

## Supplementary Online Content

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**eAppendix.** Recruitment and Screening of Subjects

**eTable 1.** Quality Control Report for Subject-Specific fMRI Data

**eTable 2.** COI Definition Based on Spatial Consistency

**eFigure.** Joint Attention Task

This supplementary material has been provided by the authors to give readers additional information about their work.

## **eAppendix. Recruitment and Screening of Subjects**

In total, 120 female right-handed subjects were recruited for participation by flyers, advertisement on social media/websites and in newspapers, as well as by referral from health care providers (clinics, outpatient care) or former participants, and provided written informed consent to the study protocol approved by the ethics committee of the University of Heidelberg (the study was part of the German Research Foundation Clinical Research Group 256<sup>1</sup>). Prior to the study, subjects underwent thorough screening and interviewing, conducted by M.Sc. level or above psychologist or MD, extensively trained for diagnostics (see Table 1 for subject-specific clinical status, comorbid diagnoses, and diagnostics data, and Table 2 for pairwise data). DSM-IV criteria were assessed by structured interviews, the Structured Clinical Interview for DSM-IV<sup>2</sup> (SCID-I, comorbid axis I) and the International Personality Disorder Examination<sup>3</sup> (IPDE, axis II; for BPD, antisocial, and avoidant personality disorder), respectively.

Volunteers were excluded if they presented general exclusion criteria for MRI measurements including current pregnancy, a lifetime history of head trauma, of neurological illness, or current alcohol abuse/drug use (pregnancy and drug use were assessed by urine tests). Furthermore, healthy subjects were excluded if a lifetime history of psychiatric disorders or substance dependency was reported (assessed using SCID-I and IPDE in  $n = 33$  HC, using thorough shortened screening in  $n = 47$  HC). For BPD patients, a lifetime history of schizophrenia or bipolar I disorder, substance dependence within one year prior to study, or reports of current alcohol or drug use lead to exclusion from the study. Subjects were included in the cBPD group according to DSM-IV criteria: A. presence of five or more BPD specific symptoms; B. at least one symptom present for 5 years or more; C. at least one symptom present at the age of 25 or earlier; and D. clinically significant suffering. Subjects were included in the rBPD group if a previous lifetime diagnosis of BPD was reported but a maximum of three DSM-IV BPD symptoms were met within two years prior to participation in the study. Current use of SSRIs but no other drugs was tolerated, and patients were asked to pause medication intake beginning two weeks prior to participation in the study (supervised by study clinicians). Current use of SSRIs at the time of data acquisition was reported by 5 cBPD patients (2 x Fluoxetine, 1 x Paroxetine, 1 x Sertraline, 1 x Escitalopram).

In addition to demographic and health related data, the following measures were acquired (Table 1): intelligence<sup>4</sup>, Global Assessment of Functioning (clinical expert rating), BPD symptom load (self-report via Borderline Symptom List; clinical expert ratings via IPDE and Zanarini Rating Scale for Borderline Personality Disorder)<sup>3,5,6</sup>, furthermore self-report ratings on non-suicidal self-injurious behavior, dissociation<sup>7</sup>, impulsivity<sup>8</sup>, difficulties in emotion regulation<sup>9</sup>, childhood trauma load<sup>10</sup>, and depressiveness<sup>11</sup>.

Inter-rater reliability was assessed for a randomly selected sub-sample of the video-taped diagnostic interviews, and according to standards rated as “excellent”<sup>12</sup> for both the IPDE and the ZAN-BPD: Intraclass correlations (ICCs) were 0.98 for the number of BPD criteria as assessed via the IPDE, and 0.97 for the total score of the ZAN-BPD.

All subjects were unknown to their partner, but met briefly immediately before the experiment exclusive of verbal communication: they were seated side-by-side, given a cotton ball for saliva oxytocin sampling (instructed to chew and saliva-dampen the cotton) and listened to a brief task instruction repetition; they were then separated to wait for the measurement at their respective scanner lab. No information regarding diagnosis of their partner was provided.

Subject-to-scanner randomization (accounting for scanner differences) as well as initial task role randomization were implemented by computer-based randomization of scanner and role assignments. Mean age of the full sample was  $25.2 \pm 4.4$  years with an average education of  $12.4 \pm 1.2$  years. A one way ANOVA revealed a significant main effect of age ( $F_{(2,117)} = 5.7, P = 0.004$ ), but not education ( $F_{(2,117)} = 1.1, P = 0.3$ ), across groups. The difference was caused by significantly/trend-level older remitted patients (t-tests for independent samples,  $T_{\text{BPD-HC/cBPD-HC}}(78) = 1.8, P = 0.07$ ;  $T_{\text{rBPD-HC/HC-HC}}(56.5) = 3.5, P = 0.001$ ;  $T_{\text{cBPD-HC/HC-HC}}(79.5) = 1.6, P = 0.11$ ). Subsequently, possible effects of age were accounted for by including age as a covariate in all analyses.

## **Task**

The task included task phases of interaction (INT, 5s duration), where one subject (sender) received information on a target location (top, bottom, left, or right corresponding, to response device buttons) via on-screen presentation in addition to a centered video image of the partner. Targets were indicated by a square shape, presented among three distractor shapes, and target location randomly switched between trials (eFigure 1). During each trial, the sender shared the information by shifting her gaze in the direction of the target location. The partner (receiver) had to follow the eye-gaze direction while viewing four plus-shaped non-targets only. A trial was successful only if both subjects indicated the correct target location (i.e., if transfer of information was successful), and subjects were instructed to engage in the interaction while responding as fast as possible. Each INT was followed by phases of no interaction (NoINT; 5s duration) where the target location was presented to both subjects, who performed the task individually, while the live video stream remained. Subjects then received feedback on their trial performance for 3s (e.g., “Successful cooperation!”). In sum, 40 trials of each type were performed. Task roles (sender/receiver) were switched after 20 alternating trials of each type and initial roles

were randomly assigned. Trial onsets were pseudo-jittered (random jitters across trials, same onsets for all subjects), resulting in a total task time of 645s.

### **Behavioral data**

The JA task was designed as a simple and early learned from of interaction for the investigation of cross-brain information flow under cooperative interactive conditions. This resulted in an average successful trial completion of 94% with SD = 6.6 (mean<sub>cBPD-HC</sub> = 93.4%, SD = 7.8; mean<sub>rBPD-HC</sub> = 93.4%, SD = 6.1; mean<sub>HC-HC</sub> = 94.0%, SD = 5.8), with a mean reaction time of 2.24s and SD = 0.3 (mean<sub>cBPD-HC</sub> = 2.18s, SD = 0.3; mean<sub>rBPD-HC</sub> = 2.33s, SD = 0.3; mean<sub>HC-HC</sub> = 2.25s, SD = 0.3). As hypothesized, we found no group differences in task performance in two main readouts of the behavioral data: neither the regression model examining the percentage of successful interaction trials ( $T = 0.3$ ,  $p = 0.76$ ), nor the same model comparing mean reaction time ( $T = 0.96$ ,  $p = 0.34$ ) indicated a significant difference, thus behavioral data was not included in further analyses of neural coupling. This highlights the importance of follow-up studies investigating different social behavior of sufficient variability to associate behavioral and neural data; possibly anchoring neural coupling in every day human life.

### **Data Acquisition and Preprocessing**

Data were acquired at two identical labs at CIMH, each equipped with a Siemens Trio 3T MRI scanner (Siemens, Erlangen, Germany), as well as a custom designed mirror box (MRC Systems GmbH, Heidelberg, Germany) holding face camera. Sites were connected via fiber optics transmitting MRI triggers, behavioral data, and video stream. The same echo-planar imaging protocols were used: TR=1550ms, TE=30ms, FOV=192mm<sup>2</sup>, 28 slices, 4mm thickness, 1mm gap, flip angle 73°, 423/420 volumes (triggering/triggered scanner<sup>13</sup>). Data were inspected for data quality measures (eTable 1). Preprocessing was conducted within Statistical Parametric Mapping software (SPM8, <http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>) within MATLAB (v.2011b, <http://www.mathworks.com/products/matlab/>) and included realignment to mean image, slice time correction, normalization to standard stereotactic space (Montreal Neurological Institute, MNI), and smoothing using a Gaussian kernel filter with 8 mm full-width-at-half-maximum.

### **Group ICA and COI Selection**

We performed a group analysis of independent components (group ICA; GIFT toolbox for MATLAB v3.0a, <http://mialab.mrn.org/software/gift/index.html>), for which preprocessed data sets were concatenated temporally and components were estimated based on the full data. The estimation was blind for task, pair, or group assignment. Task blocks were entered as sessions. Component numbering was arbitrary, and 16 components (scaled to percent signal change of the blood oxygen level dependent signal) were estimated for the full data set as defined by minimum description length criterion. In prior work, rTPJ was found as the core region driving cross-brain neural coupling<sup>13</sup>, and we selected the component representing this brain region as the component of interest (COI) to undergo further analysis<sup>13</sup>. For this, a mask was created for the rTPJ using WFU PickAtlas and IBASPM116 catalogue. Furthermore, to credit a meaningful parcellation of rTPJ regarding an anterior and a posterior share, provided spatial maps for each subregion were used<sup>14</sup>. Lastly, the components we had previously identified to display significant neural coupling in two samples<sup>13</sup> were binarized with a threshold of 0.15. As illustrated in eTable 2, all comparisons favored component #13 as the COI by means of higher regression parameters for this component than any other component within a multiple regression analysis comparing spatial consistency of components with the respective target spatial map (Figure 2a).

To assure (temporal) specificity of the COI to interaction, we performed a multiple regression analysis using the task design as the dependent variable and subject-specific time course as dependent measure, resulting in regression parameters indicating the engagement of the COI to INT and NoINT<sup>13</sup>. For an interaction-related COI, a higher association is required with INT than NoINT. To test this, subject parameters were then compared between task phases within a repeated measures ANOVA (within-subject factor task-blocks A and B, between-subject factors group and age). The direct comparison of COI association to task phases revealed a significant difference in association levels, with higher beta values for INT (0.29) as compared to NoINT (0.15;  $F_{(2,238)} = 20.1$ ,  $P < 0.001$ ). Further tests showed no main effect of group was found for the full sample ( $F_{(2,116)} = 0.5$ ,  $P = 0.6$ ), or when comparing BPD subjects only to HC-HC ( $F_{(2,76)} = 1.2$ ,  $P = 0.3$ ).

### **Temporal characteristics of neural coupling**

The within-pair contrast of tasks blocks A and B of the repeated measures ANOVA reported for the main group comparison of neural coupling examines changes of neural coupling over time (either in the form of an increase over time due to social learning with the task partner or as habituation to the task). Here, we found no differences between the first and second half of the paradigm ( $F_{(1,55)} = 2.0$ ,  $p = 0.16$ ), and no significant time\*group interaction ( $F_{(2,55)} = 2.2$ ,  $p = 0.12$ ).

## Clinical status and neural coupling

While our cBPD and rBPD did not differ in treatment variables such as the number of therapies received, types of therapies received or types of psychoactive drugs used (see Table 1) we further conducted a supplementary analysis to exclude the possibility of a hidden influence on clinical measures on the observed group differences in neural coupling.

Importantly, depressiveness (as assessed via BDI II) was observed to be significantly higher in cBPD than rBPD. In addition, patients may possibly feel much more anxious and show different levels of psychopathology in unforeseen situations, in particular in a high arousal state. We therefore took into account the arousal of patients prior to the MRI scan (quantified using the Self-Assessment Manikin, SAM)<sup>15</sup>. These measures were included in the main analysis investigating neural coupling to control for clinical status (repeated measures ANOVA; for cBPD-HC and rBPD-HC), and found our core main group effect to remain ( $F_{(1,29)} = 5.4, p = 0.03$ ). Concluding, although depressiveness and aroused state successfully captured symptom-based differences between cBPD and rBPD, these measures did not account for differences in our neural interaction-related readout.

## BPD-specific component selection

Single-subject fMRI studies have outlined a dysregulation of the Amygdala and the insula cortex as a commonly found neural abnormality in BPD<sup>16-19</sup>. In order to examine these regions in the context of neural coupling in BPD, ICA components representing amygdala (#6) and insula cortex (#15) were selected as described for the selection of the rTPJ component using predefined masks (WFU PickAtlas and IBASPM116 catalogue) and underwent the full selection procedure described in<sup>13</sup>. For Hyperscanning data analysis, we preselect ICA components that are associated with social interaction, or INT, respectively (see section Group ICA and COI Selection above). To gain a measure of association with interaction, a regression analysis was performed using task design as the dependent measure (separating INT and NoINT), and subject-specific time-courses for the respective component as the independent measure. This results in beta coefficients representing the engagement of the component with the respective task phase. For an interaction-related component of interest (COI), a significant positive engagement during task phases of interaction (INT) and compared to individual performance (NoINT) is required, which is captured by a comparison of these beta weights. We therefore compared these beta weights in a repeated measures ANOVA (within-subject factors: task phase, task block; between-subject factors: group). Similarly to considerations above, significant group differences would point to a differential engagement of the brain region to the task (i.e., either diminished activation or no difference between interaction and no interaction), which we do not expect for BPD.

For both components (amygdala #6, insula #15) we did observe higher absolute mean beta values for NoINT than for INT, a significant difference between task phases (amygdala:  $F_{(2,234)} = 6.4, p = 0.01$ ; insula: ( $F_{(2,234)} = 39.8, p < 0.001$ ), but no group\*phase interaction (amygdala:  $F_{(2,234)} = 1.2, p = 0.32$ ; insula: ( $F_{(2,234)} = 0.14, p = 0.87$ ). (see figure below). Since both amygdala as well as insula networks therefore exhibited activity that was not specifically higher during interaction, they were not considered for coupling analysis.

## Voxel-based morphometry

T1-weighted images were acquired in a magnetization-prepared rapid gradient echo sequence (TR = 2300ms, TE = 3.03ms, TI = 900ms, FOV = 256x256mm<sup>2</sup>, flip angle = 9°, plane = transaxial, slice thickness = 1mm, voxel size = 1mm<sup>3</sup> isomorph, 192 slices). Analyses were performed using default parameters within the VBM8 toolbox in SPM8. Following segmentation based on tissue probability maps, images were bias corrected, regularized by affine warping, and normalized to MNI space by applying diffeomorphic anatomic registration through exponentiated lie algebra<sup>20</sup>. Normalized images were smoothed using an 8mm Gaussian kernel (Full Width at Half Maximum).

**eTable 1. Quality Control Report for Subject-Specific fMRI Data**

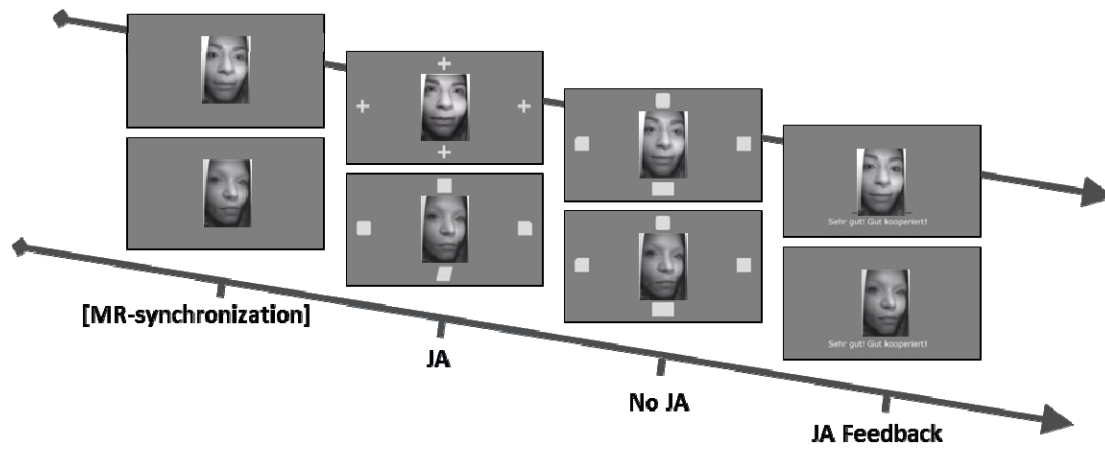
	cBPD-HC		rBPD-HC		HC-HC		ANOVA/ $\chi^2$
	mean $\pm$ SD	N	mean $\pm$ SD	N	mean $\pm$ SD	N	P
<i>Quality control measures</i>							
SNR	114.7 $\pm$ 16.5	46	107.8 $\pm$ 22.7	34	116.5 $\pm$ 12.1	40	n.s. <sup>a</sup>
Spikes	4.9 $\pm$ 14.6	46	0.7 $\pm$ 3.0	34	12.0 $\pm$ 59.2	40	n.s. <sup>b</sup>
Framewise displacement	0.9 $\pm$ 0.5	46	1.0 $\pm$ 0.7	34	0.9 $\pm$ 0.7	40	n.s. <sup>a</sup>

n.s.  $p > 0.05$ ; <sup>a</sup>, ANOVA; <sup>b</sup>,  $\chi^2$ ; N, number of available data; SD, standard deviation; ANOVA, univariate ANOVA;  $\chi^2$ , chi-squared test; BPD, Borderline Personality Disorder; cBPD, current BPD patient; rBPD, remitted BPD patient; HC, healthy control subject; SNR, signal-to-noise-ratio; Spikes, number of time points in which the signal intensity is larger than  $10 \times$ SD of the mean signal.

**eTable 2. COI Definition Based on Spatial Consistency**

	$\beta$	
	COI (#13)	2 <sup>nd</sup> highest component
COI <sub>previous study 1</sub>	2.8	0.3
COI <sub>previous study 2</sub>	2.4	0.7
rTPJ	1.5	1.4
posterior rTPJ	3.4	1.4
anterior rTPJ	2.9	1.3

COI, component of interest; rTPJ, right temporoparietal junction;  $\beta$ , beta weight gained from multiple regression analysis.



**eFigure.** Joint Attention Task

The joint attention task requires social interaction and information flow between pairs during cooperative task completion (JA), and includes phases of individual performance (No JA) for comparison.

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