accuracy (mean percentage of 'hits' and "correct rejection") were not significantly different (explicit: $t_{55} = -1.79$, $p = 0.08$; implicit: $t_{55} = 0.33$, $p = 0.74$).

For the head motion in the scanner, there was no significant group-difference on the six movement parameters (Table S1). There were no participants with movement greater than 2 mm of translation or 0.03 degrees of rotation.

Whole-brain fMRI results

The voxel-wise analysis identified the hemodynamic responses between groups in response to explicit and IF faces (Table 2; Figure S1). In response to explicit fear (EF − EN), as compared to the controls, the ASC subjects exhibited lower BOLD responses in the amygdala bilaterally, as well as in the left parahippocampus, orbitofrontal cortex (OFC), posterior cingulate cortex, and right precuneus ([EF–EN]|ASC < [EF–EN]|CTL). For implicit fear (IF−IN), as compared to the controls, the BOLD response in the ASC subjects was higher in the right amygdala ([IF−IN]|ASC < [IF−IN]|CTL).

TABLE 2 Group-wide fMRI results to explicit and implicit threat processing

Brain region	MNI coordinates Side	*x*	*y*	*z*	*t*-value
Explicit fear (EF) versus neutral (EN)					
CTL > ASC					
Hippocampus	L	−20	−9	−18	3.42
Amygdala	L	−23	−3	−16	2.56*
Amygdala	R	26	−3	−17	2.08*
Inferior frontal gyrus (orbital)	L	−29	33	−9	3.44
Parahippocampa gyrus	L	−29	−41	−9	4.01
Posterior cingulate cortex	L	−15	−50	5	3.85
Precuneus	R	12	−51	9	3.97
Caudate	L	−17	9	23	4.21
Implicit fear (IF) versus neutral (IN)					
ASC > CTL					
Amygdala	R	30	−2	−12	2.29*

Note: All clusters are significant at FWE-corrected $p < 0.05$, except those marked with an asterisk, which are taken from predefined ROIs and significant at uncorrected $p < 0.05$.
Abbreviations: ASC, autism spectrum condition; FWE, family-wise error; MNI, Montreal Neurological Institute; ROIs, regions of interest.

Amygdala reactivity

To avoid circular inferences, prior ROI-based beta estimates were extracted from the amygdala bilaterally. In order to reexamine the validity of the backwardly masked paradigm, a priori comparison was conducted to inspect the simple main effect of fear versus neutral under the implicit condition in ASC and CTL, respectively. Results showed that fear relative to neutral showed significantly stronger right amygdala reactivity in CTL ($t_{24} = 2.52$, $p = 0.019$), but not in ASC ($t_{25} = 0.71$, $p = 0.486$). For the left amygdala, neither CTL ($t_{24} = -1.3$, $p = 0.206$) nor ASC ($t_{25} = -0.67$, $p = 0.509$) yielded significance. The observed temporal signal-to-noise ratio (TSNR) in this study were calculated for each subject on a voxel-by-voxel basis by taking the mean signal of the entire realigned time series for the left and right amygdala, and dividing this mean value by their standard deviation, respectively. The mean TSNR for the left and right amygdalae was 123.5 ± 53.4 (mean ± SD, ranging from 53 to 353) and 183.9 ± 71 (ranging from 57 to 266), respectively.

Explicit versus implicit threat processing

Subsequently, an ANOVA with one within-subject variable (explicit vs. implicit) and one between-subject variable (ASC vs. CTL) on the bilateral amygdala reactivity revealed interactions of group x attention (left: $F_{1,49} = 4.09$, $p = 0.049$, $\eta^2 = 0.08$; right: $F_{1,49} = 6.09$, $p = 0.017$, $\eta^2 = 0.11$). For the left amygdala, post hoc analyses indicated that ASC relative to controls showed significantly weaker amygdala reactivity to explicit fear (EF − EN: -0.1 ± 0.19 vs. 0.48 ± 0.13; $p = 0.016$), but they were comparable in response to implicit fear (IF−IN: -0.1 ± 0.15 vs. -0.24 ± 0.16; $p = 0.55$) (Figure 1a). For the right amygdala, post hoc analyses showed a marginal trend toward significance, indicating that ASC relative to controls exhibited weaker amygdala reactivity to explicit fear (0.03 ± 0.13 vs. 0.31 ± 0.06; $p = 0.05$), but they were comparable in implicit fear (0.11 ± 0.1 vs. 0.05 ± 0.06; $p = 0.58$) (Figure 1b).

Correlation between amygdala reactivity and behavioral traits

To test if attention would dissociate the transdiagnostic nature of anxiety-related amygdala reactivity in ASC, we performed the Pearson product–moment correlation analysis between hemodynamic activity to both explicit and implicit fear and autistic trait as well as STAI (Figure 2). When the ASC and control groups were analyzed together ($N = 51$), the correlation between AQ and amygdala reactivity was negative for explicit fear ($r = -0.34$, $p = 0.015$), but positive for implicit fear ($r = 0.33$, $p = 0.018$). Fisher's z tests confirmed a significant dissociation ($z = -3.41$, $p < 0.001$). With regards to anxiety, the correlation between STAI-S and amygdala

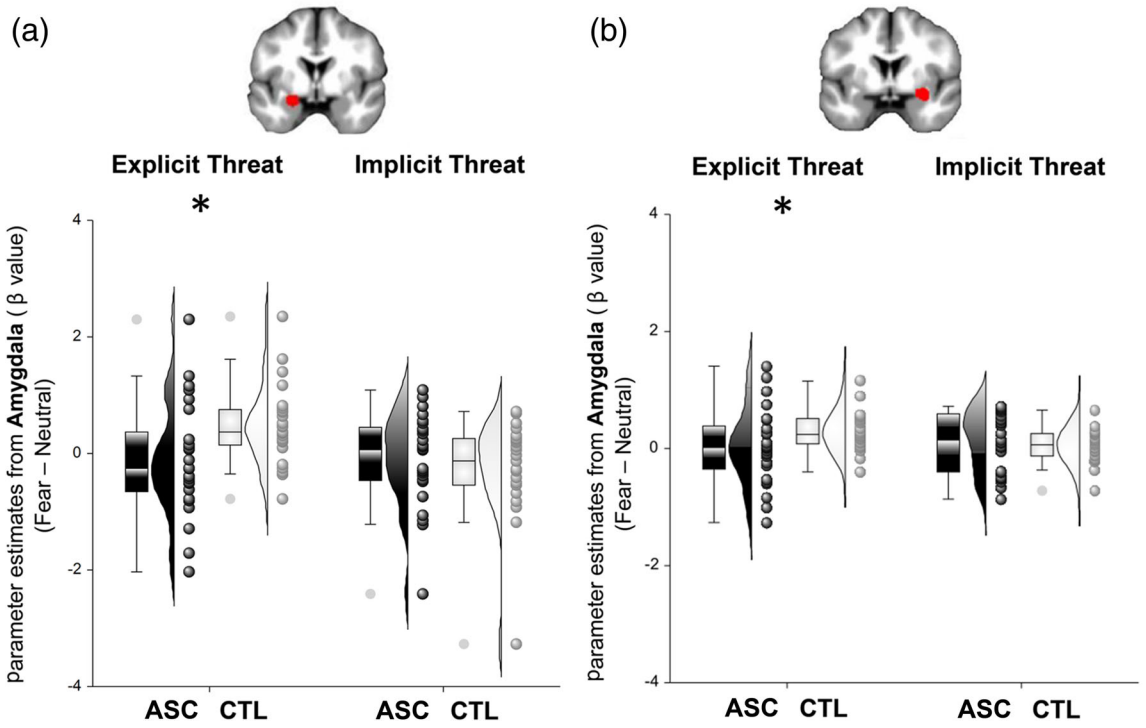

FIGURE 1 Dissociated amygdala reactivity between ASC and controls to explicit and implicit threat. (a) The left amygdala reactivity reveals an interaction of group (ASC vs. CTL) x attention (explicit vs. implicit) ($F_{1,49} = 4.09$, $p = 0.049$). Post hoc analyses indicated that, as compared to controls, ASC individuals show significantly weaker amygdala reactivity in explicit fear ($p = 0.016$), but comparable in implicit fear ($p = 0.55$). (b) The right amygdala reactivity reveals an interaction of group (ASC vs. CTL) × attention (explicit vs. implicit) ($F_{1,49} = 6.09$, $p = 0.017$). Post hoc analysis indicate that, as compared to controls, ASC individuals show significantly weaker amygdala reactivity in explicit fear ($p = 0.025$, one-tailed), but comparable in implicit fear ($p = 0.29$)

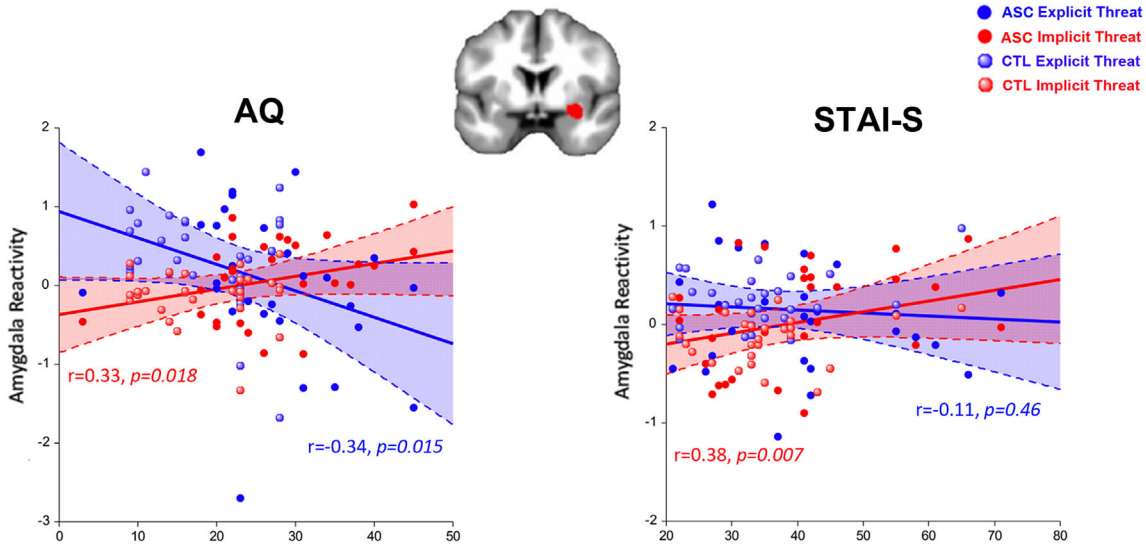

FIGURE 2 Dissociated correlations of autistic traits and anxiety with amygdala reactivity to explicit and implicit threat. The correlation between the autism quotient (AQ) and amygdala reactivity (30, 0, −26) is negative in explicit fear ($r = -0.34$, $p = 0.015$), but positive in implicit fear ($r = 0.33$, $p = 0.018$) when the ASC and control groups are recruited together ($N = 51$). Fisher's z test confirms a significant dissociation ($z = -3.41$, $p < 0.001$). The correlation between STAI-S and amygdala reactivity was positive in implicit fear ($r = 0.38$, $p = 0.007$), but none in implicit fear ($r = -0.11$, $p = 0.455$). Fisher's z tests confirmed a significant differential correlation ($z = -2.49$, $p = 0.013$). STAI, State–Trait Anxiety Inventory

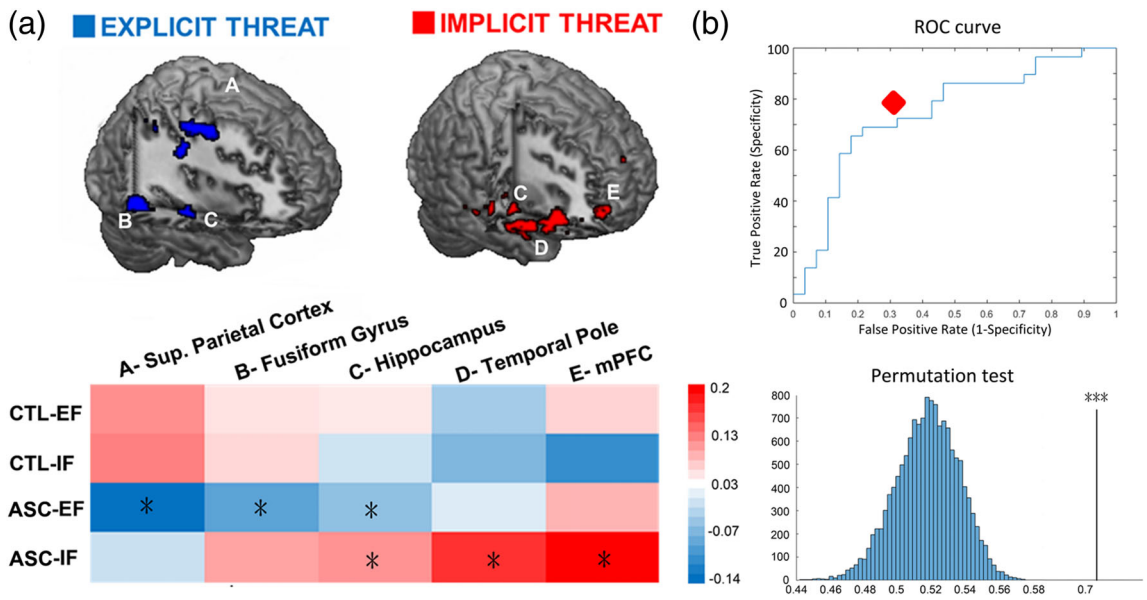

FIGURE 3 Dissociated amygdala functional connectivity by explicit and implicit threat in ASC. (a) A significance plot of the altered correlation between the seed amygdala activity and superior parietal cortex, hippocampus, and fusiform gyrus in ASC. Compared to the controls, individuals with ASC have a significantly greater negative correlations of the amygdala with the superior parietal cortex, fusiform gyrus, and hippocampus when processing explicit threat, whereas a significantly more positive connectivity of the amygdala with the medial prefrontal cortex (mPFC), temporal pole and hippocampus when processing implicit threat. *, family-wise error rate (FWE-corrected) threshold of $p < 0.05$. (b). The LibSVM machine learning model that uses amygdala-centered functional connectivity during explicit and implicit emotional processing predicted the diagnosis of autism (74%, $p < 0.0001$ against the chance level of 50%). FWE, family-wise error

reactivity was positive for implicit fear ($r = 0.379$, $p = 0.007$), but none for explicit fear ($r = -0.108$, $p = 0.455$) (Figure 2). Fisher's z tests confirmed a significant differential correlation ($z = -2.49$, $p = 0.013$).

Functional connectivity and machine learning

To further examine the extent to which ASC-related modulation in threat processing contributed to the functional coupling between different brain regions, we subsequently assessed functional connectivity in the amygdala, whose functions are related to fear processing (Figure 3a). The time series of the first eigenvariates of the BOLD response were temporally filtered, mean-corrected, and deconvolved to generate the time series of the neuronal signal for the source region –the left and right amygdala– as the physiological variable in PPI analysis. Being selected as the PPI source region, the physiological regressor was denoted by the activity in the amygdala. Fear was the psychological regressor. For explicit fear (EF–EN), as compared to the controls, the ASC showed a significantly smaller connectivity of the amygdala with the superior parietal cortex (−26, −60, 47), fusiform gyrus (38, −59, −11), and hippocampus (38, −23, −9). To further examine whether this effect was driven by a weaker positive coupling or stronger negative coupling in ASC, PPI analyses were conducted for ASC and controls separately for descriptive purposes (-Table S2 and Figure S2). There were negative couplings of the amygdala with the superior parietal cortex, fusiform gyrus, and hippocampus when ASC perceived explicit fear. For implicit fear (IF−IN), as compared to the controls, the ASC showed significantly correlations of the amygdala with the hippocampus, temporal pole, and mPFC. Furthermore, the LibSVM (Chang & Lin, 2011) machine learning model identified the predicted diagnostic value of ASC using amygdala-centered functional connectivity during explicit and implicit threat processing (74%, $p < 0.0001$ against the chance level of 50%; positive predictive value: 79%; negative predictive value: 70%; 1/norm(w) = 0.0944; model rho (ρ) = −0.0483) (Figure 3b).

DISCUSSION

In view of the conflicting literature reviewed in the introduction, this study applied machine learning algorithms on the relationship between ASC and anxiety-related amygdala response, as to test whether and how the two mechanisms –implicit and explicit processing of fear– interact in individuals with ASC and anxiety. Individuals with ASC show weaker amygdala reactivity toward explicit fear, although their activity appears to be comparable to the controls during implicit fear. While one preliminary study also uncovered significant bilateral amygdala activation with no group differences between the ASD and CTL during the subconscious presentation of anxious faces in adults with ASD (Hall et al., 2010),

no comparisons in amygdala-centered networks that varied in consciousness-dependent transmission of socially relevant information were reported. The present findings extend the existing scientific literature by demonstrating a significant dissociation dependent on explicit and implicit threat.

The correlation between AQ and amygdala reactivity is dissociated, as it is dependent on whether threat is explicit or implicit. The AQ is probably the most commonly used screening tool developed for quantifying autistic traits (Baron-Cohen, Wheelwright, Skinner, et al., 2001). The AQ has been proven useful when screening clinical samples (Woodbury-Smith et al., 2005), for predicting cognitive tasks performance (Stewart et al., 2009), social cognition (Baron-Cohen, Wheelwright, Hill, et al., 2001), facial mimicry (Hermans et al., 2009), preferences regarding gaze in relation to social stimuli (Bayliss & Tipper, 2005), as well as speech perception (Stewart & Ota, 2008).

In the same line, more severe autistic traits, as assessed by the AQ, were coupled with weaker mismatch negativity (MMN) (Fan & Cheng, 2014). It is until recently that MMN, an auditory event-related potential to a disruptive odd stimulus in a series of stimuli, has been utilized as an index for pre-attentive salience detection of emotional voice processing (Cheng et al., 2012; Hung et al., 2013; Hung & Cheng, 2014). The unpredicted presence of emotional spoken syllables embedded in a passive oddball paradigm were able to activate the amygdala, which was associated with individual differences in social orientation (Schirmer et al., 2008). The amygdala reactivity to explicit and IF faces exhibited opposite associations with MMN (Chen et al., 2017). Thus, it is no surprise to see a dissociation regarding the coupling between AQ and amygdala reactivity dependent on explicit and implicit threat.

In terms of the functional connectivity, ASC individuals exhibited dissociated patterns of amygdala-centered networks during explicit and implicit threat processing. Compared to CTL, ASC individuals with anxiety showed significantly more negative correlations of the amygdala signal with those of the superior parietal cortex, hippocampus, and fusiform gyrus during explicit threat processing, whereas more positive correlations with the mPFC, inferior frontal cortex, temporal pole, and hippocampus, during implicit threat processing. This amygdala-centered dysconnectivity could help explain the high level of anxiety comorbid with ASC and support the transdiagnostic neurobiological nature of anxiety (Chen et al., 2020; Herrington et al., 2016; Herrington, Maddox, McVey, et al., 2017; Top Jr. et al., 2016). On one hand, the existing significantly stronger negative couplings with the superior parietal cortex during explicit threat might explain the reduced amygdala reactivity toward explicit fear in ASCs. On the other hand, during implicit threat processing, an innate "amplifier" of "unseen threat" mediated by the hyperconnectivity with the mPFC, inferior frontal cortex, temporal pole, and hippocampus, dedicated to the consciousness-dependent transmission of socially relevant information may incur in the over-arousal in ASCs (Pessoa & Adolphs, 2010).

Although many research has reported that individuals with ASD have atypical brain connectivity patterns, results of more recent research do not support unanimously this traditional view, where individuals with ASD are described as having lower connectivity between distal brain regions whereas increased connectivity within proximal brain regions (Mohammad-Rezazadeh et al., 2016). For instance, literature reviews have observed a general trend in support of the hypothesis of long-range functional under-connectivity, however, the status of local connectivity still remains unclear (O'Reilly et al., 2017). Thus, further studies concerning connectivity with respect to behavior are warranted, as to probe the underlying neural networks involved in the core deficits of ASD. In the same vein, thalamocortical dysconnectivity could account for sensorimotor symptoms in ASD (Woodward et al., 2017). Restricted interest and repetitive behaviors could develop via inter- and intra-hemispheric functional dysconnectivity (Lee et al., 2016). Here, this study re-identifies the existence of attention dissociated amygdala-centered pathways toward threat (Cole et al., 2016; Friston, 2011). A previous study applying a similar paradigm showed that in healthy participants who were instructed to attend to the emotional facial expression, negative connectivity within both subcortical and cortical pathways to the amygdala, including midbrain, thalamus, visual striate cortex, and medial prefrontal cortices was identified during the explicit fear processing, suggesting a critical role of this reentrant inhibiting feedback in affording such negative affect among typically developing individuals (Williams et al., 2006). In the current study participants were asked to attend to the color of presented pictures, instead of focusing on the emotional expression, ASC individuals with high anxiety showed significantly negative correlations between the amygdala and the superior parietal cortex, hippocampus, and fusiform gyrus, as opposed to controls, indicating that ASD individuals might exert an extra effort (probably an attentional deployment strategy) in regulating emotional responsiveness to compensate for the overwhelming threat even when they were not instructed to do so (Ferri et al., 2016). Whereas poor emotional regulation (ER) and distinct patterns of neural modulation of ER were found in individuals with ASD, mPFC malfunction in regulating emotional response, in connection with limbic networks, was identified as a mechanism explaining anxiety in ASD, in which the disrupted integration in these networks underlay difficulties with the regulation of strong emotional stimuli and resulted in an enhanced perception of threat in people with ASD (Mazefsky et al., 2013; Richey et al., 2015; South & Rodgers, 2017). Similarly, during implicit fear processing, the amygdala-centered dysconnectivity,

involving the networks of the mPFC, inferior frontal cortex, temporal pole, and hippocampus, was exclusively revealed in ASC. This amygdala-mPFC dysconnectivity exclusively for ASC might help explain the over-responsiveness toward the uncertain sensory input in the perception of unaware threat (South & Rodgers, 2017). It is worth mentioning that, given the low SNR attainable in the amygdala, where susceptibility-related dropout is high, it would be advisable for future studies to include TSNR measures across the amygdala. Future studies are encouraged to further compare ASD with and without anxiety as well as controls with and without anxiety to examine the domain-general and domain-specific impairments and pathologies of ASD and anxiety.

A few limitations for this study must be addressed. First, the results' generalizability may be limited, as we only enrolled ASC subjects with IQ higher than 75; hence, the effects might be underestimated. Second, the lack of use of the standard ASD diagnostic instruments such as the Social Communication Questionnaire, the Autism Diagnostic Interview-Revised, or the Autism Diagnostic Observation Schedule (ADOS) (S. Kim & Lord, 2011; Lord et al., 1994; Rutter et al., 2003) might leave the autistic samples in this study insufficiently characterized phenotypically. Notwithstanding, given that the fairly new DSM-5 ASD severity rating system has begun to be explored in order to assess its validity and functionality, and indicating that higher severity ratings in both domains (social communication and restrictive/repetitive behavior) were significantly associated with greater calibrated severity scores of the ADOS–Second Edition (Mazurek et al., 2019), and that the assessments of the AQ and the STAI could help evaluate trait and state anxiety in individuals with autistic traits, the present findings are not to be fully ascribed to a false positive error. Third, the age distribution was relatively wide, encompassing a significantly dynamic window of brain development in this study. However, ASC and CTL individuals did not significantly differ in their age; hence, the results are not to be totally attributed to developmental changes. Finally, further studies applying longer scanning time and event-related designs are encouraged to corroborate the functional connectivity findings in ASD.

CONCLUSION

Taken together, our study identifies the dissociation of amygdala reactivity and functional connectivity dependent on explicit and implicit threat processing. Implicit anxiety in individuals with ASC could outweigh explicitly induced threat. When explicitly perceiving socio-emotional stimuli, ASC individuals with anxiety might use attentional avoidance patterns to restrict affective hyperarousal. This gives a sense of urgency for the need to develop a combined therapy to include an attention-independent behavioral/neural marker concerning anxiety in ASC.

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CONFLICT OF INTEREST

None of the authors have any conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Yu-Chun Chen, Yang-Tang Fan, and Yawei Cheng conceived and conceptualized the study. Yu-Chun Chen, Yang-Tang Fan, and Chenyi Chen collected and analyzed the data. Yu-Chun Chen, Chenyi Chen, and Yawei Cheng conducted the necessary literature reviews and drafted the first manuscript. Róger Marcelo Martínez, Chin-Yau Chen, and Chia-Chien Liu provided critical feedback and helped shape the manuscript. All authors contributed toward the revision and writing the final draft.

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