The Modulatory Effect of Oxytocin on Interoception

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Study Protocol with SAP

Participants and treatment

83 healthy male students (mean age = 21.35 years, SD = 2.48) participated in a randomized, double-blind, between-subject pharmaco-fMRI experiment where they received either intranasal OT (40 IU; Oxytocin Spray, Sichuan Meike Pharmacy Co. Ltd, China) or placebo (PLC, same ingredients other than OT). All subjects were right-handed and had normal or corrected-to-normal vision. None was currently taking any form of medication or reported a history of, or current, neurological or psychiatric symptoms. 8 subjects were excluded due to low quality of ECG data (6 subjects) or failure of behavioral data acquisition (2 subjects). Thus, 37 subjects in the OT group and 38 subjects in the PLC group were included in the final analysis.

Questionnaires

To control potential confounding effects from personality traits or current mood states, all subjects completed Chinese versions of validated psychometric questionnaires before OT/PLC administration, including the Positive and Negative Affect Schedule (Watson *et al,* 1988), Autism Spectrum Quotient (Baron-Cohen *et al,* 2001), Empathy Quotient (Baron-Cohen and Wheelwright, 2004), and NEO five-factor inventory (Costa and MacCrae, 1992). Subjects were asked to fill the Positive and Negative Affect Schedule for 3 times with the first time after they arrived the lab (pre-treatment), the second time before scanning (post-treatment), and the third time after scan (post-scan).

Experiment 1

Experiment 1 consisted of two types of task. In the heartbeat detection task participants were instructed to make a keypress each time they felt their heartbeat, while in a dot detection control task participants were instructed to make a keypress each time they detected a white dot appearing on the screen. Thus, the design allowed specific investigation of interoceptive processing by comparing attention to one's own heartbeat with attention to neutral-valenced, non-social exteroceptive visual stimuli. There were 2 functional runs in Experiment 1 and each run consisted of 6 blocks. In each run, 3 heartbeat detection blocks alternated with 3 dot detection blocks with a jittered interval of 8-12 s. Blocks were preceded by a 2-s cue indicating the task type of the subsequent block. Each block lasted 30 s and the order of the two types of block was counterbalanced across subjects. An identical, but static white dot was also presented during heartbeat detection in order to maintain visual conformity across the two tasks. Interoceptive accuracy was calculated as the mean score across allof the heartbeat detection blocks using the following transformation (Pollatos *et al,* 2007a, 2007b):

$$1/N \sum (1 - (|\text{recorded heartbeats} - \text{counted heartbeats}|)/\text{recorded heartbeats})$$

N was the number of blocks for the heartbeat detection task.

In the control task, to avoid subjects pressing the key regularly after repeated sessions the dot was presented at a varying rate of 26-40 times/30 s (mean ± standard deviation = 32.67 ± 5.65) across blocks. This rate was determined in accordance with the normal range of resting heart rate in healthy Chinese young male adults (51-96 beats/min) (Wu *et al,* 2001), with a 25% random variance (Zaki *et al,* 2012). For

example, if a subject's heart rate was 60 times/min, the dot was presented every 1.00 ± 0.25 s. To make the accuracy of the two types of task comparable, the accuracy in the control task was calculated in a similar way to the interoceptive accuracy using the following transformation:

$$1/N \sum (1 - (|\text{presented rate} - \text{tap rate}|)/\text{presented rate})$$

The presented rate was the presentation times of the dot during each 30-s control block. The tap rate was the keypress numbers made by the subject during each 30-s control block. N was the number of blocks for the control task.

Experiment 2

There were 3 functional runs in Experiment 2. Each run comprised 10 blocks with 2 blocks in each condition (happy, disgust, fearful, neutral faces and scrambled faces). The 10 blocks were presented in a pseudorandom order with a jittered interval of 8-12 s. Each block consisted of 5 faces with the same expression and each face was displayed for 6 s. Thus, each block lasted for 30 s. Interoceptive accuracy was calculated as the mean score of 6 heartbeat detection blocks for each face stimuli using the same transformation used in Experiment 1.

The emotional faces of 5 males and 5 females with happy, disgust, fearful and neutral expressions were selected from the Taiwanese Facial Expression Image Database (Chen and Yen, 2007). These faces were selected on the basis of high emotion discrimination accuracy as determined by a pilot rating study including 40 subjects not involved in the current study. All faces were carefully edited (hair, ears and background information removed) to reduce confounding effects from irrelevant

features. The scrambled mosaics were created by dividing a neutral face into 25 parts and then randomly rearranging them into a mosaic-scrambled image. All faces and images were presented in full color at 400 × 500 pixel resolution.

ECG Acquisition and Processing

ECG was obtained using an ECG100C-MRI-compatible amplifier module and was recorded using an MP150 system running AcqKnowledge v4.2 software (BIOPAC Systems, Inc.) with three MRI-compatible electrodes placed on participants' chest. The data was sampled at 2000 Hz and the ECG100c-MRI was set to a gain of 1000, with 1 Hz HP set to ON and 35 Hz LPN set to ON, in accordance with the BIOPAC manual. To remove the echo planar imaging (EPI) artifact, the raw ECG data was preprocessed in AcqKnowledge v4.2 software by performing a Comb Band Stop Filter (fixed at 18 Hz with a Q of 20), followed by a digital filter with a band pass between 0.5 and 35 Hz. Next, the RR intervals were extracted and manually corrected for artifacts. The resulting intervals were then analyzed and transformed into heartbeat number/30 s (i.e. to match the blocks in the study which were 30 s) for each condition using a custom Matlab script (Matlab 2013a).

Image Acquisition and Data Analysis

Images were collected using a 3 T, GE Discovery MR750 scanner (General Electric Medical System, Milwaukee, WI, USA). During each fMRI scan, a time series of volumes was acquired using a T2*-weighted EPI pulse sequence (repetition time, 2000 ms; echo time, 30 ms; slices, 39; thickness, 4 mm; gap, 1 mm; field of view, 240 × 240 mm; resolution, 64 × 64; flip angle, 90°). High-resolution whole-brain volume

T1*-weighted images were acquired obliquely with a three-dimensional spoiled gradient echo pulse sequence (repetition time, 6 ms; echo time, 2 ms; flip angle, 9°; field of view = 256 × 256 mm; acquisition matrix, 256 × 256; thickness, 1 mm; number of slices, 156) to control for any anatomic abnormalities and increase normalization accuracy during pre-processing.

Brain images were processed using the SPM8 software package (Wellcome Department of Cognitive Neurology, London, UK, http://www.fil.ion.ucl.ac.uk/spm/spm8) (Friston *et al,* 1994). The first five images were excluded to achieve magnet-steady images and the remaining functional images were realigned to correct for head motion based on a six-parameter rigid body algorithm. After co-registering the mean functional image and the T1 image, the T1 image was segmented to determine the parameters for normalizing the functional images to Montreal Neurological Institute (MNI) space. Next normalized images were spatially smoothed with a Gaussian kernel (8 mm full-width at half maximum, FWHM).

To further examine OT's effects during interoceptive processing, we performed exploratory psychophysiological interaction (PPI) analyses using the PPI toolbox (McLaren et al, 2012). In brief, this entails regressing responses throughout the brain on the activity of one or more seed regions. The PPI corresponds to a difference in this regression under different (psychological) experimental levels. In the present study, we asked whether OT modulates the coupling between AI and other brain regions by comparing the (physiological) effects – from the seed region – between the OT and PLC groups.

For the brain behavior analyses, parameter estimates were extracted from 6-mm sphere ROIs based on the corresponding first-level contrast representing the average activity across face types for each subject using MarsBar toolbox (Brett et al., 2002). For the connectivity analysis the mean regression coefficient for each subject was extracted as a measure of connectivity strengths using the same contrast. Pearson correlations were next calculated based on the subject-specific parameter estimates and the corresponding interoceptive accuracy score (average accuracy across all face types) from each subject.

Group Difference of Head Motion

To examine confounding effects of head motion, we used a two-sample t-test on the mean framewise displacement using Jenkinson's relative root mean square algorithm (Jenkinson et al, 2002) to examine the group difference of head motion. Results showed no significant difference of head motion between OT and PLC groups either in Experiment 1 ($t(73) = 1.47$, $p = 0.145$) or Experiment 2 ($t(73) = 0.56$, $p = 0.581$).

Results of Personality and Mood Questionnaires

There were no significant group differences on questionnaire scores measuring mood, autistic traits, empathy, and personality (Table S1). Repeated-measurement ANOVA with time (pre-treatment vs. post-treatment vs. post-scan) as within-subject factor and treatment (OT vs. PLC) as between-subject factor were performed separately on the positive and negative mood scores (Positive and Negative Affective Scale). For the positive mood, results showed a significant main effect of time (F(2,

140) = 8.02, p = 0.001), with significant decrease of the positive mood from pre-treatment (p = 0.004) and post-treatment (p = 0.038) to post-scan. For the negative mood, there was also a significant main effect of time (F(2, 140) = 34.05, p < 0.001), with significant decrease of negative mood from pre-treatment to post-treatment (p < 0.001) and post-scan (p < 0.001). The negative mood decrease from post-treatment to post-scan was also significant (p = 0.042). However, there were no significant main effects of group and its interaction with time (ps > 0.394).

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Table S1. Questionnaire scores in the OT and PLC group (mean ± sd).

Measurements	OT	PLC	t-value	*p*-value
Age	21.2±2.5	21.5±2.4	-0.63	0.529
Positive and Negative Affective Scale [1st]				
- Positive	29.9±6.0	28.7±6.4	0.88	0.383
- Negative	16.4±5.3	16.8±6.0	-0.30	0.769
Positive and Negative Affective Scale [2nd]				
- Positive	29.1±7.9	28.2±6.2	0.54	0.591
- Negative	13.4±4.3	14.2±5.1	-0.71	0.478
Positive and Negative Affective Scale [3rd]				
- Positive	27.4±7.7	25.6±7.7	1.00	0.320
- Negative	12.1±3.7	13.3±4.1	-1.32	0.191
Autism Spectrum Quotient	18.7±5.0	19.2±5.3	-0.43	0.669
Empathy Quotient	42.0±9.1	40.2±11.0	0.77	0.444
NEO five-factor inventory				
Neuroticism	29.6±7.7	31.3±6.8	-0.98	0.333
Extroversion	44.4±5.4	42.3±7.0	1.45	0.151
Openness	40.1±4.7	40.2±5.3	-0.09	0.930
Agreeableness	41.4±4.0	41.6±5.3	-0.21	0.835
Conscientiousness	43.6±5.3	42.5±4.3	1.00	0.319

95% Confidence interval.

Table S2. Regions out of the a priori ROIs in Experiment 1 (MNI coordinates).

Brain Region	BA	No. Voxels	Peak t-value	x	y	z
Interoception > control task						
L. Precuneus	7/18/19/31	12747	11.09	-4	-76	38
Precuneus			11.08	6	-74	40
Precuneus			10.55	-12	-76	46
L. Middle Frontal Gyrus	32/47/10	7072	10.17	-30	54	14
Inferior Frontal Gyrus			9.89	-46	36	8
Middle Frontal Gyrus			9.08	-46	34	18
R. Middle Frontal Gyrus	10/47/13	4150	8.00	30	54	4
Middle Frontal Gyrus			7.90	32	54	16
Posterior Insula			7.77	44	-14	14
L. Thalamus		36	6.69	-24	-28	-2
R. Thalamus		36	6.35	22	-26	-2
L. Superior Temporal Gyrus	42/22	75	6.19	-64	-26	6
L. Inferior Parietal Lobule	40	323	5.97	-50	-46	50
Inferior Parietal Lobule			5.91	-58	-36	38
R. Posterior Insula		28	5.55	38	-12	-8
R. Postcentral Gyrus	2/1	33	5.54	66	-24	36
R. Middle Frontal Gyrus	46	39	5.26	46	28	26
L. Caudate		19	5.25	-12	10	2
OT interoception > control task > PLC						
interoception > control task						
None						
OT interoception task > PLC						
interoception task						
None						

All with a $P_{FWE} < 0.05$ correction threshold and cluster > 10 voxels at whole brain level.
L indicates left; R indicates right.

Table S3. Main effect of face processing in Experiment 2 (MNI coordinates).

Brain Region	BA	No. Voxels	Peak t-value	x	y	z
L. Cuneus	18/19/37/17	6725	18.65	-20	-96	8
Middle Occipital Gyrus			18.20	20	-96	12
L. Superior Frontal Gyrus	6/8/10	4070	11.44	-12	36	52
Superior Frontal Gyrus			11.21	18	56	32
Middle Frontal Gyrus			11.01	-40	28	42
R. Middle Frontal Gyrus	9/45/46/47	2466	11.38	54	8	42
Middle Frontal Gyrus			10.72	50	38	24
R. Postcentral Gyrus	40/2	79	7.54	58	-34	52
L. Superior Temporal Gyrus	22	16	5.52	-48	2	-2
R. Superior Temporal Gyrus	22	98	6.80	52	-32	6
L. Putamen		134	6.73	-26	-4	6
R. Putamen		31	6.02	24	2	6
R. Parahippocampal Gyrus		21	6.71	26	-12	-16
R. Thalamus		31	6.47	22	-28	-2
L. Inferior Parietal Lobule	40	16	5.98	-54	-56	44
L. Medial Frontal Gyrus		26	5.82	-10	-18	62
L. Precentral Gyrus	6	13	5.61	-34	-14	48
L. Anterior Insula		114	5.50[a]	-46	2	-2
R. Anterior Insula		151	6.41[a]	46	12	2
L. Anterior Cingulate Cortex		55	4.75[a]	-4	52	12
L. Anterior Cingulate Cortex		13	4.42[a]	-4	40	26
R. Anterior Cingulate Cortex		37	4.88[a]	2	54	12
L. Amygdala		13	4.97[a]	-24	-10	-14
R. Amygdala		14	5.39[a]	26	-8	-16

All with a $P_{FWE} < 0.05$ correction threshold and cluster > 10 voxels at whole brain level.

L indicates left; R indicates right. [a]$P_{FWE} < 0.05$ small volume correction.